IntechOpen

Brain Injury

Pathogenesis, Monitoring, Recovery and Management

Edited by Amit Agrawal

BRAIN INJURY – PATHOGENESIS, MONITORING, RECOVERY AND MANAGEMENT

Edited by **Amit Agrawal**

Brain Injury - Pathogenesis, Monitoring, Recovery and Management
http://dx.doi.org/10.5772/1074
Edited by Amit Agrawal

Contributors

Rivelilson Mendes De Freitas, Jesús Devesa, Pablo Devesa, Pedro Reimunde, Víctor Arce, Bronwen Connor, Arulselvi --- Subramanian, Ravindra Mohan Pandey, Deepak Agrawal, Mohita Nimiya, Venencia Albert, Ryszard Pluta, John Weber, Aysegul Bayir, Hovhannes M Manvelyan, Jennifer Smith, Stephen Turner, Sharon Kardia, Thomas Mosley, Efthimios Dardiotis, Vaios Karanikas, Konstantinos N Paterakis, Kostas N. Fountas, Georgios Hadjigeorgiou, Giuseppe Lazzarino, Roberto Vagnozzi, Massimo Manara, Barbara Tavazzi, Roberto Floris, Angela Maria Amorini, Stefano Signoretti, Andrea Ludovici, Simone Marziali, Tracy K McIntosh, Thomas Nathaniel, Diane Haleem, Adam Brager, Effiong Otukonyong, Zamzuri Idris, Muzaimi Mustapha, Jafri Malin Abdullah, Stanislaw P. Stawicki, Steven Steinberg, Paul Beery 2nd, Johathan Wisler, Mamadou Diop, Keith St Lawrence, Jonathan T. Elliott, Ting-Yim Lee, Zhiyong Yin, Daiqin Tao, Hui Zhao, Ronald Hayes, Abhay Varma, Angela Hays, Riikka Immonen, Nick Hayward, Harlambos Gatos, Konstantinos Paterakis, Apostolos Komnos, Eftychia Kapsalaki, Austin Colohan, Dare Adewumi, Akshay Anand, Yuchuan Ding, David III Dornbos, Francis E Umesiri, Katelin Michele Haley, Leah Dziopa, Julia Glukhoy, Rahul Dani

Notice

First published in Croatia, 2012 by INTECH d.o.o.
eBook (PDF) Published by IN TECH d.o.o.
Place and year of publication of eBook (PDF): Rijeka, 2019.
IntechOpen is the global imprint of IN TECH d.o.o.
Printed in Croatia

Legal deposit, Croatia: National and University Library in Zagreb

Additional hard and PDF copies can be obtained from orders@intechopen.com

Brain Injury - Pathogenesis, Monitoring, Recovery and Management
Edited by Amit Agrawal

p. cm.

ISBN 978-953-51-0265-6

eBook (PDF) ISBN 978-953-51-5259-0

Meet the editor

Dr Agrawal completed his neurosurgery training from National Institute of Mental Health and Neurosciences, Bangalore (India) in the year 2003. He is extremely enthusiastic, and result oriented professional with over 11 years of rich experience in Research & Development & Teaching & Mentoring in the field of neurosurgery. He has attended and participated in many international & national level symposiums & conferences and delivered lectures on vivid topics. He has published more than 300 articles in the medical field covering various topics in various national and international journals. He is the Editor-in-Chief of Journal of Neurosciences in Rural Practice (www.ruralneuropractice.com). Presently he is Professor and head, Department of Neurosurgery, MM Institute of Medical Sciences and Research, Mullana (Ambala), India and promoting the concept of Neurosciences in Rural Practice through the Journal of Neurosciences in Rural Practice.

Contents

Preface

Brain injury remains one of the most difficult and challenging problems facing many researchers, clinicians and experts involved in care of these patients. The present two volume book "Brain Injury" is distinctive in its presentation and includes a wealth of updated information for professionals on the high quality research on many aspects in the field of brain injury as well as addresses the most difficult and challenging issues in the management and rehabilitation of brain injured patients. The Brain Injury - Pathogenesis, Monitoring, Recovery and Management contains 5 sections and a total 26 chapters devoted to pathogenesis of brain injury, concepts in cerebral blood flow and metabolism, investigative approaches and monitoring of brain injured, different protective mechanisms and recovery and management approach to these individuals and Book Two contains (3 sections) 12 chapters devoted to functional and endocrine aspects of brain injuries, approaches to rehabilitation of brain injured and preventive aspects of traumatic brain injuries.

Chapters in the book discus current understandings and experimental approaches, emerging role of innate and adaptive immunity, genetic effects among measures of cognitive function, compensatory neurogenesis in injured adult brain. Further the issues discussed include effects of melatonin and Alzheimer's factors on brain injury, lleukocyte response and immediate outcome in traumatic brain injury. Chapters 8 to 10 discuss the experimental models of ischemia, quantitative cerebral blood flow assessment and MRI characterization of progressive brain alterations after experimental traumatic brain injury. Chapters 11-14 address the issues in neurointensive care monitoring, dynamic visualization technology in brain deceleration injury research, experimental technology on the brain impact injuries and non-invasive bedside monitoring of cerebral blood flow and oxygen metabolism with near-infrared spectroscopy respectively. In Section IV protective mechanisms of neuroprotection in ischemia/reperfusion Injury and the issues of recovery have been discussed in details. Section V conservative as well operative management approaches to treat brain injury have been discussed. The role of decompressive craniectomy especially discussed in details.

I hope that collective contribution from experts in brain injury research area would be successfully conveyed to the readers and readers will find this book to be a valuable guide to further develop their understanding about brain injury. I am grateful to all of

the authors who have contributed their tremendous expertise to the present book, my wife and daughter for their passionate support and last but not least I wish to acknowledge the outstanding support from Mr. Bojan Rafaj, Publishing Process manager, InTech Croatia who collaborated tirelessly in crafting this book.

Dr Amit Agrawal
Professor of Neurosurgery
MM Institute of Medical Sciences & Research
Maharishi Markandeshwar University
India

Part 1

Understanding Pathogenesis

1

Current Understanding and Experimental Approaches to the Study of Repetitive Brain Injury

John T. Weber
Memorial University of Newfoundland
Canada

1. Introduction

Repetitive traumatic brain injury (TBI) occurs in a considerable number of individuals in the general population, such as athletes involved in contact sports (e.g. boxing, football, hockey and soccer), or child abuse victims. Repeated mild injuries, such as concussions, may cause cumulative damage to the brain and result in long-term cognitive dysfunction. The growing field of repetitive TBI research is reflected in the increased media attention given to reporting incidences of athletes suffering multiple blows to the head, and in several recent experimental studies of repeated mild TBI *in vivo*. Experimental reports generally demonstrate cellular and cognitive abnormalities after repetitive injury using rodent TBI models. In some cases, data suggests that the effects of a second mild TBI may be synergistic, rather than additive. In addition, some studies have found increases in cellular markers associated with Alzheimer's disease after repeated mild injuries, which demonstrates a direct experimental link between repetitive TBI and neurodegenerative disease. To complement the findings from humans and *in vivo* experimentation, my laboratory group has investigated the effects of repeated trauma in cultured brain cells using an *in vitro* model of stretch-induced mechanical injury. In these studies, cells exhibit cumulative damage when receiving multiple mild injuries. Interestingly, the extent of damage to the cells is dependent on the time between repeated injuries. Although direct comparisons to the clinical situation are difficult to make, these types of repetitive, low-level, mechanical stresses may be similar to insults received by certain athletes, such as boxers, or hockey and soccer players. As this field of TBI research continues to evolve and expand, it is essential that experimental models of repetitive injury replicate injuries in humans as closely as possible. For example, it is important to appropriately model concussive episodes versus even lower-level injuries (such as those that might occur during boxing matches or by heading a ball repeatedly in soccer). Suitable inter-injury intervals are also important parameters to incorporate into studies. Additionally, it is essential to design and utilize proper controls, which can be more of a challenge than experimental approaches to single mild TBI. These issues, as well as an overview of findings from repeated TBI research, are discussed in this chapter.

2. Overview of TBI

2.1 Occurrence and impact of TBI

Traumatic brain injury (TBI) is an insult to the brain caused by an external physical force, resulting in functional disability. Falls and motor vehicle accidents are the primary causes of TBI, while sports, assaults and gunshot wounds also contribute significantly to these types of injuries (Centre for Disease Control, 2010). TBI is one of the leading causes of death and disability worldwide, including the developing world (Reilly, 2007). In the United Kingdom, an estimated 200-300 per 100,000 people are hospitalized every year due to a TBI (McGregor & Pentland, 1997) and the incidence is reported as even higher in southern Australia and South Africa (Hillier et al., 1997; Nell & Brown, 1991). Although it has been difficult to compile reliable statistics on the prevalence and incidence of TBI in Canada (Tator, 2010), estimates in the United States suggest that between 1.4 and 1.7 million Americans sustain a TBI each year, accounting for 50,000 deaths and 80,000 to 90,000 individuals who suffer from long-term disability (Centre for Disease Control, 2010; Thurman & Guerrero, 1999). In Europe, it is estimated that at least 11.5 million individuals are suffering long-term disabilities related to a TBI (Schouten, 2007). In addition, TBI is considered to be a robust risk factor for the further development of neurodegenerative diseases, such as Alzheimer's disease (Slemmer et al., 2011), leading to additional dysfunction. Financially, the costs of TBI to society are no less distressing. Over two decades ago, an estimated 37.8 billion dollars was spent on direct costs related to hospital care in the U.S., or on indirect costs related to work loss due to disability (Max et al. 1991), and this cost has likely increased substantially. Due to the enormous impact TBI has on human health and health care systems in general throughout the world, understanding the mechanics and pathophysiology involved in TBI is essential for developing successful acute and long-term therapeutic strategies.

2.2 Repetitive mild TBI

TBI is characterized as mild, moderate or severe. Mild TBI, i.e. concussion, accounts for 70-90% of all TBI cases and 15-20% of individuals with a mild TBI have long-term dysfunction (Ryu et al, 2009). Although individuals who have experienced a moderate or severe TBI are certainly at risk of a second insult (Saunders et al., 2009), repetitive injuries occur in a considerable portion of individuals who have experienced a mild TBI. Child abuse victims, as well as victims of spousal abuse, are often subjected to multiple injuries to the head (Roberts et al., 1990; Shannon et al., 1998). Many injuries of these types go unreported, and it is difficult to assess how many insults a patient may have suffered. Arguably, athletes represent the largest group of patients that are at risk for experiencing repeated brain injuries, especially concussions (Guskiewicz et al., 2000; Kelly, 1999; Kelly & Rosenberg, 1997; Powell and Barber-Foss, 1999). Also, in comparison to child or spousal abuse victims, there is generally better documentation of how many brain injuries an individual has sustained due to recreational or sports related activities, making this population easier to study.

The idea that multiple head injuries in athletes could lead to clinical problems has long been suggested. For example, many clinicians believe that the development of *dementia pugilistica* in professional boxers is caused by the multiple hits to the head that a boxer endures over the course of their career (Jordan, 2000). Also, studies have shown that the number of concussions is inversely related to performance on several neuropsychological tests in soccer players (Matser et al., 1999; 2001), and jockeys that have experienced multiple concussions

generally display more cognitive dysfunctions than those who have had a single injury (Wall et al. 2006). An association between repetitive concussions and cognitive impairment, as well as clinical depression, has been demonstrated in professional football players in the United States (Guskiewicz et al., 2005; 2007). In Canada, the occurrence of concussion in ice hockey has been in the press substantially in recent months. The incidence of concussions in hockey appears to be on the rise not only in the National Hockey League, but also at the junior level (Ackery et al., 2009; Echlin et al., 2010). Many of these players have repeated concussions and suffer from post concussion symptoms such as memory impairment, headaches and depression (Ackery et al., 2009). As with boxers, there is evidence that repeated concussions may increase the risk of developing dementia later in life (De Beaumont et al., 2009). Therefore, it is important to understand the processes underlying the pathology of repetitive TBI.

3. Experimental approaches to the study of repetitive TBI

When studying repetitive brain trauma in athletes, we can gain much information about the pathology and progress of such injuries from the injured athletes themselves, e.g. by measuring changes in cognitive and motor performance. However, these injuries are generally at a mild level, and therefore, except in rare cases when athletes die as a result of the insult, we cannot assess the changes that have actually occurred in the brain at the cellular and sub-cellular levels. In order to compile this type of information, we must turn to experimental models of TBI.

3.1 *In vivo* studies

When discussing experimental studies of repetitive TBI *in vivo*, this does not include studies of secondary insults, such as a mechanical insult to the head followed by a defined duration of ischemia or glutamate exposure. Repeated TBI experimentation consists of an initial mechanical injury to the head followed by another mechanical insult to the head of the same or different degree. Based on these criteria, there were very few of these types of experiments conducted before the year 2000, with only a handful of repetitive injury studies being published (Kanayama et al., 1996; Olsson et al., 1976; Weitbrecht & Noetzel, 1976). Several additional *in vivo* studies of repeated injuries in rodents have now been conducted over the past decade (Allen et al., 2000; Conte et al., 2004; Creeley et al., 2004; DeFord et al., 2002; Friess et al., 2009; Huh et al., 2007; Laurer et al., 2001; Longhi et al., 2005; Raghupathi et al., 2004; Shitaka et al., 2011; Uryu et al., 2002; Yoshiyama et al., 2005). All of these repeated mild injury studies were conducted using rodent models of TBI with the exception of the studies by Friess et al (2009) and Raghupathi et al (2004), which used a pediatric model of repeated injury in pigs.

Repetitive TBI generally occurs at a mild level, therefore experimental models have been used which are minimally invasive and do not require a craniotomy, such as weight drop models or other forms of closed-skull TBI. The models must also be administered at a level that produces minimal, or preferably, no fatality. Individuals who have suffered from a mild TBI often complain of cognitive difficulties post-injury. Therefore, repeated injury studies usually evaluate cognitive function, for example using the Morris water maze (MWM) test, as well as the extent of cellular abnormalities in the cortex and hippocampus. The hippocampus in particular has received significant attention in the study of repeated mild TBI, because it plays a critical role in certain types of learning and aspects of memory

storage. Experimental and clinical data have demonstrated not only the importance of this brain region in learning and memory, but also that the hippocampus is uniquely vulnerable to injury, even after mild brain trauma (Lowenstein et al., 1992; Lyeth et al., 1990). In a study by DeFord et al. (2002), repeated mild injuries were administered to mice (four times every 24 hr), followed by MWM testing and histological analysis. Significant learning deficits were found after repeated injuries, which were not evident after a single injury. These deficits occurred even in the absence of cell death within the cortex and hippocampus. Cognitive deficits after multiple mild TBIs (using MWM analysis) were demonstrated in a similar study using a weight drop model (Creeley et al., 2004). In a recent study, Shitaka et al. (2011) used a controlled cortical impact model in mice and found that animals receiving two injuries 24 hr apart displayed MWM deficits for several weeks. In addition, although no gross histological abnormalities were noted, mice that received two insults had damaged axons in various brain areas, which could underlie the cognitive abnormalities.

In one of the early studies of repeated injury *in vivo*, Laurer et al. (2001) used an injury regimen that they described as "concussive". This model was meant to mimic the type of insult that athletes may receive, and was also used for many subsequent studies (Conte et al., 2004; Longhi et al., 2005; Uryu et al., 2002). In an assessment of cognitive and motor function after repeated injury in mice, Laurer et al. (2001) found that the brain was more vulnerable to a second insult if the second injury occurred 24 hr after the first. Even though no cognitive deficits were demonstrated in mice receiving repeated injuries, there was a decrease in motor function and neuronal loss. The authors also stated that the effects of a second mTBI could be synergistic, rather than additive. To further analyze the effects of lengthening the inter-injury interval, Longhi et al. (2005) investigated repetitive injuries three, five and seven days apart. Animals that received repeated injuries three or five days apart exhibited cognitive dysfunction not evident in sham animals or those injured only once. However, no deficits were observed when the injury interval was extended to seven days. This experimental evidence demonstrating that the brain can recover from a first injury, given sufficient amount of time, is certainly alluring, especially in relation to establishing "return-to-play" guidelines for athletes. Overall, the evidence from these *in vivo* experimental models suggests that repetitive mild TBI causes more cognitive and cellular dysfunction than a single injury, if the brain is not given a sufficient amount of time to recover.

Other *in vivo* studies have been conducted with a primary interest in discovering more about the pathology of inflicted repetitive brain injury in the pediatric population, such as 'shaken impact syndrome' (Friess et al., 2009; Huh et al., 2007; Raghupathi et al., 2004). In a study by Raghupathi et al. (2004), neonatal pigs were subjected to rapid axial rotations of the head, either once, or twice within 15 minutes. Brains were analyzed at 6 hr post-injury and animals that had received double insults exhibited a wider distribution of injured axons than animals that were injured once. In another study in piglets (Friess et al., 2009), animals were injured (by axial head rotation) either once, twice one day apart, or twice one week apart. Animals injured one day apart had the highest mortality rate. Also, animals receiving two injuries had worse neuropathology and neurobehavioral outcome than those injured only once. Huh et al. (2007) conducted experiments in young rats (11 days old) and administered one, two or three injuries spaced only 5 minutes apart. Animals receiving multiple injuries generally displayed increased axonal damage, which was evident earlier after injury than a single impact. Overall, these studies suggest a graded response to repeated injury in the pediatric brain.

3.2 Studies conducted *in vitro*

Several *in vitro* approaches have now been developed to study traumatic injury, which utilize dissociated brain cells or slices grown in culture (LaPlaca et al., 2005; Morrison et al., 1998; Noraberg et al., 2005; Spaethling et al., 2007; Weber, 2004). For many years, my laboratory group has utilized an *in vitro* model of stretch-induced mechanical injury originally developed by Ellis et al. (1995). We have characterized this stretch injury model in cell cultures composed of neurons and glia from murine hippocampus (Slemmer *et al*., 2002; Slemmer & Weber, 2005), cortex (Engel et al., 2005), and cerebellum (Slemmer *et al*., 2004), and currently in cortical cultures from rat pups.

We have previously conducted studies investigating the effects of repeated trauma on cultured hippocampal cells (Slemmer *et al*., 2002; Slemmer & Weber, 2005), which were intended to complement the findings from humans and *in vivo* experimentation. In these studies, we utilized a mild level of stretch injury that produces some measurable damage to cells when administered a single time. When mild stretch injuries were repeated at either 1-hr or 24-hr intervals, cells exhibited cumulative damage. For example, cultures that received a second insult displayed a significant loss of neurons not evident in cultures that received only one injury (see Figure 1). Additionally, cultures injured twice released a significant level of neuron specific enolase (NSE), which was not observed in cultures injured a single time. Interestingly, the extent of damage to the cells was dependent on the time between repeated injuries. For example, cultures that received a second insult 1 hr after the first injury released more S-100B protein (a biomarker of injury commonly employed in the clinic) than cultures that received a second injury at 24 hr. Cultures injured 24 hr apart also exhibited less staining with the intravital dye, propidium iodide, than those injured 1 hr apart. As demonstrated in some *in vivo* studies, these findings suggest that a level of injury producing measurable damage or dysfunction on its own, may cause cumulative damage if repeated within a certain time frame (Laurer et al., 2001; Longhi et al., 2005).

We also investigated the effects of a very low level of stretch, which produces no overt cell damage (Slemmer and Weber, 2005). This "subthreshold" level of stretch did not cause significant damage or death, even when it was repeated at a 1 hr interval. However, this low level of stretch did induce cell damage when it was repeated several times at a short interval (every 2 min), indicated by increased propidium iodide staining (a marker of cellular injury), neuronal loss, and an increase in NSE release. Although direct comparisons to the clinical situation are difficult to make, these types of repetitive, low-level, mechanical stresses may be similar to the insults received by certain athletes, such as boxers, and hockey and soccer players (Jordan, 2000; Matser et al., 1998; Matser et al., 1999; Webbe & Ochs, 2003; Wennberg & Tator, 2003). This type of *in vitro* model may provide a reliable system in which to study the mechanisms underlying cellular dysfunction following repeated injuries. In addition, this approach could provide a means for rapid screening of potential therapeutic strategies for both single and repeated mild TBI.

Another study of repeated injury *in vitro* used a model of axonal injury (Yuen et al., 2009). Low levels of strain to cortical axons in culture resulted in no obvious pathological changes. By 24 hr however, these axons exhibited increased sodium channel expression. When axons were stretched again at 24 hr, there was a significant increase in intracellular calcium, which led to degeneration of the axons. This finding suggests a possible mechanism underlying the susceptibility of the brain to a second impact within a certain temporal window.

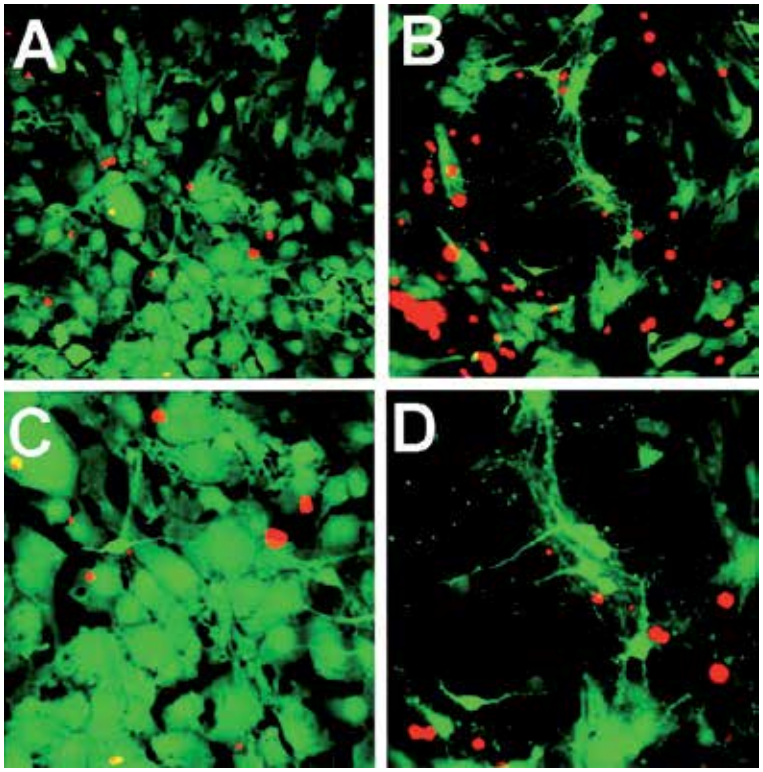


Fig. 1. Effect of repeated stretch injury on hippocampal cells in culture. Cell injury was assessed using the two dyes fluorescein diacetate (FDA) and propidium iodide (PrI). FDA stains healthy, viable cells and fluoresces green, while PrI does not pass through intact cellular membranes. If membranes are damaged, however, cells lose their ability to retain FDA and PrI will enter the cell and bind to the nucleus, fluorescing red. (A) PrI uptake following mild stretch injury at 1 h post-injury (B) A double mild insult increased PrI uptake when evaluated immediately after the second injury. Note that many cells also have beaded neurites. (A and B) Magnification: 100X. (C and D) Enlargements of A and B, respectively. Magnification: 200X. Modified from Slemmer et al. (2002). Reprinted with permission from Oxford Press, 2002.

3.3 The preconditioning phenomenon

Several studies have indicated that an initial, very mild insult to either cultured cells or to the brain itself, may provide some protection from a second, more severe insult, a finding that has been termed "preconditioning". Ischemic preconditioning, in which a brief exposure to ischemia renders the brain more resistant to subsequent longer periods of ischemia, has been well described (for review, see Schaller & Graf, 2002). There is also evidence of preconditioning cross-tolerance. For example, brief ischemia lessens damage following TBI *in vivo* (Perez-Pinzon et al., 1999). More recently, several other types of pretreatments have been demonstrated to improve outcome and pathology after experimental TBI, such as a low dose of *N*-methyl-D-aspartate (Costa et al., 2010), exposure to lipopolysaccharide (Longhi et al., 2011) or glucagon (Fanne et al., 2011), hypothermia (Lotocki et al., 2006), as well as exposure to hyperbaric oxygen (Hu et al., 2008; 2010).

Another interesting phenomenon is that heat acclimation (chronic exposure to moderate heat) can also provide resistance to subsequent TBI (Shein et al. 2007; 2008; Umschwief et al., 2010).

In our *in vitro* studies using mechanical stretch, we observed a novel form of mechanical preconditioning. When hippocampal cultures were administered a subthreshold level of stretch 24 hr prior to a mild stretch, there was a significant decrease in released S-100B protein compared to cultures that were injured at a mild level alone (Slemmer & Weber, 2005). This observation suggests some form of protection initiated by this low level of stretch. A similar finding *in vivo* was reported by Allen et al. (2000). In their study, rats received a series of mild injuries spaced three days apart using a weight drop model. Some of these animals received a severe injury after the repetitive mild injuries. Motor function deficits were evident in severely injured animals, but not in animals that received repeated mild injuries or repeated mild injuries followed by a severe injury. This last observation suggests a preconditioning effect.

An important question is how do we utilize this information for beneficial means? One can imagine the ethical implications of suggesting to people that a mild insult to their brains may in fact protect them from worse insults in the future. We still have much to learn about preconditioning. For example, what is the threshold for mechanical insults between initiating protective versus damaging mechanisms in the brain? A clear understanding of the mechanisms by which this protection is elicited holds potential for the management of mild TBI. The fact that a wide variety of stressors can protect the brain from TBI (i.e. cross-tolerance) suggests that the same, or similar mechanisms are responsible for the endogenous protection. Increasing the expression of these protective systems could not only be a reliable way for managing mild TBI, but could also provide resistance in individuals who may be at risk of sustaining an additional head injury, such as athletes. Both *in vivo* and *in vitro* models could provide reliable systems in which to study the mechanisms underlying the preconditioning phenomenon.

4. Repetitive injury and neurodegenerative disease

A correlation between the occurrence of TBI and the further development of neurodegenerative disease later in life has been recognized for several years, and TBI is considered to be one of the most robust risk factors for developing Alzheimer's disease (AD; Szczygielski, et al., 2005; Slemmer et al., 2011). There is also evidence that genetic predisposition may increase one's risk of developing AD, such as possession of the apolipoprotein E ε4 allele (Isoniemi et al., 2006). A phenomenon known as chronic TBI occurs in a significant amount of professional boxers (Jordan, 2000), with the most serious form, the neurodegenerative disorder *dementia pugilistica*, resulting in severe cognitive and motor dysfunctions. A potential link between TBI and Parkinson's disease has also been suggested (Masel and DeWitt, 2010). It is generally accepted that the pathology of AD and *dementia pugilistica* are quite similar (Geddes et al., 1999; Schmidt et al., 2001). Although epidemiological data linking TBI and neurogenerative diseases are quite strong, only a modest amount of experimental work has been conducted in order to achieve a mechanistic link between repeated mild TBI and the development of either AD or *dementia pugilistica.*

In addition to cognitive symptoms, dementias such as *dementia pugilistica* and AD are associated with specific types of neuropathological markers. In fact, AD in humans can only

be fully confirmed post-mortem via the presence of extracellular senile plaques, which are abnormal amyloid β (Aβ) protein deposits, and abnormal tau protein aggregation in specific brain regions (Price et al., 1991). The tau protein is an important functional component of the cytoskeleton in healthy neurons, but it is also a predominant component of neurofibrillary plaques found in AD and *dementia pugilistica* (Schmidt et al., 2001). Therefore, the development of abnormal tau protein pathology is a potential molecular link between TBI and dementia. In a study by Kanayama et al (1996), rats were injured with a mild impact once a day for seven days. Analysis showed an increase in abnormal tau protein deposits by one month after injury. Yoshiyama et al. (2005) used a robust injury paradigm in an attempt to model human *dementia pugilistica* in transgenic mice expressing the shortest human tau isoform (T44). Mice were subjected to four injuries a day, once a week, for four weeks, resulting in each mouse receiving a total of 16 injuries, and surprisingly, they could find only one mouse that displayed pathology of *dementia pugilistica* at nine months of age. Partly for this reason, the vast majority of animal studies have focused on the deposition of Aβ, or the intracellular processing of amyloid precursor protein (APP), from which Aβ is derived. Although high levels of Aβ have clearly been demonstrated in AD patients, the exact function of amyloid protein has not been established. Interestingly, deposition of Aβ has not been observed in the majority of nontransgenic animal studies after trauma (Laurer et al., 2001; Szczygielski, et al., 2005), and as a result, many of the current models used to investigate traumatic dementia are derived from transgenic rodents that were originally created to investigate AD. For example, the transgenic mouse Tg2576, which is characterized by AD-like amyloidosis by nine months of age, has been used in several investigations of repetitive mild TBI, and has become a popular animal model for traumatically-induced dementia.

In a study by Uryu et al. (2002), Tg2576 transgenic mice subjected to repeated, but not to single mild TBI, displayed cognitive deficits and Aβ deposition. As shown in Figure 2, Aβ deposition did not occur in these mice at either 9 or 16 weeks post-sham injury. In contrast, brain slices from Tg2576 mice that underwent repeated mild TBI displayed evident Aβ deposition (in the form of senile plaques) at 16 weeks post-injury. The appearance of senile plaques followed a delayed time-scale, which is not surprising, as dementia is often manifested in humans long after TBI. This study also demonstrated that the transgenic background alone was not sufficient to induce marked amounts of Aβ deposition in these aged mice, which is in line with a "two-hit" hypothesis proposed by Nakagawa et al. (1999). In this case, the first-hit is the genetic predisposition, which enables an individual to produce high amounts of abnormal proteins such as Aβ, and the second-hit is the TBI. However, a single mild injury alone was not enough to produce AD-like pathology. It is therefore possible that more than one mild TBI is necessary to lead to dementia later in life, whereas a single moderate or severe TBI on its own may lead to dementia. Increased incidence of dementia in humans is obviously associated with increased age, and recent evidence links aging with the overproduction of free radicals via oxidative stress (Slemmer et al., 2008). TBI is also known to dramatically increase free radicals and reactive oxygen species (Slemmer et al., 2008, Weber, 2004). Repetitive, but not single mild TBI, has been previously shown to increase oxidative stress in Tg2576 mice (Uryu et al., 2002), which could be reduced by supplementing the rodent chow with vitamin E, a known antioxidant (Conte et al., 2004). Therefore, oxidative stress may be a major contributing factor leading to the development of neurodegenerative disease following TBI.

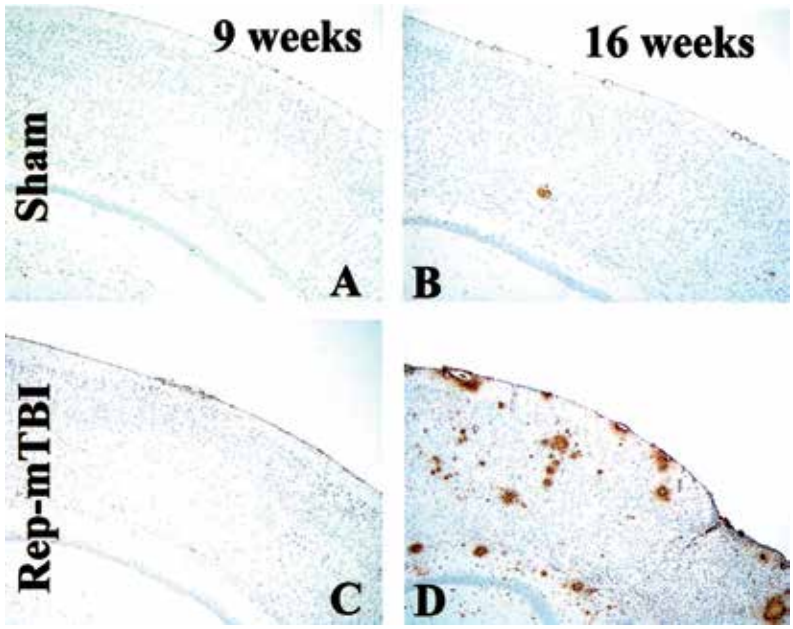


Fig. 2. Amyloid deposition in Tg2576 mice with sham (A, B) or repetitive mild TBI (C, D) with 4G8 immunohistochemistry at 9 (A, C) and 16 (B, D) weeks after mild TBI. Senile plaques increased in an age-dependent manner in both sham and injured mice, but the largest number of Aβ-positive plaques are evident in the 16-week repetitive mild TBI mice (D). Modified from Uryu et al. (2002). Reprinted with permission from the Society for Neuroscience, 2002.

The overall findings of these *in vivo* studies are quite significant, because they can demonstrate a direct experimental link between repeated mild TBI and the development of AD-like pathology, as well as other forms of dementia. Generally, it takes many years before the onset of symptoms of neurodegenerative disorders is evident, after an individual has experienced a TBI. Therefore, it requires an exceedingly long amount of time to gather this type of epidemiological data from the human population. This area of research, in particular, is where experimental models could truly help decipher the mechanisms by which neurodegenerative disease may be triggered by repetitive brain injury, and to identify potential therapeutic strategies.

5. Future directions

5.1 Potential new experimental directions

The current lines of research in repetitive TBI should certainly be continued, such as attempting to firmly establish the link to neurodegenerative disease, as well as demonstrating appropriate recovery times after a mild injury. However, new avenues also need to be explored. For example, much experimental evidence suggests that animals demonstrate cognitive deficits and cellular dysfunction after repetitive mild TBI, even though the injury may not necessarily lead to cell death (DeFord et al., 2002; Kanayama et al., 1996). Therefore, rather than trying to prevent cells from dying after repeated injuries, it may be more useful to learn how to restore normal cellular physiology after a traumatic episode. Combining studies

at the cellular and behavioral levels is crucial for attaining this goal, and one area of potential interest is the evaluation of the effects of repeated TBI on synaptic plasticity in the cortex and hippocampus. The ability of neurons to undergo changes in synaptic strength, such as long-term potentiation (LTP), is postulated to be a cellular correlate of learning and memory (Bliss & Collingridge, 1993; Malenka & Nicoll, 1999). Several studies have reported impaired hippocampal LTP after TBI *in vivo* (see Albensi, 2001; Weber, 2004). One area of future research could focus on restoring mechanisms of synaptic plasticity after injury (such as LTP), as well as correlated hippocampal-mediated behavioral tasks.

The hippocampus shares neuronal projections with areas of the cerebral cortex, which undoubtedly also contributes to memory formation and storage. Indeed, alterations in synaptic plasticity may also occur directly in the cortex after repeated mild TBI. Therefore, although the hippocampus may play a central role in the cognitive dysfunction observed after mild TBI, it is important not to overlook contributions from other brain areas as well. Since some repeated injury studies demonstrate motor impairment, it may also be appropriate to investigate cellular physiology and synaptic plasticity in the cerebellum (see Hansel et al., 2001; Weber et al., 2003; Slemmer et al., 2005) after repetitive TBI. These types of investigations could involve electrophysiology measurements as well as analysis of intracellular calcium dynamics. Intracellular calcium is extremely important to the normal function of neurons and can be considerably altered even in cells that do not go on to die (Weber, 2004; Yuen et al., 2009).

5.2 Experimental design considerations

Although deciding on appropriate research directions is of paramount importance to developing potential therapeutic strategies for repetitive TBI, the utilization of proper parameters for repeated injury studies may be just as crucial. For example, what are the best inter-injury interval, or intervals, to use? Although 24 hr between injuries is the most common (and perhaps practical) interval in the laboratory (Conte et al., 2004; Creeley et al., 2004; DeFord et al., 2002; Friess et al., 2009; Kanayama et al., 1996; Laurer et al., 2001; Shitaka et al., 2011; Uryu et al., 2002; Weitbrecht & Noetzel, 1976; Yoshiyama et al., 2005), is it the most appropriate in mimicking what occurs in humans? Also, how many injuries should a researcher administer? If one is attempting to model concussive episodes, then two or three may be enough, as this may closely mimic a true situation, especially with athletes. However, when attempting to recreate *dementia pugilistica* (Yoshiyama et al., 2005), the number of injuries should certainly be increased, and perhaps be 'subthreshold' levels of injury, i.e. a level of injury which produces no overt damage on its own.

The proper controls and endpoints to use for repeated injury studies also need to be carefully considered. For *in vivo* studies analyzing the effects of a single TBI, the issue of controls is fairly straightforward. Sham animals are treated at an equivalent time as injured animals, and the analysis, cellular or behavioral, is also performed at the same time-point. However, when comparing uninjured animals to animals that have received more than one injury, what is the proper comparison? For example, if an animal receives an injury on day one, and an additional injury on day two, and analysis takes place on day three, does one compare the data with sham animals from day one, or from day two (or both, see figure 3A)? The issue is further complicated when comparing repeatedly injured animals to animals that have received a single TBI. If the comparison concerns animals that undergo four injuries or a single injury, are the single insult animals injured at the same time as injury one in the repeated group, or at the same time as the fourth injury (see figure 3B)? This decision will affect the endpoint as well.

For example, if animals or tissue are analyzed one day after the fourth injury, then four days will have passed for the single injury group if those animals were injured on day one. This difference in time could affect the observations. One could argue that if a long enough period of time passes after the injuries, such as weeks or months, then the effect of when the single insult animals were injured will be negligible. Admittedly, this would be more proper for comparison to the human situation in which the effects of mild TBI can be manifested for weeks, months, or even years. However, this is often not practical for many laboratories, as the costs of housing animals for months can at times be prohibitive. Also, conducting long-term experiments *in vitro* is limited, since the cells generally remain viable for only a few weeks. This raises a critical point as to the relevance of repeated injury studies *in vitro*. I strongly believe that *in vitro* experiments can deliver information about the cellular mechanisms of repeated injury that are difficult to obtain *in vivo*, and that it is essential to combine data derived from *in vitro* experiments with those conducted with animals *in vivo*. However, I am unsure how to directly compare the data. For example, is a 24 hr injury interval *in vitro* equivalent to 24 hr *in vivo*? The greater consensus that exists on these issues with individuals who conduct repeated injury studies, the easier it will be to compare the data, and the stronger a case can be made for showing unequivocally, that repeated mild TBI could lead to long-term dysfunction in humans.

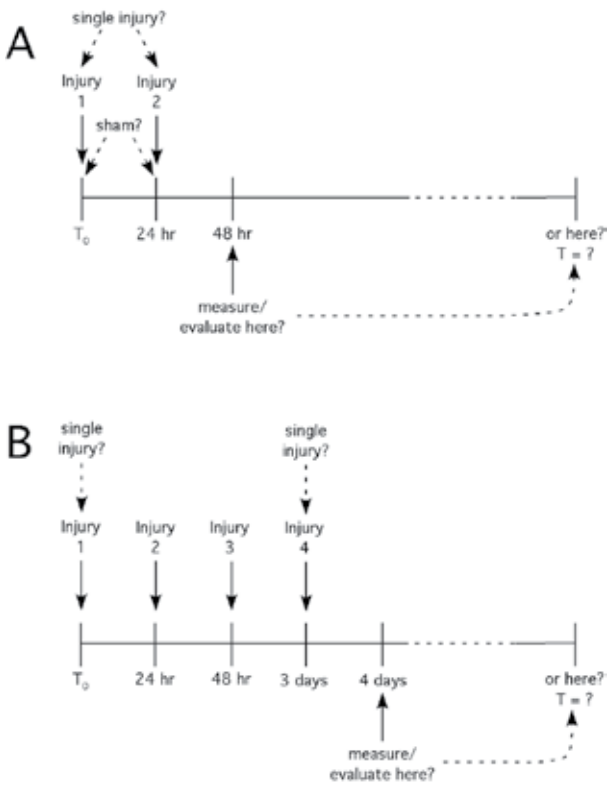


Fig. 3. Issues for consideration when designing repeated TBI experiments (i.e. choosing proper timepoints for controls and behavioral/tissue analysis). T = time.
From Weber (2007). Reprinted with permission from Elsevier, 2007.

5.3 Possible therapeutic interventions

Perhaps one of the best, and most logical, therapeutic interventions that physicians can make especially when athletes are concerned is to not allow these individuals to return to play until they seem to have fully recovered from a mild injury/concussion. This would obviously stop the individual from being in a position of acquiring a second injury in a vulnerable period. Return to play and treatment guidelines have been established by a consensus statement on concussion in sport at the 3rd International Conference on concussion in sport in Zurich in November of 2008 (McCrory et al., 2009). Diagnosis of concussion and recovery involves a wide assessment of an individual including physical signs, behavioral abnormalities, balance, sleep and cognition (Echlin et al., 2010; McCrory et al., 2009). Neuropsychological assessments and tests such as the Sideline Concussion Assessment Tool 2 (SCAT2) and the Immediate Post-Concussion Assessment and Cognitive Test (ImPACT) should also be routinely used (Echlin et al., 2010; McCrory et al., 2009), and players should have no signs of neurological deficits or syndromes before returning to play (Ackery et al. 2009). There will likely still be players that will not comply with return to play advice, but these individuals need to be made aware that lack of compliance may put them at higher risk for experiencing another concussion as well as suffering potential permanent brain damage and disability (Ackery et al., 2009).

Another potential type of intervention is genetic screening. As previously mentioned, individuals with the apolipoprotein E ε4 allele generally show poorer outcome after injury than others without this genetic polymorphism (Isoniemi et al., 2006). Also, individuals with a genetic alteration in neprilysin, which is the enzyme that degrades Aβ protein, may be at greater risk of Aβ plaque formation after TBI as well as the development of AD (Johnson et al, 2009). At present, there are no specific therapeutic interventions that are routinely used for these individuals. However, these persons could at least be advised that they may be at a much higher risk of developing AD if they sustain a TBI or repetitive mild TBIs. Therefore, they could make an informed decision about whether they would participate in activities where they may be at high risk of experience a TBI, such as specific types of sports.

TBI is known to increase free radicals and reactive oxygen species, leading to oxidative stress (Slemmer et al., 2008, Weber, 2004), and this may be a prevalent means of damage even after mild TBI. Therefore, specific agents that could be useful for treating mild TBI are antioxidants. In a study mentioned earlier (Conte et al., 2004), vitamin E, a known antioxidant, increased cognitive function and decreased Aβ deposition after repetitive concussive injury. In addition to supplementation, individuals could potentially increase the amount of antioxidant species in their body through diet, as several foods have high amounts of antioxidants (Ferrari & Torres, 2003). Of course, these compounds would have to cross the blood-brain barrier in order to provide protection from TBI. In fact, many of these species do cross into the brain. For example, Andres-Lacueva et al. (2005) demonstrated that compounds present in blueberries were found in rat brain cells after feeding them a diet with blueberry extract. In addition, Sweeney et al. (2002) showed that rats fed blueberries for six weeks were protected from stroke. This raises the possibility that an individual on a diet high in antioxidant species may be somewhat protected from a mild trauma and may have better outcome following a second mild TBI should it occur.

Another interesting prospect in the field of treating repetitive mild TBI is the potential use of cognitive enhancers, such as ampakines, which were and still are touted as therapeutic agents for neurodegenerative conditions such as Alzheimer's disease (Lynch and Gall, 2006). They are now gaining popularity as safe drugs to improve memory and concentration in

healthy individuals. Ampakines positively modulate the AMPA-type of glutamate receptors in the brain (Lynch & Gall, 2006). Glutamate receptors are known to be involved in a wide variety of processes in the nervous system, one of which is memory. Their activation appears to be imperative for memory consolidation. For example, the activation of AMPA receptors is known to facilitate LTP in the hippocampus. Ampakines are peripherally administered drugs known to cross the blood-brain barrier and can potently facilitate LTP, as demonstrated in rodents (Staubli et al., 1994). These drugs also improve memory performance in rodents and humans (Lynch, 1998; Lynch & Gall, 2006).

Ampakines have now been evaluated in clinical trials in humans. One of these drugs in particular (CX516) has demonstrated enhanced memory and cognitive performance in healthy young adults (Ingvar et al., 1997; Lynch et al., 1996). Similar positive cognitive effects were found with CX516 in healthy elderly subjects (Lynch et al., 1997). In these studies, no changes in heart rate, mood or motor performance were found. Another study in healthy elderly volunteer subjects with another ampakine (farampator) showed improvements with short-term memory (Wezenberg et al., 2007). At higher doses, farampator caused side effects such as nausea, headache and drowsiness. Overall, these drugs produce cognitive enhancement with either no, or very mild side effects. This raises the possibility of treating athletes with these drugs after they have sustained a concussion, as well as treating child and spousal abuse victims who have repetitive injuries.

6. Conclusions

Repetitive mild TBI constitutes a significant portion of all TBI cases and the incidence of repeated TBI appears to be on the rise. Overall, there has been surprisingly little attention given to experimental repetitive TBI studies. However, more researchers have conducted studies in this field in recent years. Research involving both *in vivo* and *in vitro* experimentation holds promise for unraveling the pathology of repetitive mild TBI, which may differ from that of single TBI at various levels. A greater understanding of how long the brain takes to recover after a mild injury will aid in determining return to play guidelines for athletes. In addition, further experimentation and monitoring of mild TBI sufferers will assist in developing treatment strategies for decreasing damage should a second injury occur.

7. Acknowledgements

The author would like to recognize current funding from the Natural Sciences and Engineering Research Council (NSERC) and the Canada Foundation for Innovation (CFI).

8. References

Ackery, A., Provvidenza, C. & Tator, C.H. (2009) Concussion in hockey: compliance with return to play advice and follow-up status. *Canadian Journal of Neurological Sciences*, Vol.36, No. 2, pp. 207-12, ISSN 0317-1671

Albensi, B.C. (2001) Models of brain injury and alterations in synaptic plasticity. *Journal of Neuroscience Research*, vol.65, no.4, pp. 279-283, ISSN 0360-4012

Allen, G.V., Gerami, D. & Esser, M.J. (2000) Conditioning effects of repetitive mild neurotrauma on motor function in an animal model of focal brain injury. *Neuroscience*, vol.99, no.1, pp. 93-105, ISSN 0306-4522

Andres-Lacueva, C., Shukitt-Hale, B., Galli, R.L., Jauregui, O., Lamuela-Raventos, R.M. & Joseph, J.A. (2005) Anthocyanins in aged blueberry-fed rats are found centrally and may enhance memory. *Nutritional Neuroscience*, vol.8, no.2, pp. 111-120, ISSN 1028-415X

Bliss, T.V. & Collingridge, G.L. (1993) A synaptic model of memory: long-term potentiation in the hippocampus. *Nature*, vol.361, no.6407, pp. 31-39, ISSN 0028-0836

Centers for Disease Control and Prevention. (2010) Injury Prevention & Control: Traumatic Brain Injury, Accessed April, 2011, Available from: http://www.cdc.gov /ncipc/tbi/ TBI.htm.

Conte, V., Uryu, K., Fujimoto, S., Yao, Y., Rokach, J., Longhi, L., Trojanowski, J.Q., Lee, V.M-Y., McIntosh, T.K. & Pratico, D. (2004) Vitamin E reduces amyloidosis and improves cognitive function in Tg2576 mice following repetitive concussive brain injury. *Journal of Neurochemistry*, vol.90, no.3, pp. 758-764, ISSN 0022-3042

Costa. T., Constantino, L.C., Mendonça, B.P., Pereira, J.G., Herculano, B., Tasca, C.I.& Boeck, C.R. (2010) N-methyl-D-aspartate preconditioning improves short-term motor deficits outcome after mild traumatic brain injury in mice. *Journal of Neuroscience Research,* vol.88, no.6, pp. 1329-37, ISSN 0360-4012

Creeley, C.E., Wozniak, D.F., Bayly, P.V., Olney, J.W. & Lewis, L.M. (2004) Multiple episodes of mild traumatic brain injury result in impaired cognitive performance in mice. *Academic Emergency Medicine*, vol.11, no.8, pp. 809-819, ISSN:1069-6563

De Beaumont, L., Théoret, H., Mongeon, D., Messier, J., Leclerc, S., Tremblay, S., Ellemberg, D. & Lassonde, M. (2009) Brain function decline in healthy retired athletes who sustained their last sports concussion in early adulthood. *Brain*, vol.132, no.3, pp. 695-708, ISSN 0006-8950.

DeFord, S.M., Wilson, M.S., Rice, A.C., Clausen, T., Rice, L.K., Barabnova, A., Bullock, R. & Hamm, R.J. (2002) Repeated mild brain injuries result in cognitive impairment in B6C3F1 mice. *Journal of Neurotrauma*, vol.19, no.4, pp. 427-438, ISSN: 0897-7151

Echlin, P.S., Tator ,C.H., Cusimano, M.D., Cantu, R.C., Taunton, J.E., Upshur, R.E., Hall, C.R., Johnson, A.M., Forwell, L.A. & Skopelja, E.N. (2010) A prospective study of physician-observed concussions during junior ice hockey: implications for incidence rates. *Neurosurgical Focus*, vol.29, no.5, E4, ISSN: 1092-0684

Ellis, E.F., McKinney, J.S., Willoughby, K.A., Liang, S. & Povlishock, J.T. (1995) A new model for rapid stretch-induced injury of cells in culture: characterization of the model using astrocytes. *Journal of Neurotrauma*, vol. 12, no.3, pp. 325-339, ISSN: 0897-7151

Engel, D.C., Slemmer, J.E., Vlug, A.S., Maas, A.I. & Weber, J.T. (2005) Combined effects of mechanical and ischemic injury to cortical cells: secondary ischemia increases damage and decreases effects of neuroprotective agents. *Neuropharmacology*, vol.49, no.7, pp. 985-995, ISSN 0028-3908

Fanne, R.A., Nassar, T., Mazuz, A., Waked, O., Heyman, S.N., Hijazi, N., Goelman, G. & Higazi, A.A. (2011) Neuroprotection by glucagon: role of gluconeogenesis. *Journal of Neurosurgery*, vol.114, no.1, pp. 85-91, ISSN 0022-3085

Ferrari, C.K. & Torres, E.A. (2003) Biochemical pharmacology of functional foods and prevention of chronic diseases of aging. *Biomedicine and Pharmacotherapy*, vol. 5, no. 5-6, pp. 251-60, ISSN: 0753-3322

Friess, S.H., Ichord, R.N., Ralston, J., Ryall, K., Helfaer, M.A., Smith, C. & Margulies, S.S. (2009) Repeated traumatic brain injury affects composite cognitive function in piglets. *Journal of Neurotrauma*, vol.26, no.7, pp. 1111-1121, ISSN: 0897-7151

Geddes, J.F., Vowles, G.H., Nicoll, J.A. & Revesz, T. (1999) Neuronal cytoskeletal changes are an early consequence of repetitive head injury. *Acta Neuropathol. (Berl.)*, vol.98, no.2, pp. 171-178, ISSN 0065-1435

Guskiewicz, K.M., Marshall, S.W., Bailes, J., McCrea, M., Cantu, R.C., Randolph, C. & Jordan, B.D. (2005) Association between recurrent concussion and late-life cognitive impairment in retired professional football players. *Neurosurgery*, vol.57, no.4, pp. 719-26, ISSN 0148-396X

Guskiewicz, K.M., Marshall, S.W., Bailes, J., McCrea, M., Harding, H.P. Jr., Matthews, A., Mihalik, J.R. & Cantu, R.C. (2007) Recurrent concussion and risk of depression in retired professional football players. *Medicine and Science in Sports Exercise*, vol.39, no.6, pp. 903-909, ISSN 0195-9131

Guskiewicz, K.M., Weaver, N.L., Padua, D.A. & Garrett, W.E. Jr. (2000) Epidemiology of concussion in collegiate and high school football players. *American Journal of Sports Medicine*, 2000, vol.28, no.5, pp. 643-50, ISSN: 0363-5465

Hansel, C., Linden, D.J. & D'Angelo, E. (2001) Beyond parallel fiber LTD: the diversity of synaptic and non-synaptic plasticity in the cerebellum. *Nature Neuroscience*, vol.4, no.5, pp. 467-475, ISSN 1097-6256

Hillier, S.L., Hiller, J.E. & Metzer, J. (1997) Epidemiology of traumatic brain injury in South Australia. *Brain Injury*, vol.11, no.9, pp. 649-659, ISSN: 1082-8443

Hu, S.L., Hu, R., Li, F., Liu, Z., Xia, Y.Z., Cui, G.Y. & Feng, H. (2008) Hyperbaric oxygen preconditioning protects against traumatic brain injury at high altitude. *Acta Neurochirurgica Supplement*, vol.105, pp. 191-6, ISSN: 0065-1419

Hu, S., Li, F., Luo, H., Xia, Y., Zhang, J., Hu, R., Cui, G., Meng, H. & Feng, H. (2010) Amelioration of rCBF and PbtO2 following TBI at high altitude by hyperbaric oxygen pre-conditioning. *Neurological Research*, vol. 32, no.2, pp. 173-8, ISSN 0161-6412

Huh, J.W., Widing, A.G., Raghupathi, R. (2007) Repetitive mild non-contusive brain trauma in immature rats exacerbates traumatic axonal injury and axonal calpain activation: a preliminary report. *Journal of Neurotrauma*, vol. 24, no.1, pp. 15-27, ISSN: 0897-7151

Ingvar, M., Ambros-Ingerson, J., Davis, M., Granger, R., Kessler, M., Rogers, G.A., Schehr, R.S. & Lynch G. (1997) Enhancement by an ampakine of memory encoding in humans. *Experimental Neurology*, vol. 146, no. 2, pp. 553-559, ISSN: 0014-4886

Isoniemi, H., Tenovuo, O., Portin, R., Himanen, L. & Kairisto, V. (2006) Outcome of traumatic brain injury after three decades--relationship to ApoE genotype. *Journal of Neurotrauma*, vol.23, no.11, pp. 1600-1608, ISSN: 0897-7151

Johnson, V.E., Stewart, W., Graham, D.I., Stewart, J.E., Praestgaard, A.H. & Smith, D.H. (2009) A neprilysin polymorphism and amyloid-beta plaques after traumatic brain injury. *Journal of Neurotrauma*, vol.26, no.8, pp. 1197-1202, ISSN: 0897-7151

Jordan, B.D. (2000) Chronic traumatic brain injury associated with boxing. *Seminars in Neurology*, vol.20, no.2, pp. 179-85, ISSN: 0271-8235

Kanayama, G., Takeda, M., Niigawa, H., Ikura, Y., Tamii, H., Taniguchi, N., Kudo, T., Miyamae, Y., Morihara, T. & Nishimura, T. (1996) The effects of repetitive mild brain injury on cytoskeletal protein and behavior. *Methods & Findings in Experimental & Clinical Pharmacology*, vol.18, no.2, pp. 105-115, ISSN: 0379-0355

Kelly, J.P. (1999) Traumatic brain injury and concussion in sports. *JAMA: Journal of the American Medical Association*, vol.282, no.2, pp. 989-991, ISSN 0098-7484

Kelly, J.P. & Rosenberg, J.H. (1997) Diagnosis and management of concussion in sports. *Neurology*, vol.48, no.3, pp. 575-80, ISSN: 0028-3878

LaPlaca, M.C., Cullen, D.K., McLoughlin, J.J. & Cargill, R.S. 2nd (2005) High rate shear strain of three-dimensional neural cell cultures: a new in vitro traumatic brain injury model. *Journal of Biomechanics*, vol. 38, no. 5, pp. 1093-1105, ISSN 0021-9290

Laurer, H.L., Bareyre, F.M., Lee, V.M., Trojanowski, J.Q., Longhi, L., Hoover, R., Saatman, K.E., Raghupathi, R., Hoshino, S., Grady, S.M. & McIntosh, T.K. (2001) Mild head injury increasing the brain's vulnerability to a second concussive impact. *Journal of Neurosurgery*, vol.95, no.5, pp. 859-870, ISSN 0022-3085

Longhi, L., Gesuete, R., Perego. C., Ortolano, F., Sacchi, N., Villa, P., Stocchetti, N. & De Simoni, M.G. (2011) Long-lasting protection in brain trauma by endotoxin preconditioning. *Journal of Cerebral Blood Flow and Metabolism*, Apr 6, doi: 10.1038/jcbfm.2011.42, ISSN 0271-678X

Longhi, L., Saatman, K.E., Fujimoto, S., Raghupathi, R., Meaney, D.F., Davis, J., McMillan, B.S.A, Conte, V., Laurer, H.L., Stein, S., Stocchetti, N. & McIntosh, T.K. (2005) Temporal window of vulnerability to repetitive experimental concussive brain injury. *Neurosurgery*, vol.56, no.2, pp. 364-374, ISSN 0148-396X

Lotocki, G., de Rivero Vaccari, J.P., Perez, E.R., Alonso, O.F., Curbelo, K., Keane, R.W. & Dietrich, W.D. (2006) Therapeutic hypothermia modulates TNFR1 signaling in the traumatized brain via early transient activation of the JNK pathway and suppression of XIAP cleavage. *European Journal of Neuroscience*, vol.24, no.8, pp.2283-2290, ISSN 0953-816X

Lowenstein, D.H., Thomas, M.J., Smith, D.H. & McIntosh, T.K. (1992) Selective vulnerability of dentate hilar neurons following traumatic brain injury: a potential mechanistic link between head trauma and disorders of the hippocampus. *Journal of Neuroscience*, vol.12, no.12, pp. 4846-4853, ISSN 0270-6474

Lyeth, B.G., Jenkins, L.W., Hamm, R.J., Dixon, C.E, Phillips, L.L., Clifton, G.L., Young, H.F. & Hayes, R.L. (1990) Prolonged memory impairment in the absence of hippocampal cell death following traumatic brain injury in the rat. *Brain Research*, vol.526, no.2, pp. 249-258, ISSN 0006-8993

Lynch, G. (1998) Memory and the brain: unexpected chemistries and a new pharmacology. *Neurobiology of Learning and Memory*, vol.70, no.1-2, pp. 82-100. ISSN 1074-7427

Lynch, G. & Gall, C.M. (2006) Ampakines and the threefold path to cognitive enhancement. *Trends in Neuroscience*, vol.29, no.10, pp. 554-62, ISSN 0166-2236

Lynch, G., Granger R., Ambros-Ingerson, J., Davis, C.M., Kessler, M. & Schehr, R. (1997) Evidence that a positive modulator of AMPA-type glutamate receptors improves delayed recall in aged humans. *Experimental Neurology*, vol.145, no.1, pp. 89-92, ISSN: 0014-4886

Lynch, G., Kessler, M., Rogers, G., Ambros-Ingerson, J., Granger, R. & Schehr, R.S. (1996) Psychological effects of a drug that facilitates brain AMPA receptors. *International Clinical Psychopharmacology*, vol.11, no.1, pp. 13-19, ISSN 0268-1315

Malenka, R.C. & Nicoll, R.A. (1999) Long-term potentiation—a decade of progress? *Science*, vol.285, no.5435, pp. 1870-1874, ISSN 0036-8075

Masel, B.E. & DeWitt, D.S. (2010) Traumatic brain injury: a disease process, not an event. *Journal of Neurotrauma*, vol.27, no.8, pp.1529-40, ISSN: 0897-7151

Matser, J.T., Kessels, A.G.H., Jordan, B.D., Lezak, M.D. & Troost, J. (1998) Chronic traumatic brain injury in professional soccer players. *Neurology*, vol.51, no.3, pp. 791-796, ISSN: 0028-3878

Matser, E.J., Kessels, A.G.H., Lezak, M.D., Jordan, B.D. & Troost, J. (1999) Neuropsychological impairment in amateur soccer players. *JAMA: Journal of the American Medical Association*, vol.282, no.10, pp. 971-973, ISSN 0098-7484

Matser, J.T., Kessels, A.G.H., Lezak, M.D. & Troost, J. (2001) A dose-response relation of headers and concussions with cognitive impairment in professional soccer players. *Journal of Clinical and Experimental Neuropsychology*, vol.23, no.6, pp. 770-774, ISSN 1380-3395

Max, W., Rice, D.P. & MacKenzie, E.J. (1990) The lifetime cost of injury. *Inquiry*, vol.27, no.4, pp. 332-43. ISSN: 0020-174x

McGregor, K. & Pentland, B. (1997) Head injury rehabilitation in the U.K.: an economic perspective. *Social Science and Medicine*, vol.45, no.2, pp. 295-303, ISSN 0277-9536

McCrory, P., Meeuwisse, W., Johnston, K., Dvorak, J., Aubry, M., Molloy, M. & Cantu, R. (2009) Consensus Statement on Concussion in Sport: the 3rd International Conference on Concussion in Sport held in Zurich, November 2008. *British Journal of Sports Medicine*, vol.43, Suppl 1, pp. i76-90, ISSN: 0306-3674

Morrison, B. 3rd, Saatman, K.E., Meaney, D.F. & McIntosh, T.K. (1998) In vitro central nervous system models of mechanically induced trauma: a review. *Journal of Neurotrauma*, vol.15, no.11, pp. 911-928, ISSN: 0897-7151

Nakagawa, Y., Nakamura, M., McIntosh, T.K., Rodriguez, A., Berlin, J.A., Smith, D.H., Saatman, K.E., Raghupathi, R., Clemens, J., Saido, T.C., Schmidt, M.L., Lee, V.M., Trojanowski, J.Q. (1999) Traumatic brain injury in young, amyloid-β peptide overexpressing transgenic mice induces marked ipsilateral hippocampal atrophy and diminished Aβ deposition during aging. *Journal of Comparative Neurology*, vol.411, no.3, pp.390-398, ISSN 0092-7317

Nell, V., Brown, D.S. (1991) Epidemiology of traumatic brain injury in Johannesburg—II. Morbidity, mortality and etiology. *Social Science and Medicine*, vol.33, no.3, pp. 289-96, ISSN 0277-9536

Noraberg, J., Poulsen, F.R., Blaabjerg, M., Kristensen, B.W., Bonde, C., Montero, M., Meyer, M., Gramsbergen, J.B., Zimmer, J. (2005) Organotypic hippocampal slice cultures for studies of brain damage, neuroprotection and neurorepair. *Current drug targets. CNS and neurological disorders*, vol.4, no.4, pp. 435-452, ISSN: 1568-007X

Olsson, Y., Rinder, L., Lindgren, S. & Stalhammar, D. (1971) Studies on vascular permeability changes in experimental brain concussion. 3. A comparison between the effects of single and repeated sudden mechanical loading of the brain. *Acta Neuropathologica (Berl)*, vol.19, no.3, pp. 225-233, ISSN 0001-6322

Pérez-Pinzón, M.A., Alonso, O., Kraydieh, S. & Dietrich, W.D. (1999) Induction of tolerance against traumatic brain injury by ischemic preconditioning. *NeuroReport*, vol.10, no.14, 2951-2954, ISSN 0959-4965

Powell, J.W. & Barber-Foss, K.D. (1999) Traumatic brain injury in high school athletes. *JAMA: Journal of the American Medical Association*, 282: 958-963, ISSN 0098-7484

Price, J.L., Davies, P.B., Morris, J.C., White, D.L. (1991) The distribution of tangles, plaques and related immunohistochemical markers in healthy aging and Alzheimer's disease. *Neurobiology of Aging*, vol.12, no. 4, pp. 295–312, ISSN 0197-4580

Raghupathi, R., Mehr, M.F., Helfaer, M.A. & Margulies, S.S. (2004) Traumatic axonal injury is exacerbated following repetitive closed head injury in the neonatal pig. *Journal of Neurotrauma*, vol.21, no.3, pp. 307-316, ISSN: 0897-7151

Reilly, P. (2007) The impact of neurotrauma on society: an international perspective. In: *Progress in Brain Research, vol. 161, Neurotrauma: New Insights into Pathology and Treatment*, Weber, J.T. & Maas, A.I.R. (Ed.), pp. 3-9. Elsevier, ISBN 978-0-444-53017-2, Amsterdam, The Netherlands.

Roberts, G.W., Whitwell, H.L., Acland, P.R. & Bruton, C.J. (1990) Dementia in a punch-drunk wife. *Lancet*, vol.335, no.8694, pp. 918-919, ISSN 0140-6736

Ryu, W.H., Feinstein, A., Colantonio, A., Streiner, D.L. & Dawson, D.R. (2009) Early identification and incidence of mild TBI in Ontario. *Canadian Journal of Neurological Sciences*, vol.36, no.4, pp. 429-35, ISSN 0317-1671

Schaller, B. & Graf, R. (2002) Cerebral ischemic preconditioning. An experimental phenomenon or a clinical important entity of stroke prevention? *Journal of Neurology*, vol.249, no.11, pp. 1503-1511. ISSN 0340-5354

Schmidt, M.L., Zhukareva, V., Newell, K.L., Lee, V.M-Y. & Trojanowski, J.Q. (2001) Tau isoform profile and phosphorylation state in dementia pugilistica recapitulate Alzheimer's disease. *Acta Neuropathologica (Berl)*, vol 101, no.5, pp. 518-524, ISSN 0001-6322

Schouten, J.W. (2007) Neuroprotection in traumatic brain injury: a complex struggle against the biology of nature. *Current Opinion in Critical Care*, vol.13, no. 2, pp. 134-4, ISSN 1070-5295

Shannon, P., Smith, C.R., Deck, J., Ang, L.C., Ho, M. & Becker, L. (1998) Axonal injury and the neuropathology of shaken baby syndrome. *Acta Neuropathologica (Berl)*, vol.95, no.6, pp. 95: 625-631, ISSN 0001-6322

Shein, N.A., Doron, H., Horowitz, M., Trembovler, V., Alexandrovich, A.G. & Shohami, E. (2007) Altered cytokine expression and sustained hypothermia following traumatic brain injury in heat acclimated mice. *Brain Research*, vol.1185, pp. 313-320, ISSN 0006-8993

Shein, N.A., Grigoriadis, N., Horowitz, M., Umschwief, G., Alexandrovich, A.G., Simeonidou, C., Grigoriadis, S., Touloumi, O. & Shohami, E. (2008) Microglial involvement in neuroprotection following experimental traumatic brain injury in heat-acclimated mice. *Brain Research*, vol.1244, pp. 132-41, ISSN 0006-8993

Shitaka. Y., Tran, H.T., Bennett, R.E., Sanchez, L., Levy, M.A., Dikranian, K. & Brody, D.L. (2011) Repetitive closed-skull traumatic brain injury in mice causes persistent multifocal axonal injury and microglial reactivity. *Journal of Neuropathology and Experimental Neurology,* vol.70, no.7, pp. 551-567, ISSN 0022-3069

Slemmer, J. E., De Zeeuw, C. I. & Weber, J. T. (2005) Don't get too excited: mechanisms of glutamate-mediated Purkinje cell death. In: *Progress in Brain Research, vol. 148, Creating Coordination in the Cerebellum*, De Zeeuw, C.I. & Cicirata (Ed.), pp. 367-390. Elsevier, ISBN 978-0-444-51754-8, Amsterdam, The Netherlands.

Slemmer, J. E., Hossain, M.Z. & Weber (2011) Animal Models of traumatically-induced dementia. In: Animal models of dementia, De Deyn & Van Dam (Ed.), pp. 643-662. Humana Press, ISBN 978-1-60761-897-3, New York.

Slemmer, J.E., Matser, E.J.T., De Zeeuw, C.I. & Weber, J.T. (2002) Repeated mild injury causes cumulative damage to hippocampal cells. *Brain*, vol.125, no.12, pp. 2699-2709, ISSN 0006-8950

Slemmer, J.E., Shacka, J.J, Sweeney, M.I. & Weber, J.T. (2008) Antioxidants and free radical scavengers for the treatment of stroke, traumatic brain injury and aging. *Current Medicinal Chemistry*, vol.15, pp. 404-414, ISSN 0929-8673

Slemmer, J.E. & Weber, J.T. (2005) The extent of damage following repeated injury to hippocampal cells is dependent on the severity of insult and inter-injury interval. *Neurobiology of Disease*, vol.18, no.3, pp. 421-431, ISSN 0969-9961

Slemmer, J.E., Weber, J.T. & De Zeeuw, C.I. (2004) Cell death, glial protein alterations and elevated S-100β protein in cerebellar cell cultures following mechanically induced trauma. *Neurobiology of Disease*, vol.15, no.3, pp. 563-572, ISSN 0969-9961

Spaethling, J.M., Geddes-Klein, D.M., Miller, W.J., von Reyn, C.R., Singh, P., Mesfin, M., Bernstein, S.J. & Meaney, D.F. (2007) Linking impact to cellular and molecular sequelae of CNS injury: modeling in vivo complexity with in vitro simplicity. In: *Progress in Brain Research, vol. 161, Neurotrauma: New Insights into Pathology and Treatment*, Weber, J.T. & Maas, A.I.R. (Ed.), pp. 27-39. Elsevier, ISBN 978-0-444-53017-2, Amsterdam, The Netherlands.

Staubli, U., Perez, Y., Xu, F.B., Rogers, G., Ingvar, M., Stone-Elander, S. & Lynch, G. Centrally active modulators of glutamate receptors facilitate the induction of long-term potentiation in vivo. (1994) *Proceedings of the National Academy of Sciences U.S.A.*, vol.91, no.23, pp. 11158-11162. ISSN 0027-8424

Sweeney, M.I., Kalt, W., MacKinnon, S.L., Ashby, J. & Gottschall-Pass, K.T. (2002) Feeding rats diets enriched in lowbush blueberries for six weeks decreases ischemia-induced brain damage. *Nutritional Neuroscience*, vol.5, no.6, pp. 427-31. ISSN 1028-415X

Szczygielski, J., Mautes, A., Steudel, W.I., Falkai, P., Bayer, T.A. & Wirths, O. (2005) Traumatic brain injury: cause or risk of Alzheimer's disease? A review of experimental studies. *Journal of Neural Transmission*, vol.112, no.11, pp. 1547-1564, ISSN 0300-9564

Thurman, D., Guerrero, J. (1999) Trends in hospitalization associated with traumatic brain injury. *JAMA: Journal of the American Medical Association* vol.282, no. 10, pp. 954-957, ISSN 0098-7484

Umschwief, G., Shein, N.A., Alexandrovich, A.G., Trembovler, V., Horowitz, M. & Shohami, E. (2010) Heat acclimation provides sustained improvement in functional recovery and attenuates apoptosis after traumatic brain injury. *Journal of Cerebral Blood Flow and Metabolism*, vol.30, no.3, pp. 616-27, ISSN 0271-678X

Uryu, K., Laurer, H., McIntosh, T., Pratico, D., Martinez, D., Leight, S., Lee, V.M-Y. & Trojanowski, J.Q. (2002) Repetitive mild brain trauma accelerates Aß deposition,

lipid peroxidation, and cognitive impairment in a transgenic mouse model of Alzheimer amyloidosis. *Journal of Neuroscience*, vol.22, no.2, pp. 446-454, ISSN: 0270-6474

Wall, S.E., Williams, W.H., Cartwright-Hatton, S., Kelly, T.P., Murray, J., Murray, M., Owen, A. & Turner, M. (2006) Neuropsychological dysfunction following repeat concussions in jockeys. *Journal of Neurology, Neurosurgery & Psychiatry*, vol.77, no.4, pp. 518-520, ISSN 0022-3050

Webbe, F.M. & Ochs, S.R. (2003) Recency and frequency of soccer heading interact to decrease neurocognitive performance. *Applied Neuropsychology*, vol.10, no.1, pp. 31-41, ISSN 0908-4282

Weber, J. T. (2004) Calcium homeostasis following traumatic neuronal injury. *Current Neurovascular Research*, vol.1, no.2, pp. 151-171, ISSN: 1567-2026

Weber, J.T. (2007) Experimental models of repetitive brain injuries. In: *Progress in Brain Research, vol. 161, Neurotrauma: New Insights into Pathology and Treatment*, Weber, J.T. & Maas, A.I.R. (Ed.), pp. 252-261. Elsevier, ISBN 978-0-444-53017-2, Amsterdam, The Netherlands.

Weber, J.T., De Zeeuw, C.I., Linden, D.J., Hansel, C. (2003) Long-term depression of climbing fiber-evoked calcium transients in Purkinje cell dendrites. *Proceedings of the National Academy of Sciences U.S.A.*, vol.100, no.5, pp. 2878-2883. ISSN 0027-8424

Weitbrecht, W.U. & Noetzel, H. (1976) Autoradiographic investigations in repeated experimental brain concussion (author's transl.) [Article in German]. *Archiv fur Psychiatrie und Nervenkrankheiten*, vol.223, no.1, pp. 59-68, ISSN 0003-9373

Wezenberg, E., Verkes, R.J., Ruigt, G.S., Hulstijn, W. & Sabbe, B.G. (2007) Acute effects of the ampakine farampator on memory and information processing in healthy elderly volunteers. *Neuropsychopharmacology*, vol.32, no.6, pp. 1272-1283, ISSN: 0893-133X

Wennberg, R.A. & Tator, C.H. (2003) National Hockey League reported concussions, 1986-87 and 2001-02. *Canadian Journal of Neurological Sciences* 30: 206-20, ISSN 0317-1671

Yoshiyama, Y., Uryu, K., Higuchi, M., Longhi, L., Hoover, R., Fujimoto, S., McIntosh, T., Lee, V.M-Y. & Trojanowski, J.Q. (2005) Enhanced neurofibrillary tangle formation, cerebral atrophy, and cognitive deficits induced by repetitive mild brain injury in a transgenic tauopathy mouse model. *Journal of Neurotrauma*, vol.22, no.10, pp. 1134-1141, ISSN: 0897-7151

Yuen, T.J., Browne, K.D., Iwata, A. & Smith, D.H. (2009) Sodium channelopathy induced by mild axonal trauma worsens outcome after a repeat injury. *Journal of Neuroscience Research*, vol.87, no.16, pp. 3620-3625, ISSN 0360-4012

2

Traumatic Brain Injury and Inflammation: Emerging Role of Innate and Adaptive Immunity

Efthimios Dardiotis[1,2], Vaios Karanikas[3], Konstantinos Paterakis[4], Kostas Fountas[2,4] and Georgios M. Hadjigeorgiou[1,2]
[1]*Department of Neurology, University Hospital of Larissa*
Faculty of Medicine, University of Thessaly, Larissa
[2]*Institute of Biomedical Research and Technology (BIOMED)*
Center for Research and Technology, Thessaly (CERETETH), Larissa
[3]*Department of Immunology, Faculty of Medicine, University of Thessaly, Larissa*
[4]*Department of Neurosurgery, University Hospital of Larissa*
Faculty of Medicine, University of Thessaly, Larissa
Greece

1. Introduction

Traumatic brain injury (TBI) has long been recognized as a leading cause of mortality and permanent neurological disability worldwide and has been described as a silent epidemic of modern societies. It is most common amongst young individuals, in their productive years of life, thereby causing a significant social and financial burden for them, their families and the public health system (Maas et al., 2008).

The pathophysiology of TBI is complex and multifactorial with several pathways involved in the damage of the brain. TBI has been classified into primary and secondary injury. The primary injury is the result of the external mechanical force at the moment of trauma leading to skull fractures, brain contusions, lacerations, diffuse axonal injuries, vascular tearing and intracranial hemorrhages (Maas et al., 2008). The initial impact damages directly the neuronal tissue via excitatory amino acids release and massive ionic influx referred to as traumatic depolarization (Katayama et al., 1995).

Secondary neuronal damage is induced immediately after primary injury and is mediated through several pathophysiologic mechanisms including raised intracranial pressure, disruption of blood brain barrier, brain edema, decreased cerebral blood flow, altered tissue perfusion, cerebral hypoxia, ischemia and reperfusion injury (Graham et al., 2000). Furthermore, a cascade of molecular, neurochemical, cellular and immune processes contribute to secondary damage such as disruption of calcium homeostasis, oxidative stress, excitatory mediators release, cytoskeletal and mitochondrial dysfunction, Ab-peptide deposition, inflammatory cell infiltration and neuronal cell apoptosis and death (Greve & Zink, 2009). Gene expression studies have demonstrated that several genes are implicated in the pathophysiology of secondary brain damage (Lei et al., 2009). Secondary cascade of events were found to dramatically aggregate primary neuronal damage and given that primary injury is unavoidable and irreversible, secondary processes are the targets of current therapeutic strategies and trials on neuroprotective agents (Jain, 2008).

Extensive research has indicated that cellular and humoral inflammation after TBI play a key role in the extent of brain injury and repair processes. The initiation, progression and resolution of inflammation in TBI is multifaceted involving leukocyte infiltration, activation of resident immune cells and secretion of inflammatory mediators such as pro- and anti-inflammatory cytokines, chemokines, adhesion molecules, complement factors, reactive oxygen species and other factors. Several lines of evidence support a dual role for the neuroinflammation either detrimental or beneficial depending on the extent, time and site of induction. Elucidation of the inflammatory cascade in the injured brain would offer the possibility of novel therapies.
The present article will focus on the TBI induced neuroinflammation and on the current knowledge regarding the involvement of innate and adaptive immune system in the inflammation and repair following TBI.

2. Neuroinflammation

The normal central nervous system (CNS) limits the entry of immune components and is traditionally regarded as an immune privileged organ separated from the peripheral immune system by the blood-brain-barrier (BBB). However, this concept of limited immune intervention in the CNS has been questioned, since under physiological conditions, resident brain cells are capable of immune surveillance and expression of immune mediators within the CNS. In addition, T-lymphocytes are known to enter the healthy brain parenchyma to perform surveillance in the absence of inflammatory stimulus (Hickey, 1999; Becher et al., 2000). During inflammatory brain insults the immune privileged status is compromised with an activation of innate immune cells and mobilization of specific adaptive immune responses.
A growing body of evidence suggests a pivotal role of TBI induced cerebral inflammation, including activation of resident cells, migration and recruitment of leukocytes and release of inflammatory mediators, in the extent of neuronal injury and repair. Inflammation after TBI is believed to be triggered by several factors such as extravasated blood products, tissue debris, intracellular components, complement fragments, prostaglandins, reactive oxygen and nitrogen species. The BBB is disrupted after TBI resulting in invasion of neutrophils, monocytes and lymphocytes from the periphery and activation of microglia and other resident cells and thus initiating a potent inflammatory response. A biphasic BBB breakdown after TBI has been reported with a first opening occurring immediately after the primary impact reaching a maximum permeability within a few hours and then being declined. A second-delayed opening as a result of secondary injury cascades was found to peak around 3-7 days following TBI and can last from days to years (Baskaya et al., 1997; Shlosberg et al., 2010).
The accumulation of leukocytes into the injured brain area is crucial to the extent of inflammation and secondary brain damage. Leukocytes migrate out of blood vessels into the injured brain parenchyma via binding to the endothelial selectins P and E and the intercellular adhesion molecules (ICAMs). Chemokines from the injured brain tissue contribute to the expression of these endothelial molecules in the local vasculature. Chemokines are produced by resident cells including microglia, astrocytes and neurons in response to local inflammation (Ransohoff, 2002). For instance, the chemokine CXCL8 (IL8) interacts with leukocytes, triggering the activation of the integrins LFA-1 and CR3 (Mac-1) in the surface of leukocytes. These integrins consequently interact with endothelial ICAM-1 and ICAM-2 leading to a firm adhesion, conformational changes and extravasation of

leukocytes between endothelial cells. Finally, these leukocytes migrate along the concentration gradient of chemokines to the site of TBI. Neutrophil accumulation peaks within 2 days after TBI whereas monocytes accumulate slightly later (Rhodes, 2011).

Leukocytes are believed to be important in the initiation and progression of inflammation following TBI because they contain and release a significant number of inflammatory mediators that injure neurons. Increased leukocyte infiltration has been linked to increased brain damage. Leukocytes release pro-inflammatory cytokines, proteases, prostaglandins, complement factors, free oxygen and nitrogen species which damage neuronal population and brain microvasculature and contribute to the disruption of BBB and formation of vasogenic edema (Nguyen et al., 2007). Studies in vitro have shown that mixed cultures of hippocampal neurons and neutrophils contributed to increased neuronal loss and excitotoxic damage (Dinkel et al., 2004). Also, leukocyte accumulation seemed to mediate the detrimental effects of chemokines. It was shown that increased intrathecal levels of CXCL8 were correlated to the extent of posttraumatic BBB dysfunction and mortality (Kossmann et al., 1997; Whalen et al., 2000). However, these effects were attenuated by prior depletion of the circulating leukocytes (Bell et al., 1996). Leukocytes also contribute to oxidative damage in the injured brain tissue. Free oxygen radicals released by leukocytes induce lipid and protein peroxidation, mitochondrial and DNA damage and neuronal apoptosis (Tyurin et al., 2000).

It has been hypothesized that inhibition of neutrophil function or migration would reduce the injury size and improve the functional outcome after TBI. This notion has already been proved in experimental models of ischemic brain injury. However, the beneficial role of leukocyte inhibition is less convincing in TBI experiments. Studies in animal models and humans with severe TBI have shown increased expression of the adhesion molecules selectin E and ICAM-1 in the early period following TBI (Carlos et al., 1997; McKeating et al., 1998; Pleines et al., 1998) indicating that these molecules are important in the neutrophil recruitment in the injured brain. Administration of monoclonal antibodies directed against the leukocyte adhesion molecules CD11b (aM subunit of integrin CR3) and ICAM-1 resulted in decreased neutrophil migration (Carlos et al., 1997; Weaver et al., 2000; Knoblach & Faden, 2002) and better clinical recovery (Knoblach & Faden, 2002) after experimental brain trauma. However, in the latter study the beneficial effect of anti-ICAM-1 treatment was also achieved, although to a lesser extent, with the administration of a nonspecific IgG, indicating that part of the effects may be attributed to the general properties of the antibodies. Moreover, ICAM-1 gene deficient mice with TBI did not demonstrate evidence of improved neurological function, reduced lesion volume or neutrophil accumulation compared to wild type control mice (Whalen et al., 1999), suggesting that other adhesion molecules may also play a significant role in the recruitment of neutrophils. Inhibition of neutrophil infiltration was also tested by blocking chemokine expression. Mice deficient in CXC receptor 2 which interacts with chemokines CXCL8, CXCL1 and CXCL2 and mediates the neutrophil transmigration across the BBB were reported to demonstrate significant attenuation of neutrophil infiltration, reduced tissue damage and neuronal loss, especially in the delayed phase post injury (Semple et al., 2010a). In a similar study, deletion of monocyte chemokine CCL2 gene resulted in improved neurological function, delayed reduction in lesion volume and macrophage accumulation (Semple et al., 2010b). Both the latter studies support the notion that late inhibition of leukocyte recruitment in TBI may be beneficial for the extent of brain trauma and the clinical outcome. These results were not achieved when neutrophil depletion was applied early in the course of TBI (Whalen et al., 1999), indicating that leukocyte infiltration in the early phase post injury may mediate some beneficial

physiologic processes and only delayed and prolonged leukocyte recruitment may be deleterious to the neuronal survival.

Apart from leukocyte infiltration, the humoral components of neuroinflammation were also found to play an important role in the initiation, maintenance and resolution of inflammation following TBI. The primary traumatic impact and the ensuing injury triggers the release of several cytokines which facilitate the migration of inflammatory cells, the activation of resident cells, the expression of vascular endothelial molecules and chemokines. Cellular sources of cytokines include leukocytes, lymphocytes, microglia, astrocytes, endothelial cells and neurons. Cytokines are induced shortly after primary insult and this early increase is mediated by resident brain cells. Cytokines have multiple actions and targets, and often overlapping biological effects. Cytokines exert their function either through binding to their receptors, which are expressed by both glial and neuronal cells, or through diverse pathways such as modulation of neurotransmitter receptor function, induction of nitric oxide synthase, secretion of chemokines and proteolytic enzymes (Allan & Rothwell, 2001).

Interleukin-1 (IL-1) is a pro-inflammatory cytokine that has been identified as an important mediator of the inflammation following TBI. The IL-1 family has three main members: the pro-inflammatory cytokines IL-1a and IL-1b, which exert their action by binding to the cell surface receptor IL-1RI, and the anti-inflammatory cytokine IL-1 receptor antagonist (IL-1ra) (Rothwell & Luheshi, 2000). The pro-inflammatory cytokines IL-1a and IL-1b have pleiotropic effects which are mediated by binding to the IL-1RI. IL-1 triggers inflammatory reactions, leads to recruitment of leukocytes, disruption of BBB and formation of edema, induces other interleukins, prostagladins, histamine, thromboxane, chemokines and adhesion molecules and exerts multiple effects in neuronal, glial and endothelial cells (Hopkins & Rothwell, 1995; Rothwell & Hopkins, 1995). IL-1ra is a naturally occurring competitive and highly selective inhibitor of IL-1a and IL-1b which binds to the IL-1RI without initiating signal transduction. IL-1ra plays an important role in the regulation of the inflammatory response and the balance between proinflammatory and anti-inflammatory cytokines (Arend, 1991; Dinarello, 1991).

In experimental TBI a rapid induction of IL-1b (mRNA expression and protein levels) was observed in the very early period following TBI (Fan et al., 1995; Wang & Shuaib, 2002). Similarly, IL-1ra was upregulated in response to head injury but shortly after the induction of IL-1b (Gabellec et al., 1999). Elevated levels of IL-1b were also detected intrathecally in patients with head injury (Winter et al., 2002). Moreover, these elevated levels were correlated to poorer clinical outcome (Chiaretti et al., 2005; Shiozaki et al., 2005). The proinflammatory cytokines IL-1a and IL-1b are believed to initiate inflammation and to contribute to neurodegeneration after various brain insults including TBI, whereas IL-1ra seemed to be neuroprotective. In experimental animal models, intracerebral or intraventricular administration of exogenous IL-1b markedly exacerbates brain injury (Patel et al., 2003). In contrast, administration or overexpression of IL-1ra significantly attenuates neuronal damage and inflammation (Toulmond & Rothwell, 1995; Sanderson et al., 1999; Tehranian et al., 2002). Apart from acute neuroinflammation, TBI induces long-term and persistent inflammation with elevation of IL-1 and other cytokines and increased expression of beta-amyloid protein and phosphorylated tau protein. This long-term inflammation may be the causative link between TBI and traumatic dementia (Hoshino et al., 1998; Holmin & Mathiesen, 1999). These data highlight the important role of IL-1 in the acute and chronic neuroinflammation following TBI and the possibility of beneficial effects that may ensue after its therapeutic inhibition. However, many studies have underlined the complexity of

inflammatory processes, the lack of meaningful effects after blocking a single inflammatory mediator and the duality of inflammation which means that inflammation may have either detrimental or beneficial effects depending on the site, the time of induction, the concentration of mediators and the microenvironment (Morganti-Kossmann et al., 2002). This duality was demonstrated for IL-1 which aside from its pro-inflammatory effects also seems to participate in tissue repair processes, especially when induced at later stages, via stimulation of neurotrophic factors synthesis (Spranger et al., 1990; DeKosky et al., 1994; Herx et al., 2000), astrocyte proliferation (Appel et al., 1997) and involvement in synaptic plasticity (Fagan & Gage, 1990; Bellinger et al., 1993; Ide et al., 1996).

IL-6 is another cytokine that has been studied in TBI. IL-6 was found to have also a dual role in inflammation with either regulatory, anti-inflammatory or inflammatory effects depending on the time course and extent of expression (Allan & Rothwell, 2001; Morganti-Kossmann et al., 2002). The neurotrophic properties of IL-6 are mediated by inhibition of TNFa synthesis, induction of IL-1ra and nerve growth factor and attenuation of oxidative stress (Morganti-Kossmann et al., 2001). On the contrary, IL-6 promotes inflammatory processes by stimulating the production of chemokines and adhesion molecules and the recruitment of leukocytes (Romano et al., 1997). Elevated levels of IL-6 were observed in the cerebrospinal fluid (CSF) and in the serum of patients with TBI and this increase was correlated with a favorable neurological outcome (Singhal et al., 2002; Chiaretti et al., 2008). In contrast, other studies demonstrated that IL-6 levels were correlated to the clinical severity of TBI patients (Arand et al., 2001; Minambres et al., 2003). Studies in animal models provide evidence for a neuroprotective effect of IL-6. IL-6 was found at elevated levels in experimental TBI (Shohami et al., 1994). Mice deficient for IL-6 had increased numbers of apoptotic neurons, increased oxidative stress and delayed healing of the tissue (Penkowa et al., 2000), whereas the same group demonstrated that IL-6 transgenic mice exhibited increased reduction of oxidative stress and apoptotic cell death after a cryogenic brain injury (Penkowa et al., 2003).

Tumor necrosis factor-a (TNFa) is another cytokine with a well-documented role in TBI. TNFa mRNA and protein is elevated in the early period after experimental TBI and before the infiltration of leukocytes suggesting that the early source of TNFa production are the resident cells (Riva-Depaty et al., 1994). Elevated levels were also observed in the clinical setting of TBI patients (Goodman et al., 1990; Csuka et al., 1999). TNFa has pro-inflammatory properties similar to that of IL-1 and exacerbates inflammation and secondary brain damage after TBI (Allan & Rothwell, 2001). Early upregulation of neuronal TNFa expression after TBI was found to contribute to subsequent neurological dysfunction (Knoblach et al., 1999). Inhibition of TNFa by the HU-211 compound (a novel TNFa production inhibitor), pentoxyfilline and TNF-binding protein resulted in improved neurological outcome after closed head injury (Shohami et al., 1997). However, in a phase III clinical trial, administration of the HU-211 compound in patients with TBI failed to show improved outcome 6 months after the injury, compared to the placebo group (Maas et al., 2006). These data indicate that neurodegeneration is mediated through various pathological pathways and neuroprotection cannot be achieved by blocking a single mediator as other alternative pathways may be activated leading to neuronal loss. Furthermore, as reported with IL-1, TNFa also has neuroprotective effects and can enhance recovery processes. In a very interesting study, knockout mice for the TNFa gene exhibited milder behavioral deficits compared to the wild-type mice during the acute period post-injury. However, in the long term period (4 weeks post-injury) knockout mice did not recover as well as the wild-type mice, had persistent motor deficits and greater cortical tissue loss (Scherbel et al.,

1999). These results suggest that the time, concentration and the site of TNFa induction may determine the driving of inflammatory processes towards neurodegeneration or neuroprotection.

3. Innate immunity

Microglia, the brain's resident macrophages, are the main cell type of the innate immune system of the brain. Microglia, although debatable, seem to originate from bone marrow monocytic cells which invade the CNS during embryonic development (Chan et al., 2007). Microglia provide a first line of regional defense in the CNS against various pathological insults. They are scattered throughout the CNS although some regional differences in their localization have been reported as they are more densely distributed in the gray than in the white matter and in structures like hippocampus, basal ganglia and substantia nigra (Block et al., 2007).

While resting, microglia have a highly ramified morphology with symmetrically extended, motile processes that form a network, which continuously monitor the local microenvironment of the brain parenchyma being the most susceptible sensors of brain pathology (Nimmerjahn et al., 2005; Kettenmann et al., 2011). In physiological conditions they provide surveillance of the CNS homeostasis and they sense neuronal and astrocytic activity and other physiological changes such as pH shifts, ion currents and neurotransmitter release (Farber & Kettenmann, 2005). This is achieved by the expression of numerous receptors by the microglia establishing a delicate neuron-microglia communication (McCluskey & Lampson, 2000). In an in vitro study the normal neuronal activity was found to inhibit the effects of microglia activators such as interferon-γ signifying the importance of cell to cell interactions (Neumann et al., 1996).

Various brain insults including bacterial lipopolysaccharide (LPS), cytokines, b-amyloid peptide and damaged tissue can result in activation of microglia (Nakamura, 2002). Upon activation, the cell size increases and the morphology dramatically changes to an amoeboid structure which facilitates the migration of microglial cells towards the lesion site and the phagocytosis of cellular debris and toxic substances (Raivich, 2005). In response to noxious stimuli microglia also proliferate and migrate to the lesion site. The rapidly chemotactic convergence to the site of injury is mediated by ATP, glutamate and other chemotactic agents released by the injured cells (Davalos et al., 2005; Liu et al., 2009). At this point the morphology of activated microglia cannot be discriminated from that of infiltrating macrophages using standard immunohistochemical techniques (Streit et al., 1999; Loane & Byrnes, 2010).

A significant part in the activation of microglia after inflammatory stimuli is the expression of constitutive and inducible surface receptors. Activated microglia express pattern recognition receptors, cytokine and chemokine receptors, phagocytic receptors, Fc and complement receptors, receptors for glutamate, growth factors and several other molecules (Gebicke-Haerter et al., 1996; Cho et al., 2006; Kettenmann et al., 2011). Activated microglia also express on their surface MHC class I and II molecules, making them able to present antigenic peptides and thus modulating T cell responses (Aloisi, 2001).

The specific profile of the surface receptors determine the phenotype of microglia and their functional properties. In line with macrophages phenotype, activated microglia may be neurotoxic (M1) due to the secretion of pro-inflammatory cytokines and reactive oxygen and nitrogen species. In contrast, activation of microglia may enable them to maintain and

enhance neuronal survival (M2) through the release of anti-inflammatory cytokines and neurotrophic factors. However, it is possible that M1 and M2 phenotypes may represent the two extremes of a wide spectrum of phenotypes that microglia can have in response to the type, intensity, persistence of the stimuli and the microenvironment interactions (Mantovani et al., 2004). In fact, microglia have plastic properties and at different stages of the disease can acquire diverse phenotypes and functions that can be either detrimental or beneficial.

The activation process begins when resting microglia detect the noxious stimuli or the sub-products of tissue damage. Microglia become activated, release inflammatory mediators, express surface molecules and remove cellular debris by phagocytosis. Once the toxic factors are eliminated and under the influences of the invading immune cells and the normal CNS cells activated microglia acquire a neurotrophic phenotype and release anti-inflammatory cytokines and neurotrophic factors. After a certain period of time inflammation is resolved and the activating microglia return to a resting-surveying state retaining some kind of memory of the processes. However, under not fully elucidated conditions, this delicate balance between activation-termination and neurotoxic-neurotrophic phenotype can be disrupted leading to excessive, uncontrolled or prolonged activation of microglia with destructive consequences in neuronal survival. Excessive and dysregulated microglia activation were elicited after intense and severe CNS insults. Moreover, insufficient recruitment of systemic immune cells to the CNS site of lesion may result in an inability to suppress and terminate the microglia activation or to turn them into a neuroprotective phenotype (Kempermann & Neumann, 2003; Hanisch & Kettenmann, 2007; Popovich & Longbrake, 2008; Rivest, 2009; Schwartz & Shechter, 2010). Thus, dysregulation of the innate or adaptive immune system may mediate excessive inflammatory damage following brain insults. It is obvious that a better understanding of the interaction mechanisms between immune cells may facilitate the introduction of novel therapies.

Several studies have investigated the microglia function after TBI. The release of various mediators in the extracellular space after the injured site alters the expression profile of local microglia. In a very interesting imaging study of fluorescent labeled microglia it was shown that microglia from the intact brain that are nearby the injured site extend their processes which reach the damaged site. There the processes and without cell body movement, converge and fuse together to form a spherical area of containment that separate the healthy from the injured tissue. In other words fused microglial processes act as a barrier that contains the tissue debris. This highly dynamic movement of microglial processes is under the chemotactic influence of extracellular ATP (Davalos et al., 2005).

The time of induction and the duration of microglia activation after TBI were found to be different from other brain insults such as cerebral ischemia. Studies in humans with TBI have revealed a striking delay of microglial activation and proliferation. Markers of microglial activation and proliferation were not detected until 3 days post TBI (Beschorner et al., 2000; Engel et al., 2000) whereas the same markers were expressed early in cerebral ischemia (Postler et al., 1997). This observation although in line with some findings in experimental models (Aihara et al., 1995; Holmin et al., 1997) cannot be fully understood. It is possible to reflect different activation cascades of microglia after TBI compared to other brain insults. However this window delay of microglial activation after TBI may provide a therapeutic opportunity when the target would be the microglial activation.

Several studies in TBI have also demonstrated that inflammation can persist for long period of time after the primary traumatic brain insult. Studies in rodents have revealed reactive astrocytosis for over a year following brain injury resulting in chronic progressive neuronal

tissue loss (Smith et al., 1997; Holmin & Mathiesen, 1999). In primates, microglia activation was found to persist for at least 12 months (Nagamoto-Combs et al., 2007). Post-mortem studies in humans have shown persistent elevated microglial activity several years after TBI (Gentleman et al., 2004). In addition, a recent PET imaging study in humans using a ligand that binds to activated microglia revealed increased microglial activation for up to 17 years after TBI (Ramlackhansingh et al., 2011). Persistent microglial activation is believed to underline both chronic neuroprotective and destructive processes. The precise mechanisms for the persistence of inflammatory processes after TBI are not fully understood. It is believed that a severe initial neuronal injury with excessive cytokine signals may lead to enhanced activation of microglia that further aggravate brain damage feeding a self-sustaining and self-propelling prolonged vicious circle of neurotoxicity and progressive degeneration (Gao & Hong, 2008). This dysregulated and uncontrolled microglial activation may be the key for chronic, long-lived and destructive inflammatory processes. The long-term microglia activation may also provide insights into a potential causative link between head injury and Alzheimer's disease.

4. Adaptive immunity

Adaptive immunity refers to an antigen-specific response either cell-mediated or humoral aiming at elimination of pathogenic factors. Adaptive immunity is mediated by B and T lymphocytes. In experiment TBI, T cells were found to infiltrate the brain parenchyma in a biphasic manner: immediately after the primary impact as a result of disruption of BBB and in a delayed phage with increased numbers of T cells which represents an active infiltration of specific targeting T-cells (Czigner et al., 2007). In other studies it was shown that T cell infiltration in the damaged tissue occurred 3-14 days after CNS trauma and persisted for 6 months (Kigerl et al., 2006; Beck et al., 2010). In post-mortem human studies $CD4^+$ and $CD8^+$ were identified in the injured tissue after spinal cord injury (Fleming et al., 2006).

It is known that the transformation of naive T cells into effector T cells requires an initial antigen presentation in secondary lymphoid organs and a re-activation after re-exposure to their antigen. However, the exact mechanics of adaptive immune response after brain injury have not been fully elucidated and some aspects remain obscure. It is known that naive T cells against CNS antigens are circulating in the periphery. After brain insults the cerebral auto-antigens are exposed to the peripheral blood cells. The activation of naive T cells can take place in the peripheral lymph nodes as a result of BBB disruption and release of cerebral antigens into the bloodstream alone or in conjunction with the brain APCs (Lenzlinger et al., 2001; Ling et al., 2003; Karman et al., 2004). After activation, T cells traffic to the CNS under the influence of chemokine gradient. Microglia are already activated as a result of brain tissue damage and release of inflammatory mediators. Activated microlgia release at the site of injury cytokines and chemokines, express various surface molecules including complement components and induce the expression of adhesion molecules by the endothelial cells. These alterations induce the recruitment of the T cells into the injury site. Within the CNS, T cells become re-activated against their antigen which is presented to them by local microglia and macrophages.

As already mentioned MHC expression is almost absent in normal brain parenchyma. After brain trauma activated microglia upregulate the expression of MHC class I and class II and the expression of adhesion molecules and costimulatory factors. Activated microglia can act as antigen-presenting cells to T cells by phagocytosis of the tissue debris,

processing of the relevant antigen and its subsequent presentation on class I or class II MHC (Ankeny et al., 2006).

Another hypothesis that has been introduced is that the initial activation of T cells takes place within CNS. However, data have shown that the interaction between microglia and T cells is incomplete and insufficient to support a full activation and proliferation of T cells. In an ex vivo study this interaction resulted in increased T cell apoptosis which may reflect a regulatory function of microglia upon T cell responses (Ford et al., 1996). It seems possible that activated microglia may influence the function and maintenance of auto-reactive T cells in the CNS either directly by presenting the antigen causing re-activation of the T cells or indirectly by determining the local inflammatory/anti-inflammatory microenvironment and the recruitment of T cells.

The peripheral activation of T cells against cerebral antigens is supported by a number of studies. Several studies have provided evidence for a traveling of the antigen from the CNS to the peripheral lymph nodes. Fluorescent substances injected into the CNS of animal models were detected in the cervical lymph nodes in a few hours after the injection. In addition, intracerebrally infused protein antigen was found to elicit the accumulation of antigen-specific CD8+ T cells 3 days after the injection (Ling et al., 2003). Moreover, experimental and human studies of CNS trauma have revealed the presence of auto-reactive T cells against cerebral antigens. Isolated T cells from an animal model of spinal cord injury when injected intravenously into naive recipients were found to induce spinal cord neuroinflammation and transient hind limb paralysis and ataxic gait (Popovich et al., 1996). Additionally auto-reactive T cells against myelin basic protein were found in increased frequencies in patients with spinal cord injury compared to multiple sclerosis patients and normal controls (Kil et al., 1999).

Adaptive immunity may mediate either pathogenic or reparative processes based of the inflammatory conditions of the microenvironment during activation of T-cells. It has been hypothesized that Th1 immune response may aggravate brain damage by pro-inflammatory actions whereas Th2 response may alleviate brain damage by anti-inflammatory and neurotrophic effects. In a study of freeze cerebral injury activated T cells were found to exacerbate brain damage when transferred to rats 24h before the injury (Fee et al., 2003). In contrast, in an very intriguing study in animals with injury in the optic nerve it was shown that intraperitoneally injected anti-myelin basic protein specific T cells prevented the secondary degeneration of retinal ganglion cells (Moalem et al., 1999). These significant data were also replicated in an animal model of spinal cord injury. It was shown that autoreactive T cells against myelin basic protein or active immunization with myelin basic protein improved the recovery by promoting neuroprotective and regenerative processes (Hauben et al., 2000). Furthermore, it was showed that Th2 cells specific for myelin basic protein had protective effects on neuronal survival. This neuroprotective effect of the antigen-specific T cells was influenced by the extent of non-specific activation of the T cells (Wolf et al., 2002). In another study of peripheral facial nerve injury $CD4^+$ T cells were found to mediate facial motor neurons survival (Serpe et al., 2003).

These observations of beneficial effects of autoreactive T cells led to the introduction of the term "protective autoimmunity" implying that the recognition of an exposed self antigen by T cells can result in protection, repair and maintenance of the functional integrity of a tissue rather than the initiation of an autoimmune process. (Schwartz et al., 2003; Schwartz & Shechter, 2010). Regulatory T cells ($CD4^+CD25^+Foxp3^+$) that suppress autoimmune activity seem to play a central role in protective autoimmunity. The concept of protective

autoimmunity raises some interesting therapeutics options. A vaccination with weak CNS specific antigens or a modulation of regulatory T cells activity that enhance protective autoimmunity would be a novel approach for neuroprotection and repair after CNS trauma (Schwartz et al., 2009).

In conclusion, neuroinflammation seems to play a key role in the pathophysiology of brain damage following TBI. A complex interaction between several components and mediators of the innate and adaptive immunity appear to determine the extent of inflammation and its nature, either destructive or reparative. A better understanding of these mechanisms that are implicated in the initiation, progression and termination of the inflammation and the communication between immune cells is required for the development of new and effective therapeutic strategies.

5. References

Aihara, N., et al. (1995). Altered immunoexpression of microglia and macrophages after mild head injury. *J Neurotrauma*, 12, 1, pp. 53-63.

Allan, S. M. and Rothwell, N. J. (2001). Cytokines and acute neurodegeneration. *Nat Rev Neurosci*, 2, 10, pp. 734-44.

Aloisi, F. (2001). Immune function of microglia. *Glia*, 36, 2, pp. 165-79.

Ankeny, D. P., et al. (2006). Spinal cord injury triggers systemic autoimmunity: evidence for chronic B lymphocyte activation and lupus-like autoantibody synthesis. *J Neurochem*, 99, 4, pp. 1073-87.

Appel, E., et al. (1997). Regulation of GDNF expression in cultured astrocytes by inflammatory stimuli. *Neuroreport*, 8, 15, pp. 3309-12.

Arand, M., et al. (2001). Early inflammatory mediator response following isolated traumatic brain injury and other major trauma in humans. *Langenbecks Arch Surg*, 386, 4, pp. 241-8.

Arend, W. P. (1991). Interleukin 1 receptor antagonist. A new member of the interleukin 1 family. *J Clin Invest*, 88, 5, pp. 1445-51.

Baskaya, M. K., et al. (1997). The biphasic opening of the blood-brain barrier in the cortex and hippocampus after traumatic brain injury in rats. *Neurosci Lett*, 226, 1, pp. 33-6.

Becher, B., et al. (2000). Brain-immune connection: immuno-regulatory properties of CNS-resident cells. *Glia*, 29, 4, pp. 293-304.

Beck, K. D., et al. (2010). Quantitative analysis of cellular inflammation after traumatic spinal cord injury: evidence for a multiphasic inflammatory response in the acute to chronic environment. *Brain*, 133, Pt 2, pp. 433-47.

Bell, M. D., et al. (1996). Overriding the brain's intrinsic resistance to leukocyte recruitment with intraparenchymal injections of recombinant chemokines. *Neuroscience*, 74, 1, pp. 283-92.

Bellinger, F. P., et al. (1993). Interleukin 1 beta inhibits synaptic strength and long-term potentiation in the rat CA1 hippocampus. *Brain Res*, 628, 1-2, pp. 227-34.

Beschorner, R., et al. (2000). Differential regulation of the monocytic calcium-binding peptides macrophage-inhibiting factor related protein-8 (MRP8/S100A8) and allograft inflammatory factor-1 (AIF-1) following human traumatic brain injury. *Acta Neuropathol*, 100, 6, pp. 627-34.

Block, M. L., et al. (2007). Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. *Nat Rev Neurosci*, 8, 1, pp. 57-69.

Carlos, T. M., et al. (1997). Expression of endothelial adhesion molecules and recruitment of neutrophils after traumatic brain injury in rats. *J Leukoc Biol*, 61, 3, pp. 279-85.

Chan, W. Y., et al. (2007). The origin and cell lineage of microglia: new concepts. *Brain Res Rev*, 53, 2, pp. 344-54.

Chiaretti, A., et al. (2008). Interleukin-6 and nerve growth factor upregulation correlates with improved outcome in children with severe traumatic brain injury. *J Neurotrauma*, 25, 3, pp. 225-34.

Chiaretti, A., et al. (2005). Interleukin 1beta and interleukin 6 relationship with paediatric head trauma severity and outcome. *Childs Nerv Syst*, 21, 3, pp. 185-93; discussion 194.

Cho, B. P., et al. (2006). Pathological dynamics of activated microglia following medial forebrain bundle transection. *Glia*, 53, 1, pp. 92-102.

Csuka, E., et al. (1999). IL-10 levels in cerebrospinal fluid and serum of patients with severe traumatic brain injury: relationship to IL-6, TNF-alpha, TGF-beta1 and blood-brain barrier function. *J Neuroimmunol*, 101, 2, pp. 211-21.

Czigner, A., et al. (2007). Kinetics of the cellular immune response following closed head injury. *Acta Neurochir (Wien)*, 149, 3, pp. 281-9.

Davalos, D., et al. (2005). ATP mediates rapid microglial response to local brain injury in vivo. *Nat Neurosci*, 8, 6, pp. 752-8.

DeKosky, S. T., et al. (1994). Upregulation of nerve growth factor following cortical trauma. *Exp Neurol*, 130, 2, pp. 173-7.

Dinarello, C. A. (1991). Interleukin-1 and interleukin-1 antagonism. *Blood*, 77, 8, pp. 1627-52.

Dinkel, K., et al. (2004). Neurotoxic effects of polymorphonuclear granulocytes on hippocampal primary cultures. *Proc Natl Acad Sci U S A*, 101, 1, pp. 331-6.

Engel, S., et al. (2000). Dynamics of microglial activation after human traumatic brain injury are revealed by delayed expression of macrophage-related proteins MRP8 and MRP14. *Acta Neuropathol*, 100, 3, pp. 313-22.

Fagan, A. M. and Gage, F. H. (1990). Cholinergic sprouting in the hippocampus: a proposed role for IL-1. *Exp Neurol*, 110, 1, pp. 105-20.

Fan, L., et al. (1995). Experimental brain injury induces expression of interleukin-1 beta mRNA in the rat brain. *Brain Res Mol Brain Res*, 30, 1, pp. 125-30.

Farber, K. and Kettenmann, H. (2005). Physiology of microglial cells. *Brain Res Brain Res Rev*, 48, 2, pp. 133-43.

Fee, D., et al. (2003). Activated/effector CD4+ T cells exacerbate acute damage in the central nervous system following traumatic injury. *J Neuroimmunol*, 136, 1-2, pp. 54-66.

Fleming, J. C., et al. (2006). The cellular inflammatory response in human spinal cords after injury. *Brain*, 129, Pt 12, pp. 3249-69.

Ford, A. L., et al. (1996). Microglia induce CD4 T lymphocyte final effector function and death. *J Exp Med*, 184, 5, pp. 1737-45.

Gabellec, M. M., et al. (1999). Expression of interleukin-1 genes and interleukin-1 receptors in the mouse brain after hippocampal injury. *Neurosci Res*, 33, 4, pp. 251-60.

Gao, H. M. and Hong, J. S. (2008). Why neurodegenerative diseases are progressive: uncontrolled inflammation drives disease progression. *Trends Immunol*, 29, 8, pp. 357-65.

Gebicke-Haerter, P. J., et al. (1996). Molecular mechanisms of microglial activation. A. Implications for regeneration and neurodegenerative diseases. *Neurochem Int*, 29, 1, pp. 1-12.

Gentleman, S. M., et al. (2004). Long-term intracerebral inflammatory response after traumatic brain injury. *Forensic Sci Int*, 146, 2-3, pp. 97-104.

Goodman, J. C., et al. (1990). Elevation of tumor necrosis factor in head injury. *J Neuroimmunol*, 30, 2-3, pp. 213-7.

Graham, D. I., et al. (2000). Recent advances in neurotrauma. *J Neuropathol Exp Neurol*, 59, 8, pp. 641-51.

Greve, M. W. and Zink, B. J. (2009). Pathophysiology of traumatic brain injury. *Mt Sinai J Med*, 76, 2, pp. 97-104.

Hanisch, U. K. and Kettenmann, H. (2007). Microglia: active sensor and versatile effector cells in the normal and pathologic brain. *Nat Neurosci*, 10, 11, pp. 1387-94.

Hauben, E., et al. (2000). Passive or active immunization with myelin basic protein promotes recovery from spinal cord contusion. *J Neurosci*, 20, 17, pp. 6421-30.

Herx, L. M., et al. (2000). Central nervous system-initiated inflammation and neurotrophism in trauma: IL-1 beta is required for the production of ciliary neurotrophic factor. *J Immunol*, 165, 4, pp. 2232-9.

Hickey, W. F. (1999). Leukocyte traffic in the central nervous system: the participants and their roles. *Semin Immunol*, 11, 2, pp. 125-37.

Holmin, S. and Mathiesen, T. (1999). Long-term intracerebral inflammatory response after experimental focal brain injury in rat. *Neuroreport*, 10, 9, pp. 1889-91.

Holmin, S., et al. (1997). Delayed cytokine expression in rat brain following experimental contusion. *J Neurosurg*, 86, 3, pp. 493-504.

Hopkins, S. J. and Rothwell, N. J. (1995). Cytokines and the nervous system. I: Expression and recognition. *Trends Neurosci*, 18, 2, pp. 83-8.

Hoshino, S., et al. (1998). Emergence of immunoreactivities for phosphorylated tau and amyloid-beta protein in chronic stage of fluid percussion injury in rat brain. *Neuroreport*, 9, 8, pp. 1879-83.

Ide, C. F., et al. (1996). Cellular and molecular correlates to plasticity during recovery from injury in the developing mammalian brain. *Prog Brain Res*, 108, pp. 365-77.

Jain, K. K. (2008). Neuroprotection in traumatic brain injury. *Drug Discov Today*, 13, 23-24, pp. 1082-9.

Karman, J., et al. (2004). Initiation of immune responses in brain is promoted by local dendritic cells. *J Immunol*, 173, 4, pp. 2353-61.

Katayama, Y., et al. (1995). Role of excitatory amino acid-mediated ionic fluxes in traumatic brain injury. *Brain Pathol*, 5, 4, pp. 427-35.

Kempermann, G. and Neumann, H. (2003). Neuroscience. Microglia: the enemy within? *Science*, 302, 5651, pp. 1689-90.

Kettenmann, H., et al. (2011). Physiology of microglia. *Physiol Rev*, 91, 2, pp. 461-553.

Kigerl, K. A., et al. (2006). Comparative analysis of lesion development and intraspinal inflammation in four strains of mice following spinal contusion injury. *J Comp Neurol*, 494, 4, pp. 578-94.

Kil, K., et al. (1999). T cell responses to myelin basic protein in patients with spinal cord injury and multiple sclerosis. *J Neuroimmunol*, 98, 2, pp. 201-7.

Knoblach, S. M. and Faden, A. I. (2002). Administration of either anti-intercellular adhesion molecule-1 or a nonspecific control antibody improves recovery after traumatic brain injury in the rat. *J Neurotrauma*, 19, 9, pp. 1039-50.

Knoblach, S. M., et al. (1999). Early neuronal expression of tumor necrosis factor-alpha after experimental brain injury contributes to neurological impairment. *J Neuroimmunol*, 95, 1-2, pp. 115-25.

Kossmann, T., et al. (1997). Interleukin-8 released into the cerebrospinal fluid after brain injury is associated with blood-brain barrier dysfunction and nerve growth factor production. *J Cereb Blood Flow Metab*, 17, 3, pp. 280-9.

Lei, P., et al. (2009). Microarray based analysis of microRNA expression in rat cerebral cortex after traumatic brain injury. *Brain Res*, 1284, pp. 191-201.

Lenzlinger, P. M., et al. (2001). Markers for cell-mediated immune response are elevated in cerebrospinal fluid and serum after severe traumatic brain injury in humans. *J Neurotrauma*, 18, 5, pp. 479-89.

Ling, C., et al. (2003). In situ processing and distribution of intracerebrally injected OVA in the CNS. *J Neuroimmunol*, 141, 1-2, pp. 90-8.

Liu, G. J., et al. (2009). Glutamate induces directed chemotaxis of microglia. *Eur J Neurosci*, 29, 6, pp. 1108-18.

Loane, D. J. and Byrnes, K. R. (2010). Role of microglia in neurotrauma. *Neurotherapeutics*, 7, 4, pp. 366-77.

Maas, A. I., et al. (2006). Efficacy and safety of dexanabinol in severe traumatic brain injury: results of a phase III randomised, placebo-controlled, clinical trial. *Lancet Neurol*, 5, 1, pp. 38-45.

Maas, A. I., et al. (2008). Moderate and severe traumatic brain injury in adults. *Lancet Neurol*, 7, 8, pp. 728-41.

Mantovani, A., et al. (2004). The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol*, 25, 12, pp. 677-86.

McCluskey, L. P. and Lampson, L. A. (2000). Local neurochemicals and site-specific immune regulation in the CNS. *J Neuropathol Exp Neurol*, 59, 3, pp. 177-87.

McKeating, E. G., et al. (1998). Leukocyte adhesion molecule profiles and outcome after traumatic brain injury. *Acta Neurochir Suppl*, 71, pp. 200-2.

Minambres, E., et al. (2003). Correlation between transcranial interleukin-6 gradient and outcome in patients with acute brain injury. *Crit Care Med*, 31, 3, pp. 933-8.

Moalem, G., et al. (1999). Autoimmune T cells protect neurons from secondary degeneration after central nervous system axotomy. *Nat Med*, 5, 1, pp. 49-55.

Morganti-Kossmann, M. C., et al. (2001). Role of cerebral inflammation after traumatic brain injury: a revisited concept. *Shock*, 16, 3, pp. 165-77.

Morganti-Kossmann, M. C., et al. (2002). Inflammatory response in acute traumatic brain injury: a double-edged sword. *Curr Opin Crit Care*, 8, 2, pp. 101-5.

Nagamoto-Combs, K., et al. (2007). Prolonged microgliosis in the rhesus monkey central nervous system after traumatic brain injury. *J Neurotrauma*, 24, 11, pp. 1719-42.

Nakamura, Y. (2002). Regulating factors for microglial activation. *Biol Pharm Bull*, 25, 8, pp. 945-53.

Neumann, H., et al. (1996). Neuronal control of MHC class II inducibility in rat astrocytes and microglia. *Eur J Neurosci*, 8, 12, pp. 2582-90.

Nguyen, H. X., et al. (2007). Polymorphonuclear leukocytes promote neurotoxicity through release of matrix metalloproteinases, reactive oxygen species, and TNF-alpha. *J Neurochem*, 102, 3, pp. 900-12.

Nimmerjahn, A., et al. (2005). Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. *Science*, 308, 5726, pp. 1314-8.

Patel, H. C., et al. (2003). Interleukin-1 in the brain: mechanisms of action in acute neurodegeneration. *Ann N Y Acad Sci*, 992, pp. 39-47.

Penkowa, M., et al. (2003). Astrocyte-targeted expression of interleukin-6 protects the central nervous system during neuroglial degeneration induced by 6-aminonicotinamide. *J Neurosci Res*, 73, 4, pp. 481-96.

Penkowa, M., et al. (2000). Impaired inflammatory response and increased oxidative stress and neurodegeneration after brain injury in interleukin-6-deficient mice. *Glia*, 32, 3, pp. 271-85.

Pleines, U. E., et al. (1998). Soluble ICAM-1 in CSF coincides with the extent of cerebral damage in patients with severe traumatic brain injury. *J Neurotrauma*, 15, 6, pp. 399-409.

Popovich, P. G. and Longbrake, E. E. (2008). Can the immune system be harnessed to repair the CNS? *Nat Rev Neurosci*, 9, 6, pp. 481-93.

Popovich, P. G., et al. (1996). Concept of autoimmunity following spinal cord injury: possible roles for T lymphocytes in the traumatized central nervous system. *J Neurosci Res*, 45, 4, pp. 349-63.

Postler, E., et al. (1997). Expression of the S-100 proteins MRP-8 and -14 in ischemic brain lesions. *Glia*, 19, 1, pp. 27-34.

Raivich, G. (2005). Like cops on the beat: the active role of resting microglia. *Trends Neurosci*, 28, 11, pp. 571-3.

Ramlackhansingh, A. F., et al. (2011). Inflammation after trauma: Microglial activation and traumatic brain injury. *Ann Neurol*.

Ransohoff, R. M. (2002). The chemokine system in neuroinflammation: an update. *J Infect Dis*, 186 Suppl 2, pp. S152-6.

Rhodes, J. (2011). Peripheral immune cells in the pathology of traumatic brain injury? *Curr Opin Crit Care*, 17, 2, pp. 122-30.

Riva-Depaty, I., et al. (1994). Contribution of peripheral macrophages and microglia to the cellular reaction after mechanical or neurotoxin-induced lesions of the rat brain. *Exp Neurol*, 128, 1, pp. 77-87.

Rivest, S. (2009). Regulation of innate immune responses in the brain. *Nat Rev Immunol*, 9, 6, pp. 429-39.

Romano, M., et al. (1997). Role of IL-6 and its soluble receptor in induction of chemokines and leukocyte recruitment. *Immunity*, 6, 3, pp. 315-25.

Rothwell, N. J. and Hopkins, S. J. (1995). Cytokines and the nervous system II: Actions and mechanisms of action. *Trends Neurosci*, 18, 3, pp. 130-6.

Rothwell, N. J. and Luheshi, G. N. (2000). Interleukin 1 in the brain: biology, pathology and therapeutic target. *Trends Neurosci*, 23, 12, pp. 618-25.

Sanderson, K. L., et al. (1999). Interleukin-1 receptor antagonist attenuates regional neuronal cell death and cognitive dysfunction after experimental brain injury. *J Cereb Blood Flow Metab*, 19, 10, pp. 1118-25.

Scherbel, U., et al. (1999). Differential acute and chronic responses of tumor necrosis factor-deficient mice to experimental brain injury. *Proc Natl Acad Sci U S A*, 96, 15, pp. 8721-6.

Schwartz, M., et al. (2009). Boosting T-cell immunity as a therapeutic approach for neurodegenerative conditions: the role of innate immunity. *Neuroscience*, 158, 3, pp. 1133-42.

Schwartz, M., et al. (2003). Protective autoimmunity against the enemy within: fighting glutamate toxicity. *Trends Neurosci*, 26, 6, pp. 297-302.

Schwartz, M. and Shechter, R. (2010). Systemic inflammatory cells fight off neurodegenerative disease. *Nat Rev Neurol*, 6, 7, pp. 405-10.

Semple, B. D., et al. (2010b). Role of CCL2 (MCP-1) in traumatic brain injury (TBI): evidence from severe TBI patients and CCL2-/- mice. *J Cereb Blood Flow Metab*, 30, 4, pp. 769-82.

Semple, B. D., et al. (2010a). Deficiency of the chemokine receptor CXCR2 attenuates neutrophil infiltration and cortical damage following closed head injury. *Neurobiol Dis*, 40, 2, pp. 394-403.

Serpe, C. J., et al. (2003). CD4+ T, but not CD8+ or B, lymphocytes mediate facial motoneuron survival after facial nerve transection. *Brain Behav Immun*, 17, 5, pp. 393-402.

Shiozaki, T., et al. (2005). Cerebrospinal fluid concentrations of anti-inflammatory mediators in early-phase severe traumatic brain injury. *Shock*, 23, 5, pp. 406-10.

Shlosberg, D., et al. (2010). Blood-brain barrier breakdown as a therapeutic target in traumatic brain injury. *Nat Rev Neurol*, 6, 7, pp. 393-403.

Shohami, E., et al. (1997). Cytokine production in the brain following closed head injury: dexanabinol (HU-211) is a novel TNF-alpha inhibitor and an effective neuroprotectant. *J Neuroimmunol*, 72, 2, pp. 169-77.

Shohami, E., et al. (1994). Closed head injury triggers early production of TNF alpha and IL-6 by brain tissue. *J Cereb Blood Flow Metab*, 14, 4, pp. 615-9.

Singhal, A., et al. (2002). Association between cerebrospinal fluid interleukin-6 concentrations and outcome after severe human traumatic brain injury. *J Neurotrauma*, 19, 8, pp. 929-37.

Smith, D. H., et al. (1997). Progressive atrophy and neuron death for one year following brain trauma in the rat. *J Neurotrauma*, 14, 10, pp. 715-27.

Spranger, M., et al. (1990). Regulation of Nerve Growth Factor (NGF) Synthesis in the Rat Central Nervous System: Comparison between the Effects of Interleukin-1 and Various Growth Factors in Astrocyte Cultures and in vivo. *Eur J Neurosci*, 2, 1, pp. 69-76.

Streit, W. J., et al. (1999). Reactive microgliosis. *Prog Neurobiol*, 57, 6, pp. 563-81.

Tehranian, R., et al. (2002). Improved recovery and delayed cytokine induction after closed head injury in mice with central overexpression of the secreted isoform of the interleukin-1 receptor antagonist. *J Neurotrauma*, 19, 8, pp. 939-51.

Toulmond, S. and Rothwell, N. J. (1995). Interleukin-1 receptor antagonist inhibits neuronal damage caused by fluid percussion injury in the rat. *Brain Res*, 671, 2, pp. 261-6.

Tyurin, V. A., et al. (2000). Oxidative stress following traumatic brain injury in rats: quantitation of biomarkers and detection of free radical intermediates. *J Neurochem*, 75, 5, pp. 2178-89.

Wang, C. X. and Shuaib, A. (2002). Involvement of inflammatory cytokines in central nervous system injury. *Prog Neurobiol*, 67, 2, pp. 161-72.

Weaver, K. D., et al. (2000). Effect of leukocyte-endothelial adhesion antagonism on neutrophil migration and neurologic outcome after cortical trauma. *J Trauma*, 48, 6, pp. 1081-90.

Whalen, M. J., et al. (1999). Effect of traumatic brain injury in mice deficient in intercellular adhesion molecule-1: assessment of histopathologic and functional outcome. *J Neurotrauma*, 16, 4, pp. 299-309.

Whalen, M. J., et al. (1999). Neutrophils do not mediate blood-brain barrier permeability early after controlled cortical impact in rats. *J Neurotrauma*, 16, 7, pp. 583-94.

Whalen, M. J., et al. (2000). Interleukin-8 is increased in cerebrospinal fluid of children with severe head injury. *Crit Care Med*, 28, 4, pp. 929-34.

Winter, C. D., et al. (2002). A microdialysis method for the recovery of IL-1beta, IL-6 and nerve growth factor from human brain in vivo. *J Neurosci Methods*, 119, 1, pp. 45-50.

Wolf, S. A., et al. (2002). Neuroprotection by T-cells depends on their subtype and activation state. *J Neuroimmunol*, 133, 1-2, pp. 72-80.

3

Shared Genetic Effects among Measures of Cognitive Function and Leukoaraiosis

Jennifer A. Smith[1], Thomas H. Mosley, Jr.[2],
Stephen T. Turner[3] and Sharon L. R. Kardia[1]
[1]*Department of Epidemiology, University of Michigan, Ann Arbor, MI*
[2]*Department of Medicine, University of Mississippi Medical Center, Jackson, MS*
[3]*Department of Internal Medicine, Mayo Clinic, Rochester, MN*
USA

1. Introduction

The aging process influences cognitive and physical functioning through a variety of biological mechanisms. Multiple facets of cognitive function decline with age, including executive function, memory, language, visuomotor coordination, and information processing speed. Strong epidemiological trends show that areas of brain injury due to ischemic damage also increase with age. Areas of ischemic damage known as leukoaraiosis appear as hyperintense spots on MRI of the white matter of the brain. Leukoaraiosis is a strong predictor of ischemic stroke and vascular dementia, independent of other known risk factors (Markus et al., 2005). It is also strongly associated with cognitive impairment and cognitive decline in individuals who have not yet progressed to dementia (Pantoni et al., 2007; Schmidt et al., 2007).

Few studies have examined the genetic contribution to later-age cognitive changes in relationship to markers of subclinical ischemic brain injury such as leukoaraiosis. After increasing age, the main risk factors for leukoaraiosis are elevated blood pressure and lack of hypertension control (van Dijk et al., 2004). However, there is a significant amount of inter-individual variation in leukoaraiosis among subjects with similar duration and severity of hypertension (Schmidt et al., 2004; Szolnoki & Melegh, 2006). Cognitive functioning is also highly variable, and it is likely that genetic variability accounts for a significant portion of the variation in both structural characteristics of the brain such as leukoaraiosis and measures of cognitive function (Deary et al., 2004).

In this chapter, we present a review of the biological mechanisms that influence leukoaraiosis and cognitive function, discuss the public health implications of the clinical manifestations of cerebrovascular disease, and explore the broad genetic attributes that explain inter-individual variation and covaraition (i.e., pleiotropy) among these brain traits.

1.1 Biological mechanisms that influence leukoaraiosis and cognitive function

1.1.1 Physiology and pathology of cerebrovascular disease and leukoaraiosis

The human brain is composed of gray matter (the cerebral cortex) that is responsible for consciousness, movement, and cognition and white matter that consists of nerve fibers that

transmit impulses among cerebral areas and to the central nervous system. Leukoaraiosis is visible as bright spots in the white matter on T2-weighted MRIs (Markus, 2008; O'Sullivan, 2008). Leukoaraiosis ranges in severity from small, distinct areas of white matter hyperintensity (punctuate lesions) to large regions of white matter hyperintensity (early confluent or confluent lesions) (O'Sullivan, 2008). Leukoaraiosis is thought to be a marker of cerebral small vessel disease (cerebrovascular disease) in the long, narrow penetrating arterioles that supply the white matter with blood (Markus, 2008). This type of small vessel disease is defined by areas of diffuse arteriolosclerosis with deposits of a proteinaceous substance that includes fibrin, amyloid, and collagen, which results in thickening of the vessel and chronic ischemia that leads to demyelination, axonal loss, and gliosis (Markus, 2008; O'Sullivan, 2008). It occurs in regions of the brain that have low perfusion pressure, such as the deep white matter, and results in chronic ischemia and multiple diffuse infarctions due to small vessel occlusions (lacunar infarctions), both of which are visible as hyperintensity on MRIs (Markus, 2008). In regions of leukoaraiosis, there appears to be decreased blood flow (hypoperfusion) and impaired ability to regulate blood flow (autoregulation) (Markus, 2008).

Recently, it has been suggested that endothelial dysfunction, characterized by the inability of endothelial cells to perform tasks such as mediation of coagulation, platelet adhesion, and immune response, may be the intermediate process between hypertension and the alterations in blood flow observed in areas of leukoaraiosis (Hassan et al., 2003; Markus, 2008). Circulating endothelial markers may show a pro-coagulant pattern of endothelial function (e.g. higher circulating levels of thrombomodulin (*TM*) and lower circulating levels of tissue factor pathway inhibitor (*TFPI*)) that is specific to leukoaraiosis (Hassan et al., 2003) and may be related to progression of leukoaraiosis (Markus et al., 2005). Further support for endothelial dysfunction comes from the strong association between leukoaraiosis and elevated homocysteine level, which is hypothesized to be a mediator of endothelial damage (Hassan et al., 2004).

1.1.2 Hypertension as a predictor of leukoaraiosis

Development of leukoaraiosis is thought to be a marker of one of the major mechanistic pathways between hypertension and clinical endpoints such as ischemic stroke and vascular dementia, and is a known risk factor for both of these endpoints (Markus et al., 2005; O'Sullivan, 2008). Inadequately controlled hypertension gives rise to ischemic damage of the brain that is thought to be the manifestation of underlying cerebrovascular disease (Turner & Boerwinkle, 2000). Several studies have also demonstrated an association between hypertension in midlife and cognitive decline in later life (Launer et al., 2000), and it has been hypothesized that this is due to the cumulative effects of subclinical damage due to small vessel disease (Knopman et al., 2001) with leukoaraiosis as a detectable sign of one of the main mechanistic pathways implicated (Sierra & Coca, 2006). Hypertension is a leading risk factor for ischemic stroke (Roger et al., 2011) and for cognitive decline leading to vascular dementia (Launer et al., 2000). Hypertension affects approximately 1 in 3 American adults (76.4 million people), and accounts for $43.5 billion in yearly direct and indirect costs in the United States (Roger et al., 2011).

1.1.3 The relationship between leukoaraiosis and cognitive function

In a review of studies pertaining to leukoaraiosis and cognition, Pantoni et al. (2007) conclude that despite different study characteristics, there is almost invariably evidence of

an effect of leukoaraiosis on cognition. In particular, leukoaraiosis is more strongly associated with decreasing executive function than memory and is also associated with a decline in motor performance such as gait disturbances (Pantoni et al., 2007; Schmidt et al., 2007). The rate of progression of leukoaraiosis over time is also related to cognitive decline, and the severity of leukoaraiosis at baseline is a significant predictor of progression (Schmidt et al., 2007). It is also important to keep in mind, however, that other factors may affect the association between leukoaraiosis and cognitive decline such as brain atrophy and stroke (Pantoni et al., 2007; Schmidt et al., 2007).

1.1.4 Cognitive decline and vascular dementia

Dementia is a heterogeneous group of disorders with variable etiology that involves impairment in cognitive domains such as memory, executive function, and language as well as specific physical impairments such as gait abnormalities that cause significant impairment in social or occupational function and represent a decline from a previous level of functioning (American Psychiatric Association, 2000). The differential diagnosis of vascular dementia (VaD), incorporates the underlying vascular cause as well as the cognitive and physical symptomology (Pohjasvaara et al., 2000), specifically "focal neurological signs and symptoms or laboratory evidence indicative of cerebrovascular disease (multiple infarctions involving cortex and underlying white matter) that are judged to be etiologically related to the disturbance" (American Psychiatric Association, 2000). Leukoaraiosis and multiple lacunar (small vessel) strokes, both caused by cerebrovascular disease, are the primary markers of VaD (Geldmacher & Whitehouse, 1997) and are thought to be contributors to cognitive impairment in individuals who have not yet progressed to dementia (Pantoni et al., 2007; Schmidt et al., 2007). Several studies have also shown that leukoaraiosis is predictive of incident VaD (Prins et al., 2004).

1.1.5 Complexity in the inter-relationships among clinical outcomes and sub-clinical measures

A complex relationship exists among hypertension, leukoaraiosis, cognitive decline, dementia, and stroke. The figure below illustrates what is currently known about the inter-relationships among these clinical outcomes and subclinical measures, including:

- Leukoaraiosis is thought to be a manifestation (sub-clinical marker) of cerebrovascular disease.
- Hypertension is a primary risk factor for leukoaraiosis.
- Leukoaraiosis is a risk factor for cognitive decline and ischemic stroke.
- Leukoaraiosis, ischemic stroke, and cognitive decline are included in the clinical criteria for the diagnosis of vascular dementia.
- Ischemic stroke (without additional evidence of leukoaraiosis) is a risk factor for cognitive decline.

1.2 Public health implications of the clinical manifestations of cerebrovascular disease

The clinical outcomes that are associated with cerebrovascular disease have large public health implications. Ischemic stroke accounts for 87% of all strokes, a leading cause of morbidity, mortality, and economic burden in the US (Roger et al., 2011). Stroke is the second most common cause of death and disability-adjusted life-years in industrialized

countries (Lopez et al., 2001) and the third most common cause of death in the US, accounting for approximately 1 in 18 deaths in 2007 (Roger et al., 2011). Over 7 million Americans currently living with the cognitive and physical consequences of stroke (Roger et al., 2011), and it has been estimated that stroke account for approximately 4% of all direct health care costs in the US (Donnan et al., 2008). The risk of first-ever stroke is almost twice as high for African Americans as for white Americans, which may in part be due to the higher prevalence of hypertension in this group (Roger et al., 2011).

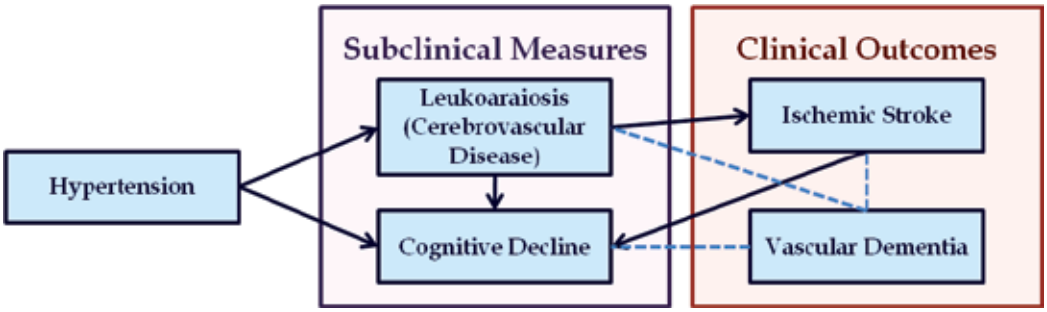


Fig. 1. Relationship between subclinical measures and clinical outcomes. Dark blue arrows represent risk factor relationships, and dashed blue lines represent clinical criteria.

Dementia is also an important public health burden in the U.S. and abroad (Haan & Wallace, 2004), and the World Health Organization predicts that there will be approximately 29 million people affected by all forms of dementia by the year 2020 (Essink-Bot et al., 2002). Alzheimer's disease (AD) and other dementias affect over 5.2 million Americans, including between 200,000 and 500,000 people under the age of 65. Dementias place a heavy economic burden on the health care system, with each Medicare patient with dementia accounting for more than three times as much spending than the average beneficiary (Alzheimer's Association, 2008). The aging population of the U.S. is expected to dramatically increase the prevalence of dementia, which is thought to affect 3%-11% of people older than 65 and 25%-47% of people older than 85 (Boustani et al., 2003). Older African Americans are about twice as likely to develop AD and other dementias as older white Americans, which may in part be due to the higher prevalences of hypertension and diabetes and lower average socioeconomic status of this group (Alzheimer's Association, 2010).

1.3 Role of genetics in leukoaraiosis and cognitive function

Genetic factors are likely to account for a significant amount of the inter-individual variation in cognitive functioning and brain structure (Deary et al., 2004). Heritability studies, candidate gene studies, and genome-wide association studies are beginning to shed light on the biological processes involved in the progression from hypertension to the development of leukoaraiosis and cognitive decline that are indicators of increased risk of stroke and dementia.

1.3.1 Genetics of leukoaraiosis and cognitive function

Estimates of heritability for leukoaraiosis range from 0.45-0.71, indicating that genetic factors account for a large proportion of the inter-individual variation in this trait (Atwood et al., 2004; Carmelli et al., 1998; Turner et al., 2004). Candidate gene studies for

leukoaraiosis have primarily concentrated on genes in pathways known to be involved in hypertension, vasculature, and endothelial damage. Although initial findings have been encouraging, no specific genetic factors have been unequivocally shown to be associated with this trait (Paternoster et al., 2009). Genome-wide association studies (GWAS) for this trait have also been limited, but are currently being conducted in several ethnic groups. To date, the most promising evidence for association is with a region on chromosome 17q25 in European Americans (Fornage et al., 2011).

For cognitive decline, the most promising candidate genes include those that are associated with hypertension, leukoaraiosis, Alzheimer's Disease (AD), normal cognitive functioning, cardiovascular function, oxidative stress, and inflammation (Deary et al., 2004), though candidate gene studies have not yet established any specific genetic factors that definitively affect cognitive decline. GWAS for a variety of cognitive traits are currently underway. GWASs that examine the change of cognitive traits over time in multiple cohorts will be particularly useful for identifying genetic factors associated with cognitive decline.

1.3.2 Role of pleiotropy

Pleiotropy is most simply defined as the condition in which variation in a single gene affects multiple traits (Hodgkin, 1998). Defined in this manner, pleiotropic genes range from those that encode proteins involved in a single biological pathway that influences multiple disease processes and/or organ systems to those that play entirely different roles in multiple biological pathways. In some instances, pleiotropy is "the phenomenon in which a single gene controls several distinct, seemingly unrelated, phenotypic effects" (Zou et al., 2008). A well-known example of pleiotropy in humans is the ε4 allele of the apolioprotein E (*APOE*) gene (Dickstein et al., 2010). *APOE* is a plasma cholesterol transport molecule that resides primarily on very low density lipoproteins, and the ε4 allele has been shown to be a risk factor for coronary heart disease and stroke through mechanisms related directly to lipid transport. However, the ε4 alelle is also a risk factor for AD and cognitive decline, with those carrying the allele having a younger age of onset as well as an accelerated pace of cognitive decline. Though the precise mechanism by which ε4 leads to cognitive decline is not known, the main hypotheses are through pathways not directly related or only tangentially related to lipid transport. *APOE* appears to affect brain traits through its role as a chaperone for the amyloid beta protein and/or mediation of the phosphorylation of the tau protein.

The study of pleiotropy in model organisms and humans serves several functions. In model organisms, it serves to further the understanding and elucidation of the complex biological pathways that regulate the development of traits, providing information about normal cellular function, normal development and function at the organismal level, connections between previously unrecognized biological processes, and increased predictive ability in breeding programs (Hodgkin, 1998). It also it provides insight into the mechanisms of evolution, as effects on multiple traits due to a single genetic variant may pose severe evolutionary constraints (Cheverud et al., 2004). The findings from pleiotropy studies in model organisms, particularly the high degree of connectivity among transcriptional modules, have strong implications for understanding the pleiotropic genetic mechanisms that are also operating in humans. A greater understanding of the underlying pleiotropic mechanisms contributing to human health and disease has the potential to allow for earlier identification of individuals at increased risk for disease, the development of more efficacious treatments, and the tailoring of particular treatments to people most likely to respond positively.

1.3.3 Bivariate variance component analysis to assess pleiotropy

Studies of pleiotropy in humans have generally consisted of bivariate genetic analysis using variance decomposition techniques and linkage analysis in biologically related groups of traits. Variance decomposition techniques are used to parse the total phenotypic correlation in a pair of traits into the correlation due to genetic influences (genetic correlation) and the correlation due to environmental influences (environmental correlation) using family relationships.

Bivariate variance decomposition techniques have been used to study pleiotropy in humans for a variety of purposes. Comuzzie et al. (1994) estimated the genetic and environmental correlations among eight measures of skinfolds in order to inform epidemiologic studies that examine these measures as risk factors for heart disease and diabetes. The authors argue that studying pleiotropy in risk factors is important because shared genetic or environmental effects may confound analyses using these traits if these effects are unrecognized. Bivariate genetic analysis is has also been used to identify measureable endophenotypes that can be used to study the genetic underpinnings of complex diseases with multiple etiologies. For example, Charlesworth et al. (2010) examined the genetic correlations between several quantitative characteristics of the eye and primary open-angle glaucoma in order to determine the most appropriate endophenotypes to focus on in genetic association studies.

1.4 Motivation for studying pleiotropy of leukoaraiosis and cognitive function

In this chapter, we focus on estimating the heritabilities, genetic correlations, and environmental correlations between leukoaraiosis and seven measures of neurocognitive function. In a sample of 759 whites and 720 African Americans, we examine patterns of pleiotropy using a bivariate variance components approach. Findings of this work help inform the understanding of the genetic relationships among leukoaraiosis and measures of cognitive function. A deeper understanding the genetics of leukoaraiosis development and its impact on cognitive decline in individuals free of overt neurocognitive disorders may help to inform pharmacogenomic drug development and preventive strategies for identifying individuals at increased risk of stroke and dementia. Research into the genetic architecture of leukoaraiosis and cognitive function in samples that are presymptomatic is particularly important because preventive interventions for dementia would need to start early, preferably before any brain damage occurs (DeKosky & Marek, 2003).

2. Methods

2.1 Sample

The National Heart, Lung and Blood Institute established the Family Blood Pressure Program (FBPP) in 1996 from four existing research networks that were investigating the genetics of hypertension and its sequelae (FBPP Investigators, 2002), including The Genetic Epidemiology Network of Arteriopathy (GENOA). GENOA recruited hypertensive sibships from Rochester, Minnesota and Jackson, Mississippi for linkage and association studies to investigate the genetic underpinnings of hypertension and target organ damage related to hypertension (Daniels et al., 2004).

In the initial phase of the GENOA study (Phase I: 1996-2001), all members of sibships containing ≥ 2 individuals with essential hypertension clinically diagnosed before age 60 were invited to participate, including both hypertensive and normotensive siblings (1,583

non-Hispanic whites and 1,841 African Americans). The diagnosis of essential hypertension was established based on blood pressure levels measured at the study visit (>140 mmHg average systolic BP or >90 mmHg average diastolic BP) or a prior diagnosis of hypertension and current treatment with antihypertensive medications. Exclusion criteria were secondary hypertension, alcoholism or drug abuse, pregnancy, insulin-dependent diabetes mellitus, or active malignancy. In the second phase of the GENOA study (Phase II: 2000-2004), 1,241 white and 1,482 African American participants were successfully re-recruited to measure potential target organ damage due to hypertension. Phase I and II GENOA data consist of demographic information, medical history, clinical characteristics, lifestyle factors, and blood samples for genotyping and biomarker assays. Written informed consent was obtained from all subjects and approval was granted by participating institutional review boards. All reported phenotype and covariate data used for this analysis was collected during the Phase II exam.

The Genetics of Microangiopathic Brain Injury (GMBI) study (2001-2006) is an ancillary study of GENOA undertaken to investigate susceptibility genes for ischemic brain injury. Phase II GENOA participants that had a sibling willing and eligible to participate in the GMBI study underwent a neurocognitive testing battery to assess several domains of cognitive function including learning, memory, attention, concentration, and language. Ischemic brain damage to the subcortical and periventricular white matter (leukoaraiosis) was quantified by magnetic resonance imaging (MRI) in subjects who had no history of stroke or neurological disease and no implanted metal devices. Participants were excluded from this analysis if they were less than 45 years of age or had evidence of silent stroke (transient ischemic attack) upon examination of their MRI. The analysis sample was comprised of 762 whites in 378 sibships and 720 African Americans in 413 sibships.

2.2 Leukoaraiosis

Leukoaraiosis volume (cm^3) was obtained via MRI in a separate clinical visit. All MRI scans were performed on identically equipped Signa 1.5 T MRI scanners (GE Medical Systems, Waukesha, WI, USA) and images were centrally processed at the Mayo Clinic. Symmetric head positioning with respect to orthogonal axes was verified by a series of short scout scans. Total intracranial volume (head size) was measured from T1-weighted spin echo sagittal images, each set consisting of 32 contiguous 5 mm thick slices with no interslice gap, field of view = 24 cm, matrix = 256 x 192, obtained with the following sequence: scan time = 2.5 min, echo time = 14 ms, repetitions = 2, replication time = 500 ms (Jack et al., 1989). Total brain and leukoaraiosis volumes were determined from axial fluid-attenuated inversion recovery (FLAIR) images, each set consisting of 48 contiguous 3-mm interleaved slices with no interslice gap, field of view = 22 cm, matrix = 256 x 160, obtained with the following sequence: scan time = 9 min, echo time = 144.8 ms, inversion time = 2,600 ms, repetition time = 26,002 ms, bandwidth = +/- 15.6 kHz, one signal average. A FLAIR image is a T2-weighted image with the signal of the cerebrospinal fluid nulled, such that brain pathology appears as the brightest intracranial tissue. Interactive imaging processing steps were performed by a research associate who had no knowledge of the subjects' personal or medical histories or biological relationships. A fully automated algorithm was used to segment each slice of the edited multi-slice FLAIR sequence into voxels assigned to one of three categories: brain, cerebrospinal fluid, or leukoaraiosis. The mean absolute error of this method is 1.4% for brain volume and 6.6% for leukoaraiosis volume, and the mean test-

retest coefficient of variation is 0.3% for brain volume and 1.4% for leukoaraiosis volume (Jack et al., 2001). White matter hyperintensities in the corona-radiata and periventricular zone, as well as central gray infarcts (ie, lacunes) were included in the global leukoaraiosis measurements. Brain scans with cortical infarctions were excluded from the analyses because of the distortion of the leukoaraiosis volume estimates that would be introduced in the automated segmentation algorithm.

2.3 Neuropsychological testing battery

Neuropsychological tests were conducted in a private room that was free of noise and other distractions by trained interviewers. In order to assure accuracy and comparability in test administrator performance, a portion (approximately 5%) of all interviews were tape recorded and evaluated for accuracy to provide feedback to test administrators. The neuropsychological outcome measures used for this analysis are presented in Table 1 along with the cognitive functions assessed.

Neurocognitive Test	*Outcome Measure*	*Cognitive Functions*
Rey's Auditory Verbal Learning Test (RAVLT)	RAVLT delayed recall	Learning Delayed memory Vulnerability to interference
Rey's Auditory Verbal Learning Test (RAVLT)	RAVLT total learning	Learning Immediate memory
Digit Symbol Substitution Test (DSST)	DSST	Psychomotor speed Visual attention Concentration
Controlled Oral Word Association Test (COWA)	COWA FAS	Language Verbal fluency (phonetic association)
Controlled Oral Word Association Test (COWA)	COWA animals	Language Category fluency (semantic association)
Stroop Color Word Test	Stroop color word	Concentration effectiveness Ability to shift perceptual sets in response to novel stimuli
Stroop Color Word Test	Stroop interference	Ability to shift perceptual sets in response to novel stimuli

Table 1. Measures of cognitive function

2.3.1 Rey's Auditory Verbal Learning Test (RAVLT)

Rey's Auditory Verbal Learning Test (RAVLT) is a brief test that assesses learning and memory through multiple learning trials and a 30-minute delayed recall (Rey, 1964). Specifically, the measure assesses immediate memory span, new learning, vulnerability to interference in learning, and recognition memory. RAVLT testing norms for individuals aged 55 and older were developed through Mayo's Older Americans Normative Studies (MOANS), and the testing procedure followed in GMBI was identical to that used in MOANS (Ivnik et al., 1992).

The examiner begins by reading a list of 15 common words aloud, and participants are asked to recall as many of the words as possible in any order. The same procedure is repeated four more times using the same list of 15 words. The total number of words that the participant remembers correctly over the five trials is recorded and forms the basis of the RAVLT total learning outcome measure for this analysis, which assesses immediate memory. Delayed recall is assessed by asking the participant to again name as many words as he/she remembers after a 30-minute delay, forming the basis of the RAVLT delayed learning outcome measure for this analysis. During the 30-minute interim, an interference task is performed in which the interviewer reads another set of words aloud, and the participant is asked to recall them. Thus, the delayed learning outcome assesses both delayed memory as well as vulnerability to interference.

2.3.2 Wechsler Adult Intelligence Scale Revised (WAIS-R) Digit Symbol Substitution Task (DSST)

The Digit Symbol Substitution task (DSS) from the Wechsler Adult Intelligence Scale Revised (WAIS-R) (Wechsler, 1981) is a timed translation test designed to measure complex visual attention, sustained and focused concentration, response speed, and visuomotor coordination (Lezak, 1995). In this test, participants are given a key in which each number corresponds to a special symbol. The task consists of filling in empty boxes below a series of random numbers with the symbol corresponding to the appropriate number (translating the numbers to symbols). After a practice session to ensure that the participant understands the task, participants were given a 90 second time limit to complete as many items as possible. The DSST outcome measure for this analysis was the number of correct symbols completed in 90 seconds.

2.3.3 Controlled Oral Word Association Test (COWA) of the Multilingual Aphasia Examination

The Multilingual Aphasia Examination was developed to diagnose the presence of aphasic disorders (any type of acquired language impairment), and the Controlled Oral Word Association Test (COWA) is a subset of this examination designed to measure verbal fluency (Lezak, 1995). Two measures of verbal fluency were used as outcomes for the present study, one of letter fluency (Word Fluency Test (WFT)) and one of category fluency (Animal Naming).

The Word Fluency Test of the COWA assesses letter fluency (phonetic association) by asking subjects to generate words orally that begin with a specific letter of the alphabet ("F", "A", and "S") for a period of 60 seconds. These letters were chosen because they have been demonstrated to allow more vocabulary choices overall than other letters. Scoring of this test consisted of adding the total number of admissible words generated for each of the three letters. Inadmissable words include proper nouns as well as variations, plurals, and repetitions of previously stated words.

The Animal Naming portion of the COWA assesses category fluency (semantic association) by asking subjects to name as many animals as possible in a period of 60 seconds (Lezak, 1995). Scoring of this test is the sum of all admissible animals. Inadmissable animals include extinct, imaginary, or magical animals, proper names, and variations of previously stated animals.

2.3.4 Stroop Color Word (CW) Test

The Stroop Color Word (CW) Test is primarily a measure of concentration effectiveness, specifically the ability to shift perceptual sets to correspond with changing demands and the

ability to inhibit a customary response to stimulus in favor of a more novel one (Lezak, 1995; Stroop, 1935). Administration and scoring of the test to GMBI participants followed procedures outlined in the standardized version of the CW test developed by Golden (Golden, 1978).

This test consists of three pages: the word page, the color page, and the color-word page. The word page consists of the words "RED", "GREEN", and "BLUE" arranged randomly and printed in black ink. The color page consists of sets of "XXXX" printed in red, green, or blue ink. The color-word page consists of the words from the word page printed in the colors on the color page, but no word matches the color in which it is printed (for example, the word "RED" is printed in either green or blue ink). For this study, the participant was first asked to read the word page as fast as he/she could for 45 seconds, and the total number of correct words was recorded. If the participant stated an incorrect word, the interviewer said, "No," and the participant was instructed to read the same word again to correct their error. The same procedure was followed for naming the colors on the color page. The participant was then asked to state the colors of the words on the color-word page as fast as he/she could in 45 seconds, and the total number of correctly stated colors were recorded.

Two measures from this test are used as outcomes in the present study. The color-word (CW) score is the total number of correctly stated colors out of 100 from the color-word page. The Stroop interference score is the difference in scores between the color page and the color-word page.

2.4 Statistical analysis

2.4.1 Descriptive statistics

Data management and statistical analyses were conducted primarily in R version 2.8.0 (R Core Development Team, 2008). Distributional plots indicated that the measures of leukoaraiosis volume are severely right-skewed, so this variable was transformed by taking the natural log of (leukoaraiosis + 1). The cognitive traits appeared to have relatively normal distributions; thus, no variable transformations were applied to these variables. T-tests were conducted for the outcome measures to test whether there were significant differences in the white and African American study participants.

2.4.2 Covariates

Biometrical modeling of leukoaraiosis and cognitive function included age at cognitive testing, gender, and education as covariates. Relative performance on cognitive tests is determined using age- and gender-specific population-based norms, since both of these variables are known to affect cognitive function. Age is also a very strong independent predictor for leukoaraiosis. Education affects performance on some cognitive tests, as people with higher educational attainment tend to perform better (Valenzuela & Sachdev, 2006). For this analysis, education was categorized as follows: 0) less than high school, 1) completed high school (GED), 2) some college, and 3) completed college (4+ years). To account for differences in brain size, intracranial volume was also included in models of leukoaraiosis. We conducted multivariable linear mixed models with covariates as predictor variables for each outcome measure to explore the relationships between these variables and each outcome of interest in GENOA whites and African Americans.

2.4.3 Biometrical genetic modeling

The expected covariance of a trait between a pair of individuals can be modeled as a function of the variance parameters and the expected correlation between the individuals

for genetic effects, based on family relationships (Sing et al., 1987). In this study, SOLAR (Sequential Oligogenic Linkage Analysis Routines) (Almasy & Blangero, 1998) was used to implement a variance component regression based on maximum likelihood estimation to estimate the proportion of phenotypic variance that is due to additive genetic effects, giving an estimate of heritability. Shared residual (non-genetic) effects are assumed to be zero because the siblings in this study are all adults and have reported living in separate households.

Heritabilities were estimated for the outcome variables (all cognitive traits and ln(leukoaraiosis+1)) both with and without covariates (age, sex, education, and TIV) included in the biometric models. When covariates are included in the model, the heritability estimate is given by [(1-proportion of variance explained by covariates)*h^2]*100, and represents the hertiability the residual variance of the trait that is not accounted for by the covariates.

The heritability of the traits were tested for significance by comparing the log-likelihood of the model in which heritability is estimated to that of the model in which heritability is fixed to 0. The null distribution of the likelihood ratio test statistic is a 50:50 mixture of a Chi-square distribution with one degree of freedom and a point mass at zero.

2.4.4 Phenotypic, genetic, and environmental correlations

The phenotypic, genetic, and environmental correlations among all pairs of traits in both ethnic groups were estimated in SOLAR, both with and without covariates included in the biometric models. The estimated genetic and environmental correlations, ρ_g and ρ_e, were tested for significance by comparing the log-likelihood of the model in which the parameter of interest is estimated to that of the model in which the parameter is fixed to 0.

The test for pleiotropy, or evidence of shared genetic influences, is as follows: $\mathbf{H_o}$: $\rho_g = 0$ vs. $\mathbf{H_a}$: $\rho_g \neq 0$. The null distribution of the likelihood ratio test statistic statistic is a Chi-square distribution with one degree of freedom. Rejection of the null hypothesis provides evidence of pleiotropy.

The presence of shared environmental influences (non-genetic influences beyond the covariates included in the model) is tested similarly: $\mathbf{H_o}$: $\rho_e = 0$ vs. $\mathbf{H_a}$: $\rho_e \neq 0$. The null distribution of the likelihood ratio test statistic statistic is a Chi-square distribution with one degree of freedom. Rejection of the null hypothesis provides evidence that there are shared environmental influences on the traits.

3. Results

3.1 Descriptive statistics

Descriptive statistics of the outcome measures and covariates for the 762 white and 720 African American participants, as well as T-tests comparing the samples, are presented in Tables 2 and 3. GENOA whites are 58.1% female, have a mean age at the time of cognitive testing of 61.3 years (range= 45-84 years), and have a mean leukoaraiosis volume of 8.11cm^3 (range=1.2-62 cm^3). GENOA African Americans have a much larger percentage of females (72.6%), have a higher mean age of cognitive testing (63.3 years, range = 45-91 years)), and have a higher mean volume of leukoaraiosis with greater variability (9.56cm^3, range=2.0-126 cm^3). Approximately half of both whites and African Americans attended at least some college; however, only 5.2% of white participants did not graduate from high school or obtain a GED while this was true for 28.3% of African American participants. The mean

values for all outcome measures were significantly different in whites and African Americans except for Stroop interference. Leukoaraiosis was strongly right skewed in both populations, but had a relatively normal distribution after taking the natural logarithm.

		Whites		*African Americans*	
Trait	**Category**	**N**	**Percentage**	**N**	**Percentage**
Education	0 (Less than HS)	40	5.2%	204	28.3%
	1 (HS/GED)	329	43.2%	205	28.5%
	2 (Some College)	246	32.3%	127	17.6%
	3 (Grad/Professional)	147	19.3%	184	25.6%
Gender	Male	319	41.9%	197	27.4%
	Female	443	58.1%	523	72.6%

Table 2. Descriptive characteristics of the samples

	Whites		*African Americans*		
Trait	**N**	**Mean (±SD)**	**N**	**Mean (±SD)**	**T-test P-value[a]**
Leukoaraiosis volume, cm³	714	8.11 (6.83)	574	9.56 (9.89)	0.0028
Ln (leukoaraiosis+1)	714	2.06 (0.05)	574	2.16 (0.55)	0.0002
RAVLT delayed recall	758	9.08 (3.27)	708	6.80 (3.36)	<2.2E-16
RAVLT total learning	759	47.7 (9.8)	712	40.1 (9.4)	<2.2E-16
DSST	758	50.2 (12.4)	697	32.9 (13.6)	<2.2E-16
COWA FAS	760	32.4 (13.6)	687	28.6 (11.7)	1.96E-08
COWA animals	762	19.3 (4.9)	716	14.9 (4.5)	<2.2E-16
Stroop color word	740	34.5 (9.3)	648	22.3 (10.1)	<2.2E-16
Stroop interference	740	32.8 (9.3)	648	33.6 (11.8)	0.4450

[a] T-test p-value for a test of equality of trait means in whites and African Americans.

Table 3. Comparison of outcome measures

3.2 Associations between covariates and outcome measures

In order to explore the relationship between covariates and each outcome of interest, we conducted multivariable linear mixed models with covariates as predictor variables for each outcome measure. In both whites and African Americans, age, gender, and education were significant predictors for all cognitive measures except that education was not a significant predictor of Stroop interference in whites and gender was not a significant predictor of Stroop color word in African Americans after accounting for the other covariates. As expected, increasing age was associated with lower cognitive scores, while increasing education was associated with higher cognitive scores. Female gender also showed a trend of being associated with higher cognitive scores. In both groups, increasing age and total intracranial volume were associated with increasing leukoaraiosis volume, while gender and education were not associated with this measure.

The amount of variance explained by the covariates, as measured by R^2, showed a consistent pattern between the two groups. R^2 was lowest for Stroop interference (0.018 in whites and

0.074 in African Americans) and highest for DSST (0.411 in whites and 0.529 in African Americans). The amount of variance explained by covariates in the remainder of the cognitive measures ranged from 0.122 to 0.309 in whites and from 0.207 to 0.294 in African Americans. Variance of ln(leukoaraiosis+1) explained by covariates was higher in whites (0.309) than in African Americans (0.213).

3.3 Genetic variance (heritability)

In order to examine the contribution of genetic factors to the observed variation in the traits, we used a biometrical approach to estimate the proportion of variance in the traits explained by genetic factors (heritabilities) both with and without inclusion of covariates in the models (Table 4). Heritabilities of all traits were highly significant in both whites and African Americans (p-value<0.001) with the exception of Stroop interference in African Americans that showed only a marginally significant heritability, illustrating that all of the traits under study are influenced by genetic factors. Similar patterns of heritability were observed between the two groups, though African Americans tended to have lower heritabilities than whites for most traits.

Trait	h^2 for Trait Modeled without Covariates	h^2 for Trait Modeled with Covariates	Proportion of Variance Explained by Covariates	Percent Variation Due to Genetic Factors After Accounting for Covariates[a]
Whites				
Ln(leukoaraiosis+1)	0.656***	0.529***	0.311	36.45
RAVLT delayed recall	0.602***	0.526***	0.222	40.92
RAVLT total learning	0.627***	0.516***	0.308	35.71
DSST	0.774***	0.843***	0.403	50.33
COWA FAS	0.441***	0.366***	0.139	31.51
COWA animals	0.503***	0.349***	0.152	29.60
Stroop color word	0.586***	0.429***	0.276	31.06
Stroop interference	0.302***	0.275***	0.023	26.87
African Americans				
Ln(leukoaraiosis+1)	0.485***	0.432***	0.217	33.83
RAVLT delayed recall	0.494***	0.390***	0.203	31.08
RAVLT total learning	0.560***	0.440***	0.279	31.72
DSST	0.810***	0.556***	0.525	26.41
COWA FAS	0.710***	0.536***	0.300	37.52
COWA animals	0.551***	0.329***	0.260	24.35
Stroop color word	0.532***	0.440***	0.232	33.79
Stroop interference	0.135***	0.154*	0.075	14.25

[a] [(1 – Proportion of variance explained by adjustment covariates)*h^2]*100

For all traits modeled with covariates, biometric models included age, sex, and education. The biometric model for ln(leukoaraiosis+1) also included TIV.

Null hypothesis of tests: $h^2 = 0$

* 0.01 < p-value < 0.05, ** 0.001 < p-value < 0.01, *** p-value < 0.001

Table 4. Trait heritabilities

Heritabilities in traits modeled without covariates were lowest for Stroop interference (0.302 in whites, 0.135 in African Americans) and highest for DSST (0.774 in whites, 0.81 in African Americans), with the majority of heritabilities in the range of 0.45 to 0.6. Leukoaraiosis had a higher heritability in whites (0.656) than in African Americans (0.485). After including covariates in the biometric models, heritabilities for the traits were generally lower but remained highly significant. Again, Stroop interference had the lowest heritability in both groups (0.275 in whites, 0.154 in African Americans) and DSST had the highest (0.843 in whites, 0.556 in African Americans), with the remaining traits ranging between 0.33 and 0.54. Leukoaraiosis still showed higher heritability in whites (0.529) than in African Americans (0.432).

The proportion of the observed trait variance accounted for by covariates estimated with biometric modeling mirrored the relationships we observed in multivariate linear mixed modeling, described above. The lowest proportion of variance explained was for Stroop interference (0.023 in whites, 0.075 in African Americans) and the highest was for DSST (0.403 in whites, 0.525 in African Americans). For the remainder of the traits, the proportion of variance explained by covariates ranged from 0.139 (COWA FAS) to 0.311 (leukoaraiosis) in whites and from 0.203 (RAVLT delayed recall) to 0.3 (COWA FAS) in African Americans.

In order to determine the proportion of variation in the traits explained by genetic factors, we multiplied the proportion of variation not explained by the covariates by the heritability. Expressed as a percentage of total variation, genetic factors explain the lowest amount of variation in Stroop interference in both groups (26.87% in whites, 14.25% in African Americans). The largest amount of variation explained by genetic factors in whites was for DSST (50.33%) followed by RAVLT delayed recall (40.92%) and leukoaraiosis (36.45%). In African Americans, genetic factors explained the largest percentage of variation in COWA FAS (37.52%) followed by leukoaraiosis (33.83%) and Stroop color word (33.79%). For the majority of traits, the amount of variation explained by genetic factors was lower in African Americans than in whites, but most traits in both groups had at least 25% of variation explained by genetic factors, showing that genetics has an important influence on these traits.

3.4 Phenotypic correlations between trait pairs

The patterns observed in the correlations estimated biometrically in SOLAR are presented in Table 5. The strongest correlation in the traits modeled with covariates was between the two RAVLT measures (0.755 in whites, 0.729 in African Americans), and the weakest correlations were between leukoaraiosis and all cognitive traits (ranging from -0.001 to -0.083). In general, multiple measures from the same test exhibited stronger correlations than measures across tests, which is intuitive since measures from the same test are assessing different but closely related cognitive functions. Patterns of correlation in whites and African Americans were very similar, though whites generally tended to exhibit somewhat stronger correlations.

3.5 Genetic and environmental correlations between trait pairs

In order to begin to understand the extent to which pleiotropic genetic effects may be contributing to each pair of traits, we used a biometrical approach to estimate genetic and environmental correlations (Table 6). Overall, there were far more significant genetic correlations (pleiotropic effects) between trait pairs than environmental correlations, indicating that shared genetic effects were more common in these pairs of traits than shared

	Leuko	RAV-DR	RAV-TL	DSST	C-FAS	C-AN	Str-CW	Str-Int
Whites								
Leuko	0.529***	-0.253	-0.295	-0.294	-0.072	-0.174	-0.232	-0.110
RAV-DR	-0.083	0.526***	0.816	0.442	0.274	0.373	0.376	0.052
RAV-TL	-0.063	0.755	0.516***	0.523	0.346	0.429	0.441	0.147
DSST	-0.064	0.213	0.269	0.843***	0.376	0.334	0.600	0.268
C-FAS	-0.023	0.163	0.234	0.265	0.366***	0.440	0.345	0.160
C-AN	-0.039	0.261	0.311	0.175	0.367	0.349***	0.309	0.134
Str-CW	0.003	0.186	0.226	0.424	0.262	0.160	0.429***	-0.134
Str-Int	-0.071	-0.010	0.089	0.228	0.128	0.107	-0.233	0.275***
African Americans								
Leuko	0.432***	-2.10	-0.281	-0.270	-0.213	-0.172	-0.197	-0.108
RAV-DR	-0.007	0.390***	0.790	0.416	0.307	0.285	0.275	0.153
RAV-TL	-0.079	0.729	0.440***	0.492	0.401	0.358	0.308	0.257
DSST	-0.075	0.188	0.220	0.556***	0.557	0.489	0.534	0.311
C-FAS	-0.081	0.136	0.020	0.298	0.536***	0.540	0.347	0.311
C-AN	-0.031	0.143	0.188	0.245	0.395	0.329***	0.357	0.204
Str-CW	-0.056	0.145	0.132	0.332	0.170	0.161	0.440***	-0.189
Str-Int	-0.021	0.046	0.139	0.194	0.219	0.129	-0.339	0.154*

Leuko = Ln(leukoaraios+1), RAV-DR = RAVLT delayed recall, RAV-TL = RAVLT total learning, C-FAS = COWA FAS, C-AN = COWA animals, Str-CW = Stroop color word, Str-Int = Stroop interference
Above diagonal: phenotypic correlations, ρ_P, for traits modeled without covariates
Below diagonal: phenotypic correlations, ρ_P, for traits modeled with covariates
Diagonal: heritabilities from polygenic analysis, h^2, for traits modeled with covariates
For all traits modeled with covariates, biometric models included age, sex, and education. The biometric model for ln(leukoaraiosis+1) also included TIV.
Null hypothesis of tests: $h^2 = 0$ (diagonal)
* 0.01 < p-value < 0.05, ** 0.001 < p-value < 0.01, *** p-value < 0.001

Table 5. Biometrically derived phenotypic correlations among traits

environmental effects. For all estimates of genetic and environmental correlations, adjustment covariates were included in the biometric models.

The majority of significant genetic correlations observed were in whites. In whites, significant genetic correlations ranged from 0.263 (RAVLT total learning and DSST) to 0.918 (RAVLT total learning and RAVLT delayed recall). Other highly significant genetic correlations (p-value<0.001) were between DSST and Stroop color word (0.7) and between RAVLT total learning and COWA animals (0.55). Many of the pairs involving RAVLT, COWA, and DSST also showed significant genetic correlations, ranging from 0.263 to 0.476. Leukoaraiosis and RAVLT had a marginally significant negative genetic correlation (-0.28), indicating that genes shared between these two traits have opposite effects on the traits (for example, a certain genetic variation may increase leukoaraiosis volume while decreasing learning scores). No other evidence of pleiotropic effects was found between leukoaraiosis and cognitive measures.

In contrast to the relative abundance of genetic correlations between these measures, there were very few significant environmental correlations in whites. The most significant

environmental correlation was between the trait pair that also had the highest genetic correlation, RAVLT total learning and RAVLT delayed recall (0.586), although the environmental correlation was substantially less than the genetic correlation. This indicates that for this trait pair, shared genetic effects have a stronger influence than shared environmental effects, though both contribute to the observed strong phenotypic correlation. The only other highly significant environmental correlation was between Stroop color word and Stroop interference (-0.427). This correlation is negative since poor cognitive performance is indicated by a low score on Stroop color word but by a high score on Stroop interference. Only two other trait pairs exhibited even marginally significant environmental correlations in whites. Leukoaraiosis had a negative environmental correlation with Stroop interference (-0.274) and the two measures from the COWA had a positive environmental correlation (0.293).

	Leuko	RAV-DR	RAV-TL	DSST	C-FAS	C-AN	Str-CW	Str-Int
Whites								
Leuko	0.529***	0.078	0.178	-0.008	-0.092	-0.136	-0.066	-0.274*
RAV-DR	-0.233	0.526***	0.586***	-0.015	-0.072	0.185	0.087	-0.024
RAV-TL	-0.280*	0.918***	0.516***	0.354	0.149	0.142	0.128	0.092
DSST	-0.092	0.329**	0.263*	0.843***	0.107	0.015	0.021	0.276
C-FAS	0.062	0.476**	0.350*	0.418**	0.366***	0.293*	0.163	0.185
C-AN	0.084	0.372*	0.550**	0.310*	0.495*	0.349***	0.023	0.157
Str-CW	0.078	0.296	0.336*	0.700***	0.413*	0.371*	0.429***	-0.427***
Str-Int	0.230	0.010	0.091	0.280	0.007	-0.004	0.146	0.275***
African Americans								
Leuko	0.432***	-0.161	-0.261	-0.232	-0.169	-0.127	-0.194	0.130
RAV-DR	0.215	0.390***	0.596***	0.327*	0.167	0.166	0.040	0.035
RAV-TL	0.158	0.915***	0.440***	0.401**	0.322*	0.128	0.017	0.035
DSST	0.078	0.036	0.045	0.556***	0.442**	0.270*	-0.005	0.237
C-FAS	0.010	0.102	0.075	0.179	0.536***	0.313*	-0.019	0.113
C-AN	0.124	0.101	0.286	0.228	0.533**	0.329***	0.158	0.020
Str-CW	0.128	0.293	0.278	0.698***	0.363*	0.167	0.440***	-0.541***
Str-Int	-0.422	0.010	0.432	0.164	0.507	0.522	0.099	0.154*

Leuko = Ln(leukoaraios+1), RAV-DR = RAVLT delayed recall, RAV-TL = RAVLT total learning, C-FAS = COWA FAS, C-AN = COWA animals, Str-CW = Stroop color word, Str-Int = Stroop interference
Above diagonal: environmental correlations, ρ_e
Below diagonal: genetic correlations, ρ_g
Diagonal: heritabilities from univariate polygenic analysis, h^2, for adjusted traits
For all adjusted traits, biometric models included age, sex, and education. The biometric model for ln(leukoaraiosis+1) also included TIV.
Null hypothesis of tests: $\rho_e = 0$ (above diagonal)
Null hypothesis of tests: $\rho_g = 0$ (below diagonal)
Null hypothesis of tests: $h^2 = 0$ (diagonal)
* 0.01 < p-value < 0.05, ** 0.001 < p-value < 0.01, *** p-value < 0.001

Table 6. Genetic and enviromental correlations among traits

The overall patterns of genetic and environmental correlations in African Americans were strikingly similar to the patterns observed in whites. However, many of the genetic correlations in African Americans did not reach statistical significance due to larger standard errors in their estimates, and there was slightly more evidence of shared environmental effects. Two of the four highly significant genetic correlations observed in whites were also observed in African Americans. RAVLT total learning and RAVLT delayed recall were the most strongly genetically correlated (0.915) followed by DSST and Stroop color word (0.698). The only other significant genetic correlations were between the two measures of COWA (0.533) and between COWA FAS and Stroop color word (0.363). There were no significant genetic correlations between leukoaraiosis and any of the cognitive traits.

As with whites, the most highly significant environmental correlation was between the two measures of RAVLT (0.596) and between Stroop color word and Stroop interference (-0.541). The direction and magnitudes of the correlations for these traits were also the same in African Americans as they were in whites. The other strongly significant environmental correlations observed in African Americans were between DSST and RAVLT total learning (0.401) and DSST and COWA FAS (0.442). Four additional pairs of traits also exhibited marginally significant environmental correlations, including the two COWA measures (0.313) that also showed a marginally significant environmental correlation in whites.

4. Discussion

4.1 Heritability of leukoaraiosis

In our study, the heritability of leukoaraiosis was 0.529 in whites and 0.432 in African Americans, after adjustment for age, sex, and total intracranial volume. This finding is consistent with heritability estimates from comparable studies. Heritability was estimated to be 0.71 in study of white male twins (mean age 73 years) after adjustment for age and head size (Carmelli et al., 1998) and 0.55 in a sample of stroke- and dementia-free subjects (mean age 61.0 years) after adjustment for sex, age, age^2, and total cranial volume (Atwood et el., 2004). Turner et al. (2004) showed that leukoaraiosis has a consistently high heritability even after adjustment for blood pressure. These high heritabilities imply that much of the inter-individual differences in variation of leukoaraiosis are due to differences in genetics.

4.2 Heritability of cognitive function

Our study estimates that the majority of heritabilities of seven measures of cognitive function in whites and African Americans are between ~0.35 and ~0.55. Previous studies of the heritability of cognitive functioning has been conducted primarily in twin studies that use factor analysis to identify a common factor to act as a proxy for overall cognitive function, and the findings point to a higher heritability for this measure than was estimated in our study. For example, McGue and Christensen (2002) estimated the heritability of general cognitive function as measured by five cognitive tasks comprised of fluency, digit span, and recall to be 0.70. Finkel et al. (1995) peformed quantitative genetic analysis on four measures of cognitive function (verbal, spatial, perceptual speed, and memory) and showed that heritability for a general cognitive factor was between 0.54 and 0.81 in two samples across several age groups of adults (from young to elderly).

Our results show that there are differences in heritability across cognitive measures, with processing speed having the highest heritability in whites (0.843) and African Americans

(0.556). Other studies have also shown that processing speed has the strongest genetic influence among measures of executive function (Carmelli et al., 2002). The heritability of executive function generally tends to be higher than that of memory, with heritabilities for executive functions between 0.34-0.70 (Carmelli et al., 2002; Sleegers et al., 2007) and heritabilities for memory function closer to 0.2-0.4 (McGue & Christensen, 2001; Plomin et al., 1994; Sleegers et al., 2007). In GENOA whites, memory measures (RAVLT) had slightly higher heritabilities than most of the executive function tests except for DSST. However, in African Americans, heritability of memory measures tended to be similar to those of executive funtion.

While existing research supports true differences in the heritabilities of cognitive domains, a portion of the observed differences may be due to the nature of the tests and scoring procedures. More complex tests such as Stroop may be more susceptible to measurement error than less complex tests such as DSST, and test scores that are mathematically manipulated (e.g., Stroop interference) are particularly susceptible to this type of error. Increased measurement error may lead to lower heritability estimations as well as weaker genetic and environmental correlations among trait pairs.

4.3 Bivariate variance component analysis in leukoaraiosis and cognitive traits

We found that in GENOA whites, measures of cognitive function exhibit substantial evidence of genetic correlation (pleiotropy), with measures of similar tests showing stronger genetic correlation. Evidence of phenotypic correlation due to shared environment was largely limited to a smaller number of cognitive trait pairs in African Americans. However, we detected no evidence of genetic or environmental correlations between measures of cognitive function and leukoaraiosis.

The lack of evidence of genetic correlation between leukoaraiosis and cognitive function in this study stands in contrast to a study conducted by Carmelli, et al. (2002), which used maximum likelihood nested modeling techniques to estimate the proportion of variance in leukoaraiosis and executive control function due to genetic, shared environmental, and non-shared environmental effects in 142 pairs of elderly twins (mean age = 73 years). The total phenotypic correlation between leukoaraiosis and executive function was -0.20, and they found that 70% of the total phenotypic correlation was accounted for by shared genes while 30% was accounted for by shared environments. While this is substantial, the contribution of overlapping genes (genes shared between the executive control factor and leukoaraiosis) to the genetic variance in executive function was only 8% due to the relatively low phenotypic correlation. The lack of detecting shared genetic or enviornmental effects between leukoaraiosis and measures of cognitive function in the GENOA study was partially due to the very low phenotypic correlations among leukoaraiosis and the measures of cognitive function, which may be a function of the younger average age of GENOA participants.

Other studies of the shared genetic components of cognitive traits tend to focus on change in cognition over time. Variance components analysis of the relationship between cognitive change and perception speed in a study of 292 twins aged 40-84 revealed that 90% of the age-related variance and 70% of the genetic variance in cognitive function was shared with perception speed, demonstrating that there is a genetic component to processing speed which also influences general cognitive functioning (Finkel & Pedersen, 2000). This finding was supported by the GENOA study, as processing speed (DSST) exhibited genetic correlation with nearly all of the other cognitive measures in whites. Although GENOA

African Americans showed little evidence for genetic correlation among cognitive traits, a significant genetic correlation was found for DSST and Stroop color word.

4.4 Differences in the genetic parameters for whites and African Americans

Heritabilities of leukoaraiosis and cognitive measures showed a consistent pattern in whites and African Americans. DSST had the highest heritability (0.883 in whites, 0.556 in African Americans), Stroop interference had the lowest heritability (0.275 in whites, 0.154 in African Americans), and leukoaraiosis and the other cognitive measures had mid-range heritabilities (0.35-0.53 in whites, 0.33-0.54 in African Americans). However, heritabilities showed a clear trend of being lower in African Americans, with COWA FAS being the only notable exception (heritability = 0.366 in whites, 0.536 in African Americans). A similar trend was observed for overall phenotypic correlations and genetic correlations, with African Americans showing a similar but weaker correlational structure.

There are several reasons that could account for the lower observed heritabilities in African Americans. Since heritability is the fraction of the total variability of the trait accounted for by additive genetic factors, lower heritabilities could result from greater trait variation (a larger denominator) or from a smaller contribution from additive genetic effects (a smaller numerator). Differences in either the numerator or the demoninator could be due to true population differences between whites and African Americans, or they could simply be artefacts of the GENOA samples, such as differences in age or family structure. We examined the possibility that age or family structure may be responsible for the differences in heritability by re-estimating heritabilities in sub-samples of each ethnic group that were of equivalent age or had equivalent family compositions (data not shown). Trends in the heritabilities remained consistent across sub-samples, so we concluded that the lower heritabilities observed in the African American GENOA sample reflect a true difference in the population parameters of the groups studied and is not an artefact of the GENOA sample structure. However, it is possible that the same factors hypothesized to contribute to variability in test scores among ethnic groups (e.g., cultural variability in familiarity with testing response sets, motivation and attitudes toward test-taking) may have increased the measurement error of the cognitive function tests in the African American sample, resulting in lower heritability estimates for this group.

Lower heritabilities of leukoaraiosis and the seven cognitive traits as well as differences in the genetic and environmental correlations between GENOA whites and African Americans suggest that non-genetic factors have a greater effect in African Americans than in whites for all of the brain traits studied. Though similar patterns were observed in whites and African Americans, genetic correlations (evidence of shared genetic effects) among cognitive traits tended to be higher and more significant in whites. While very little evidence of environmental correlation (shared environmental effects) between cognitive traits was observed in whites, eight of the 21 pairs of cognitive traits (38%) had evidence of significant environmental correlation in African Americans. Therefore, it is likely that non-genetic factors are indeed playing a larger role in affecting variation in brain traits in African American GENOA sample.

4.5 The effect of age on genetic parameter estimation

Most studies of the genetic and environmental factors associated with leukoaraiosis have had samples composed of individuals who have already experienced clinical endpoints such

as stroke or severe cognitive decline. The relatively young age range of our sample provided the unique opportunity to examine the relationships between leukoaraiosis and cognitive phenotypes in asymptomatic individuals, at a time when preventive treatment would be most effective. However, the young age of our sample also imposed constraints, including the limited variability in the leukoaraiosis phenotype. In addition, it has been shown that heritabilities of cognitive traits tend to vary with age (Knopman et al., 2001; Mattay, 2008). Since this study was cross-sectional, we did not have the ability to examine how heritabilities and genetic correlations change over time. Differential heritabilities across age groups also implies that there may be genetic factors that show age-related changes in penetrance with respect to cognitive traits. It is therefore plausible that the genetic correlations between leukoaraiosis and cognitive traits may also change with age.

5. Conclusion

The complex relationship between subclinical measures and clinical outcomes underscores the importance of studying leukoaraiosis and cognitive decline as the predecessors of stroke and dementia in order to better elucide the shared and unique contributions of genetic factors to the disease pathologies that result from hypertension and other risk factors. This study illustrates that the genetic and environmental influences on the subclinical measures of stroke and dementia vary substantially between measures of structural injury (leukoaraiosis) and performance on cognitive tests, and that unique patterns of genetic and environmental correlation exist across cognitive domains (memory vs. executive function).
All of the traits studied demonstrate significant heritability, indicating that genetic factors account for a substantial portion of the variability in leukoaraiosis as well as multiple measures of cognitive function. Some of the cognitive measures, particularly those that assessed similar cognitive domains, demonstrated a significant degree of shared genetic effects, while others demonstrated a greater degree of shared environmental effects. Leukoaraiosis, while heritable, does not share any genetic or environmental influences with the cognitive measures. Patterning of heritability and genetic/environmental correlations showed definitive trends that were consistent across ethic groups, but one clear difference was that cognitive measures tended to have more shared genetic effects in whites and more shared enviornmental effects in African Americans. These results indicate that the environmental and genetic factors that predispose individuals to leukoaraiosis and cognitive decline are likely to be best understood and studied at the family and community levels. Integrating genetic information with family history and cultural or socio-economic stressors and supports is likely to contribute to new knowledge of the unique and shared risk factors for these traits, and thus lead to more effective preventive strategies.

6. Acknowledgements

Data collection and statistical analysis was supported by the National Institutes of Health research grants U10 HL54457, U01 HL054481, and R01 NS041558. We would like to thank Eric Boerwinkle and Myriam Fornage (University of Texas Health Science Center), Clifford Jack, Jr. (Mayo Clinic), Wei Zhao and Patricia Peyser (University of Michigan), Reagan Kelly (Z-Tech at National Center for Toxological Research), the research staff at the Mayo Clinic and University of Mississippi field centers, and the families that participated in the GENOA study.

7. References

Almasy, L., & Blangero, J. (1998). Multipoint Quantitative-Trait Linkage Analysis in General Pedigrees. *American Journal of Human Genetics*, Vol. 62, No. 5, pp. 1198-1211.

Alzheimer's Association. (2008). Alzheimer's Disease Facts and Figures 2008: 10 Million U.S. Baby Boomers Will Develop Alzheimer's Disease, Vol. 4, No. 2, pp. 110-133.

American Psychiatric Association. (2000). *Diagnostic and Statistical Manual of Mental Disorders-IV-TR*. Washington, D.C.

Atwood, L.D., Wolf, P.A., Heard-Costa, N.L., Massaro, J.M., Beiser, A., D'Agostino, R.B., & DeCarli, C. (2004). Genetic Variation in White Matter Hyperintensity Volume in the Framingham Study. *Stroke*, Vol. 35, No. 7, pp. 1609-1613.

Boustani, M., Peterson, B., Hanson, L., Harris, R., Lohr, K.N., & U.S. Preventive Services Task Force. (2003). Screening for Dementia in Primary Care: A Summary of the Evidence for the U.S. Preventive Services Task Force. *Annals of Internal Medicine*, Vol. 138, No. 11, pp. 927-937.

Carmelli, D., DeCarli, C., Swan, G.E., Jack, L.M., Reed, T., Wolf, P.A., & Miller, B.L. (1998). Evidence for Genetic Variance in White Matter Hyperintensity Volume in Normal Elderly Male Twins. *Stroke*, Vol. 29, No. 6, pp. 1177-1181.

Carmelli, D., Reed, T., & DeCarli, C. (2002). A Bivariate Genetic Analysis of Cerebral White Matter Hyperintensities and Cognitive Performance in Elderly Male Twins. *Neurobiology of Aging*, Vol. 23, No. 3, pp. 413-420.

Charlesworth, J., Kramer, P.L., Dyer, T., Diego, V., Samples, J.R., Craig, J.E., Mackey, D.A., Hewitt, A.W., Blangero, J., & Wirtz, M.K. (2010). The Path to Open-Angle Glaucoma Gene Discovery: Endophenotypic Status of Intraocular Pressure, Cup-to-Disc Ratio, and Central Corneal Thickness. *Investigative Ophthalmology and Visual Science*, Vol. 51, No. 7, pp. 3509-3514.

Cheverud, J.M., Ehrich, T.H., Vaughn, T.T., Koreishi, S.F., Linsey, R.B., & Pletscher, L.S. (2004). Pleiotropic Effects on Mandibular Morphology II: Differential Epistasis and Genetic Variation in Morphological Integration. *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution*, Vol. 302, No. 5, pp. 424-435.

Comuzzie, A.G., Blangero, J., Mahaney, M.C., Mitchell, B.D., Stern, M.P., & MacCluer, J.W. (1994). Genetic and Environmental Correlations among Skinfold Measures. *International Journal of Obesity and Related Metabolic Disorders*, Vol. 18, No. 6, pp. 413-418.

Daniels, P.R., Kardia, S.L., Hanis, C.L., Brown, C.A., Hutchinson, R., Boerwinkle, E., Turner, S.T., & Genetic Epidemiology Network of Arteriopathy study. (2004). Familial Aggregation of Hypertension Treatment and Control in the Genetic Epidemiology Network of Arteriopathy (GENOA) Study. *American Journal of Medicine*, Vol. 116, No. 10, pp. 676-681.

Deary, I.J., Wright, A.F., Harris, S.E., Whalley, L.J., & Starr, J.M. (2004). Searching for Genetic Influences on Normal Cognitive Ageing. *Trends in Cognitive Sciences*, Vol. 8, No. 4, pp. 178-184.

DeKosky, S.T., & Marek, K. (2003). Looking Backward to Move Forward: Early Detection of Neurodegenerative Disorders. *Science*, Vol. 302, No. 5646, pp. 830-834.

Dickstein, D.L., Walsh, J., Brautigam, H., Stockton, S.D.,Jr, Gandy, S., & Hof, P.R. (2010). Role of Vascular Risk Factors and Vascular Dysfunction in Alzheimer's Disease. *Mount Sinai Journal of Medicine*, Vol. 77, No. 1, pp. 82-102.

Donnan, G.A., Fisher, M., Macleod, M., & Davis, S.M. (2008). Stroke. *Lancet*, Vol. 371, No. 9624, pp. 1612-1623.

Essink-Bot, M.L., Pereira, J., Packer, C., Schwarzinger, M., & Burstrom, K. (2002). Cross-National Comparability of Burden of Disease Estimates: The European Disability Weights Project. *Bulletin of the World Health Organization*, Vol. 80, No. 8, pp. 644-652.

Family Blood Pressure Program Investigators. (2002). Multi-Center Genetic Study of Hypertension: The Family Blood Pressure Program (FBPP). *Hypertension*, Vol. 39, No. 1, pp. 3-9.

Finkel, D., Pedersen, N.L., McGue, M., & McClearn, G.E. (1995). Heritability of Cognitive Abilities in Adult Twins: Comparison of Minnesota and Swedish Data. *Behavior Genetics*, Vol. 25, No. 5, pp. 421-431.

Finkel, D., & Pedersen, N.L. (2000). Contribution of Age, Genes, and Environment to the Relationship between Perceptual Speed and Cognitive Ability. *Psychology and Aging*, Vol 15, No. 1, pp. 56-64.

Fornage, M.F., Debette, S., Bis, J.C., Schmidt, H., Ikram, M.A., Dufouil, C., Sigurdsson, S., Lumley, T., DeStefano, A.L., Fazekas, F., et. al. (2011). Genome-Wide Association Studies of Cerebral White Matter Lesion Burden. *Annals of Neurology*, Vol. 69, No. 6, pp. 928-939.

Geldmacher, D.S., & Whitehouse, P.J.,Jr. (1997). Differential Diagnosis of Alzheimer's Disease. *Neurology*, Vol. 48, No. 5, Supplement 6, pp. S2-S9.

Golden, C.J. (1978). *Stroop Color and Word Test: A Manual for Clinical and Experimental Uses*, Shoelting Company, Wood Dale, IL.

Haan, M.N., and Wallace, R. (2004). Can Dementia be Prevented? Brain Aging in a Population-Based Context. *Annual Review of Public Health*, Vol. 25, pp. 1-24.

Hassan, A., Hunt, B.J., O'Sullivan, M., Parmar, K., Bamford, J.M., Briley, D., Brown, M.M., Thomas, D.J., & Markus, H.S. (2003). Markers of Endothelial Dysfunction in Lacunar Infarction and Ischaemic Leukoaraiosis. *Brain*, Vol. 126, No. 2, pp. 424-432.

Hassan, A., Hunt, B.J., O'Sullivan, M., Bell, R., D'Souza, R., Jeffery, S., Bamford, J.M., & Markus, H.S. (2004). Homocysteine is a Risk Factor for Cerebral Small Vessel Disease, Acting Via Endothelial Dysfunction. *Brain*, Vol. 127, No. 1, pp. 212-219.

Hodgkin, J. (1998). Seven Types of Pleiotropy. *International Journal of Developmental Biology*, Vol. 42, No. 3, pp. 501-505.

Ivnik, R., Malec, J., and Smith, G. (1992). Mayo's Older Americans Normative Studies: Updated AVLT Norms for Ages 56 to 97. *The Clinical Neuropsychologist*, Vol. 6, Supplement, pp. 83-104.

Jack, C.R.,Jr, Twomey, C.K., Zinsmeister, A.R., Sharbrough, F.W., Petersen, R.C., & Cascino, G.D. (1989). Anterior Temporal Lobes and Hippocampal Formations: Normative Volumetric Measurements from MR Images in Young Adults. *Radiology*, Vol. 172, No. 2, pp. 549-554.

Jack, C.R.,Jr, O'Brien, P.C., Rettman, D.W., Shiung, M.M., Xu, Y., Muthupillai, R., Manduca, A., Avula, R., & Erickson, B.J. (2001). FLAIR Histogram Segmentation for Measurement of Leukoaraiosis Volume. *Journal of Magnetic Resonance Imaging*, Vol. 14, No. 6, pp. 668-676.

Knopman, D., Boland, L.L., Mosley, T., Howard, G., Liao, D., Szklo, M., McGovern, P., Folsom, A.R., & Atherosclerosis Risk in Communities (ARIC) Study Investigators.

(2001). Cardiovascular Risk Factors and Cognitive Decline in Middle-Aged Adults. *Neurology*, Vol. 56, No. 1, pp. 42-48.

Launer, L.J., Ross, G.W., Petrovitch, H., Masaki, K., Foley, D., White, L.R., & Havlik, R.J. (2000). Midlife Blood Pressure and Dementia: The Honolulu-Asia Aging Study. *Neurobiology of Aging*, Vol. 21, No. 1, pp. 49-55.

Lezak, M. (1995). *Neuropsychological Assessment.* Oxford University Press, New York, NY.

Lopez, A.D., Mathers, C.D., Ezzati, M., Jamison, D.T., & Murray, C.J. (2001). Global and Regional Burden of Disease and Risk Factors, 2001: Systematic Analysis of Population Health Data. *The Lancet*, Vol. 367, No. 9524, pp. 1747-1757.

Markus, H.S., Hunt, B., Palmer, K., Enzinger, C., Schmidt, H., & Schmidt, R. (2005). Markers of Endothelial and Hemostatic Activation and Progression of Cerebral White Matter Hyperintensities: Longitudinal Results of the Austrian Stroke Prevention Study. *Stroke*, Vol. 36, No. 7, pp. 1410-1414.

Markus, H.S. (2008). Genes, Endothelial Function and Cerebral Small Vessel Disease in Man. *Experimental Physiology*, Vol. 93, No. 1, pp. 121-127.

Mattay, V.S., Goldberg, T.E., Sambataro, F., & Weinberger, D.R. (2008). Neurobiology of Cognitive Aging: Insights from Imaging Genetics. *Biological Psychology*, Vol. 79, No. 1, pp. 9-22.

McGue, M., & Christensen, K. (2001). The Heritability of Cognitive Functioning in very Old Adults: Evidence from Danish Twins Aged 75 Years and Older. *Psychology and Aging*, Vol. 16, No. 2, pp. 272-280.

McGue, M., & Christensen, K. (2002). The Heritability of Level and Rate-of-Change in Cognitive Functioning in Danish Twins Aged 70 Years and Older. *Experimental Aging Research*, Vol. 28, No. 4, pp. 435-451.

O'Sullivan, M. (2008). Leukoaraiosis. *Practical Neurology*, Vol. 8, No. 1, pp. 26-38.

Pantoni, L., Poggesi, A., & Inzitari, D. (2007). The Relation between White-Matter Lesions and Cognition. *Current Opinion in Neurology*, Vol. 20, No. 4, 390-397.

Paternoster, L., Chen, W., & Sudlow, C.L. (2009). Genetic Determinants of White Matter Hyperintensities on Brain Scans: A Systematic Assessment of 19 Candidate Gene Polymorphisms in 46 Studies in 19,000 Subjects. *Stroke*, Vol. 40, No. 6, pp. 2020-2026.

Plomin, R., Pedersen, N.L., Lichtenstein, P., & McClearn, G.E. (1994). Variability and Stability in Cognitive Abilities are Largely Genetic Later in Life. *Behavior Genetics*, Vol. 24, No. 3, pp. 207-215.

Pohjasvaara, T., Mantyla, R., Ylikoski, R., Kaste, M., & Erkinjuntti, T. (2000). Comparison of Different Clinical Criteria (DSM-III, ADDTC, ICD-10, NINDS-AIREN, DSM-IV) for the Diagnosis of Vascular Dementia. National Institute of Neurological Disorders and Stroke-Association Internationale Pour La Recherche Et l'Enseignement En Neurosciences. *Stroke*, Vol. 31, No. 12, pp. 2952-2957.

Prins, N.D., van Dijk, E.J., den Heijer, T., Vermeer, S.E., Koudstaal, P.J., Oudkerk, M., Hofman, A., & Breteler, M.M. (2004). Cerebral White Matter Lesions and the Risk of Dementia. *Archives of Neurology*, Vol. 61, No. 10, pp. 1531-1534.

R Core Development Team. (2008). R: A Language and Environment for Statistical Computing.

Rey, A. (1964). *L'Examen Clinique en Psychologie.* Presses Universitaires de France.

Roger, V.L., Go, A.S., Lloyd-Jones, D.M., Adams, R.J., Berry, J.D., Brown, T.M., Carnethon, M.R., Dai, S., de Simone, G., Ford, E.S., et al. (2011). Heart Disease and Stroke Statistics 2011 Update: A Report From the American Heart Association. *Circulation*, Vol. 123, pp. e18-e209.

Schmidt, R., Scheltens, P., Erkinjuntti, T., Pantoni, L., Markus, H.S., Wallin, A., Barkhof, F., & Fazekas, F. (2004). White Matter Lesion Progression: A Surrogate Endpoint for Trials in Cerebral Small-Vessel Disease. *Neurology*, Vol. 63, No. 1, pp. 139-144.

Schmidt, R., Petrovic, K., Ropele, S., Enzinger, C., & Fazekas, F. (2007). Progression of Leukoaraiosis and Cognition. *Stroke*, Vol. 38, No. 9, pp. 2619-2625.

Sierra, C., & Coca, A. (2006). White Matter Lesions and Cognitive Impairment as Silent Cerebral Disease in Hypertension. *Scientific World Journal*, Vol. 6, pp. 494-501.

Sing, C.F., Boerwinkle, E., Moll, P.P., & Templeton, A.R. (1987). Characterization of Genes affecting Quantitative Traits in Humans. In *Proceedings of the Second International Conference on Quantitative Genetics,* B. Weir, E.J. Eisen, M.M. Goodman, and G. Namkoong, eds., pp. 250-269, Sinauer Associates, Inc., Raliegh, NC,.

Sleegers, K., de Koning, I., Aulchenko, Y.S., van Rijn, M.J., Houben, M.P., Croes, E.A., van Swieten, J.C., Oostra, B.A., & van Duijn, C.M. (2007). Cerebrovascular Risk Factors do Not Contribute to Genetic Variance of Cognitive Function: The ERF Study. *Neurobiology of Aging*, Vol. 28, No. 5, pp. 735-741.

Stroop, J. (1935). Studies of Inference in Serial Verbal Reactions. *Journal of Experimental Psychology*, Vol. 18, pp. 643-662.

Szolnoki, Z., & Melegh, B. (2006). Gene-Gene and Gene-Environment Interplay Represent Specific Susceptibility for Different Types of Ischaemic Stroke and Leukoaraiosis. *Current Medicinal Chemistry*, Vol. 13, No. 14, pp. 1627-1634.

Turner, S.T., & Boerwinkle, E. (2000). Genetics of Hypertension, Target-Organ Complications, and Response to Therapy. *Circulation*, Vol. 102, No. 20, Supplement 4, pp. 40-45.

Turner, S.T., Jack, C.R., Fornage, M., Mosley, T.H., Boerwinkle, E., & de Andrade, M. (2004). Heritability of Leukoaraiosis in Hypertensive Sibships. *Hypertension*, Vol. 43, No. 2, pp. 483-487.

Valenzuela, M.J., & Sachdev, P. (2006). Brain Reserve and Cognitive Decline: A Non-Parametric Systematic Review. *Psychological Medicine*, Vol. 36, No. 8, pp. 1065-1073.

van Dijk, E.J., Breteler, M.M., Schmidt, R., Berger, K., Nilsson, L.G., Oudkerk, M., Pajak, A., Sans, S., de Ridder, M., Dufouil, C. et al. (2004). The Association between Blood Pressure, Hypertension, and Cerebral White Matter Lesions: Cardiovascular Determinants of Dementia Study. *Hypertension*, Vol. 44, No. 5, pp. 625-630.

Wechsler, D. (1981). *The Wechsler Adult Intelligence Scale-Revised.* Psychological Corporation, New York.

Zou, L., Sriswasdi, S., Ross, B., Missiuro, P.V., Liu, J., & Ge, H. (2008). Systematic Analysis of Pleiotropy in C. Elegans Early Embryogenesis. *PLoS Computational Biology*, Vol. 4, No. 2, e1000003.

4

Compensatory Neurogenesis in the Injured Adult Brain

Bronwen Connor
Department of Pharmacology and Clinical Pharmacology, Centre for Brain Research
Faculty of Medical and Health Sciences, The University of Auckland
New Zealand

1. Introduction

The occurrence of neurogenesis, defined as the generation of new neurons, has become well established in the adult mammalian brain, including the human brain over the last two decades. Neurogenesis in the adult brain can be divided into four phases: (a) progenitor cell proliferation; (b) migration of progenitor cells towards a target area; (c) terminal differentiation into a specific phenotype, and; (d) integration into established networks. Neural stem/progenitor cells generate neurons throughout life in the mammalian forebrain subventricular zone (SVZ)–olfactory bulb (OB) pathway and the hippocampal dentate gyrus [for review see (Whitman, *et al.*, 2009)]. Neural progenitor cells can be isolated from these two regions and cultured *in vitro* as self-renewable neurospheres in epidermal growth factor (EGF) and fibroblast growth factor-2 (FGF2) containing media (Reynolds, *et al.*, 1992, Reynolds, *et al.*, 1996). Upon withdrawal of growth factors, they differentiate into the three main neural lineages; neurons, astrocytes and oligodendrocytes (Reynolds & Weiss, 1996).
There are four major cell types within the adult SVZ-OB pathway; ependymal cells, Type B, Type C and Type A cells (Doetsch, *et al.*, 1997). The true neural stem cells in this region are the Type B cells which have the characteristics of radial glial cells, including the expression of GFAP (Doetsch, *et al.*, 1997, Doetsch, *et al.*, 1999). Type B cells proliferate slowly to generate Type C cells, which are the most rapidly proliferating cell type in the SVZ. The bipotent Type C cells are able to divide either symmetrically or asymmetrically to generate glial or neural precursor cells. The SVZ-OB pathway is organized as an extensive network of chains of migrating neural precursor cells (neuroblasts; Type A cells) that travel through glial tubes formed by GFAP positive radial glial-like cells (Lois, *et al.*, 1994, Doetsch, *et al.*, 1996, Lois, *et al.*, 1996, Doetsch, *et al.*, 1997). SVZ- derived neuroblasts migrate long distances via a restricted forebrain pathway known as the rostral migratory stream (RMS) to their final destination in the olfactory bulb. This is achieved through a unique form of tangential chain migration. Migrating neuroblasts (Type A cells) in the SVZ – OB pathway can be identified by their expression of characteristic markers such as the polysialylated form of neural cell adhesion molecule (PSA-NCAM), neuron-specific βIII-tubulin and doublecortin (Dcx). Once the neuroblasts reach the subependymal region of the olfactory bulb, they disperse radially and differentiate into granule and periglomerular neurons (Luskin, 1993, Lois&Alvarez-Buylla, 1994, Lois, *et al.*, 1996, Thomas, *et al.*, 1996, Curtis, *et al.*,

2007). Studies have shown that olfactory granule and periglomerular cells are continuously added to the olfactory bulb to both increase total cell number over time in these layers as well as replace pre-existing cells (Lagace, *et al.*, 2007, Imayoshi, *et al.*, 2008). The function of persistent olfactory bulb neurogenesis is largely unknown, but increasing evidence supports a role for the new neurons in olfactory memory and odour discrimination (Gheusi, *et al.*, 2000, Petreanu, *et al.*, 2002, Rochefort, *et al.*, 2002).

In contrast to the extensive migration undertaken by neurons destined for the olfactory bulb, dentate gyrus granule neurons are born locally in the subgranular zone (SGZ), a germinal layer between the dentate gyrus and the hilus (Altman, *et al.*, 1965, Kaplan, *et al.*, 1977, Eriksson, *et al.*, 1998, Kornack, *et al.*, 1999, Gould, *et al.*, 2001). Within the SGZ, GFAP positive cells (Type B cells) divide to give rise to immature Type D cells, which then generate granule neurons (Palmer, *et al.*, 2000, Seri, *et al.*, 2001). Interestingly, Type D cells divide less frequently and are more differentiated than the transit amplifying Type C cells in the SVZ. SGZ-derived neural progenitor cells generate new neurons that make and receive functional synapses (Palmer, *et al.*, 2000, Song, *et al.*, 2002, Van Praag, *et al.*, 2002). Ongoing hippocampus neurogenesis is known to facilitate long-term potentiation and stimulate learning and memory (Van Praag, *et al.*, 1999, Wang, *et al.*, 2005, Imayoshi, *et al.*, 2008) with ablation of adult-born dentate granule cells impairing certain forms of hippocampal-dependent learning (Dupret, *et al.*, 2008, Imayoshi, *et al.*, 2008, Clelland, *et al.*, 2009).

Adult neurogenesis is not static, but its rate may fluctuate in response to environmental change. Evidence from *in vitro* and *in vivo* studies have demonstrated that neurogenesis can be regulated by a range of growth and neurotrophic factors, neurotransmitters and hormones [for review see (Parent, 2003, Lie, *et al.*, 2004)]. Neurogenesis has also been shown to be altered by the presence of cell death induced by brain injury or disease [for review see (Peterson, 2002, Parent, 2003, Lie, *et al.*, 2004, Goldman, 2005)]. A critical issue of neurogenesis, both during development and in adulthood, is the appropriate integration of different cell types to form mature neural cells. This means that progenitor cells need to migrate from their places of birth to their final positions. Such a highly regulated process is mediated by a number of environmental cues like substrates, chemoattractive/ chemorepulsive factors, and detachment/stop signals. Although some of these factors have been identified, many remain to be discovered [for review see (Cayre, *et al.*, 2009)]. Progenitor cell migration is most extensive in the developing and immature brain. In the adult brain, neural cell migration still continues, although in a more limited capacity with the most extensive region of migration observed in the SVZ-OB pathway. It is not yet clear why new neurons are not born in the place they need to reside. While the maintenance of stem cell niches in the adult brain may provide a potential source of cells for brain repair and cell replacement, these regions may be costly for the organism and may also require specific features that restrict the structures where they can persist. As a result, in both normal and pathological conditions cells need to be able to migrate from these discrete niches to their final destination. During pathological processes, such as brain injury, the brain demonstrates spontaneous attempts at repair and regeneration. These processes result in a distinct profile of cell proliferation and migration not observed in the normal adult brain, which appear to be mediated by an independent set of environmental cues. This chapter will discuss what is known about the response of endogenous adult neural progenitor cells to brain injury including stroke, traumatic brain injury, epilepsy and excitotoxic injury, the mechanisms by which this response may occur, and how this knowledge may be translated to effective therapeutic strategies.

2. The response of progenitor cells to the injured brain

2.1 Temporal lobe epilepsy

Epilepsy, characterized by periodic and unpredictable occurrence of seizure activity, affects ~50 million people worldwide and temporal lobe epilepsy (TLE) is among the most frequent types of intractable epilepsy. Abnormal hippocampal neurogenesis has emerged as an important pathophysiology of TLE over the past decade [for review see (Kuruba, *et al.*, 2009)]. Initial studies on neurogenesis in animal models of TLE by Parent and colleagues (Parent, *et al.*, 1997, Parent, *et al.*, 1998) and Bengzon and colleagues (Bengzon, *et al.*, 1997) provided the first evidence for increased hippocampal neurogenesis following acute seizures. In these studies, an increase in the production of new cells was observed in the SGZ of the dentate gyrus following pilocarpine-induced status epilepticus (SE) (Parent, *et al.*, 1997, Gray, *et al.*, 1998) or kindling stimulations (Bengzon, *et al.*, 1997, Parent, *et al.*, 1998). However, by 3-4 weeks after seizure induction, neurogenesis returned to baseline levels. In normal animals, proliferating cells labeled with the mitotic marker bromodeoxyuridine (BrdU) are restricted to the SGZ of the hippocampus. In contrast, following seizure activity BrdU+ cells were found extensively in the dentate hilus and/or dentate molecular layer of the hippocampus, indicating aberrant migration of dividing cells in response to seizure-induced cell loss (Parent, *et al.*, 1997, Scharfman, *et al.*, 2000, Scharfman, *et al.*, 2002, Scharfman, *et al.*, 2003, Parent, *et al.*, 2006). Similarly, displaced granule cells have been observed in hippocampal tissues obtained from patients with TLE (Houser, 1990, Thom, *et al.*, 2002, Liu, *et al.*, 2008). This suggests that acute seizure-induced dentate gyrus neurogenesis promotes aberrant circuitry development, which likely contributes to the evolution of initial seizure-induced hippocampal injury into chronic epilepsy (Kuruba, *et al.*, 2009).

In addition to the neurogenic response observed in the hippocampus, progenitor cells in the SVZ also respond to seizure activity in the adult rodent brain. Within 1-2 weeks following pilocarpine-induced seizure activity, Parent and colleagues (Parent, *et al.*, 2002) observed an increase in BrdU labeling and Nissl staining in the RMS. These changes were associated with an increase in expression of the Type A neuroblast marker Dcx 2 – 3 weeks following prolonged seizures. At these same time points the RMS expanded and contained more proliferating cells and immature neurons. BrdU labeling and retroviral tracing showed that prolonged seizures also increased neuroblast migration to the olfactory bulb. Importantly, a large number of labeled cells were found adjacent to the RMS instead of within its realms (most prominent at 14 days following seizure induction), indicating that seizure activity induces aberrant migration of SVZ-derived progenitor cells into surrounding regions of the brain (Parent, *et al.*, 2002).

Increased neurogenesis observed following acute seizure activity returns to baseline by about 2 months after the initial seizure episode in rats. The extent of neurogenesis has then been shown to decline significantly in the chronic phase of epilepsy when significant numbers of spontaneous seizures manifest [for review see (Hattiangady, *et al.*, 2008)]. A 64-81% decrease in neurogenesis was reported at 5 months post-SE with an inverse relationship evident between the frequency of spontaneous seizures and the extent of neurogenesis (Hattiangady, *et al.*, 2004). The severe reduction in hippocampal neurogenesis observed in chronic TLE is not however associated with either decreased production of new cells or reduced survival of newly born cells in the dentate gyrus. Rather, it is due to a decline in the neuronal fate-choice decision of newly generated cells with the majority of newly born

cells differentiating to a glial rather than a neuronal lineage in response to chronic TLE (Hattiangady, *et al.*, 2010). Thus, diminished hippocampal neurogenesis might contribute to the persistence of spontaneous seizures, learning and memory deficits, and depression prevalent in chronic TLE.

2.2 Traumatic brain injury

Traumatic brain injury (TBI) is characterized by both neuronal and white matter loss, with resultant brain atrophy and functional neurological impairment. Injury may be in the form of focal damage, or it may be diffuse with widespread delayed neuronal loss. In addition to local neuronal loss resulting from the mechanical primary insult, TBI also induces a cascade of delayed secondary events that contribute to neuronal death, including ischemia, Wallerian degeneration secondary to diffuse axonal injury, excitotoxicity, dysregulation of calcium homeostasis, mitochondrial dysfunction and free radical-mediated damage. Among the diffuse injury sites, the hippocampus is known to be especially vulnerable in humans and shows the earliest evidence of TBI-induced degeneration in experimental models. The most frequently used experimental models of TBI include the controlled cortical impact (CCI) and lateral fluid percussion (FPI) models (Wang, *et al.*, 2010). The lateral FPI model can reproduce multiple types of human TBI, including focal contusion, intraparenchymal and subarachnoid hemorrhage, tissue tears and axonal damage, and has been widely adopted as a combined model of focal and diffuse brain injury. The CCI model generally has been found to produce a more focused injury compared to lateral FPI; the severity of injury is also significantly greater in the gray matter relative to the underlying white matter. In both injury models there is an acute neurogenic response with an increase in hippocampal progenitor cell proliferation observed from 24hr to 1-2 weeks following TBI (Dash, *et al.*, 2001, Chirumamilla, *et al.*, 2002, Emery, *et al.*, 2005). Newly generated neurons in the dentate gyrus integrate into the existing hippocampal circuitry following TBI, potentially resulting in cognitive recovery (Sun, *et al.*, 2005). Transgenic approaches have demonstrated that following TBI, the nestin-expressing progenitor cells are first activated by injury, whereas the later Dcx-expressing committed neuroblasts appear to be eliminated (Miles, *et al.*, 2008, Yu, *et al.*, 2008). Later, the Dcx-expressing cells within the dentate gyrus reemerge and are likely contributors to stable neurogenesis (Yu, *et al.*, 2008).

Adult SVZ neurogenesis has also been investigated in the CCI model of TBI (Goings, *et al.*, 2002, Ramaswamy, *et al.*, 2005). In these studies, SVZ progenitor cell proliferation was observed either to be reduced (Goings, *et al.*, 2002), or to exhibit a delayed increase in proliferation (Ramaswamy, *et al.*, 2005) following TBI. In the lateral FPI model, an increase in SVZ progenitor cell incorporation of BrdU was observed between 2 – 8 days post injury (Chirumamilla, *et al.*, 2002). Interestingly, in the CCI model progenitor cell migration within the SVZ - OB pathway, as demonstrated by PSA-NCAM expression, was not enhanced until 25-35 days post TBI (Goings, *et al.*, 2002). Retroviral labeling of SVZ progenitor cells and examination of the location of labeled cells at 4 days and 3 weeks post injury in adult mice determined that very few cells migrated into the cerebral cortex in the normal brain, whereas a large number of labeled cells migrated into the lesioned area following cortical impact (Goings, *et al.*, 2004). Migration of progenitor cells into the lesioned cortex appeared to be at the expense of migration to the olfactory bulb; in control animals approximately half of the labeled SVZ cells were found in the olfactory bulb, whereas only a quarter of labeled cells migrated there following cortical injury (Goings, *et al.*, 2004). However, the majority of adult-born cells located in the lesioned area appear to be newly generated glial cells, with

limited to no neuronal differentiation observed (Chirumamilla, *et al.*, 2002, Goings, *et al.*, 2004). Finally, an increase in proliferative markers and the number of proliferative neural progenitor cells was recently observed to be increased in the perilesion cortex of the human brain following TBI (Zheng, *et al.*, 2011), indicating that TBI may also induce compensatory neurogenesis in the human brain. Thus, it appears that TBI results in compensatory neurogenesis in response to both hippocampal and cortical damage, with progenitor cell migration focused on recruitment to areas of neural injury. Further studies however are required to determine the fate and survival of adult-born cells in areas of TBI-induced injury.

2.3 Focal ischemia

Ischemic stroke involves an interruption in blood supply to the brain and results in the death of neural cells and corresponding loss of brain function. Focal ischemia is generated through the blockage of blood vessels which supply specific regions of the brain, and is commonly modeled by the occurrence of transient middle cerebral artery occlusion (tMCAo) which results in damage to the cortex and striatum. Studies of experimental stroke in rodents over the past decade indicate that focal ischemia potently stimulates SVZ cell proliferation and neurogenesis (Jin, *et al.*, 2001, Zhang, *et al.*, 2001, Arvidsson, *et al.*, 2002, Parent, *et al.*, 2002, Ohab, *et al.*, 2006). Although initial studies suggested that the increase in SVZ neurogenesis after stroke is transient (Arvidsson, *et al.*, 2002, Parent, *et al.*, 2002), more recent work indicates that it persists for at least 4 months after ischemia (Thored, *et al.*, 2006). SVZ progenitor cells have also been observed to migrate in chains into the ischemic striatum and cortex (Arvidsson, *et al.*, 2002, Parent, *et al.*, 2002, Jin, *et al.*, 2003, Ohab, *et al.*, 2006, Yamashita, *et al.*, 2006, Zhang, *et al.*, 2009). As with TBI, this appears to be at the expense of olfactory bulb migration. Recent evidence suggests that a similar long-distance migration of neuroblasts may occur in peri-infarct tissue in human stroke (Jin, *et al.*, 2006). Although a large number of neuroblasts reach regions of striatal damage after stroke, few of them differentiate into mature neurons. Most adult-born neurons appear to die (Arvidsson, *et al.*, 2002, Parent, *et al.*, 2002), perhaps from a failure to integrate or due to inflammatory milieu. However, the persistence of SVZ neuroblast migration to the injured striatum for up to a year after ischemia (Thored, *et al.*, 2006) suggests that the SVZ may serve as a constant reservoir of new neurons that offers an extended window for therapeutic manipulation. In most stroke models, many of the surviving cells differentiate into neurons, but the precise nature of the neurons that persist long term in the striatum is controversial. The generation of neurons expressing markers of the striatal medium spiny neurons including DARPP-32 and calbindin after tMCAo in adult rats has been reported by two groups (Arvidsson, *et al.*, 2002, Parent, *et al.*, 2002). More recently however, Liu and colleagues (Liu, *et al.*, 2009) used retroviral reporters to label SVZ progenitor cells prior to inducing stroke in adult rats and found that adult-born neurons exclusively differentiated into calretinin-expressing interneurons. This may be due to differences in the location and extent of focal ischemic injury, selectivity of ischemic-induced neural cell loss, or alternatively the response of the specific population of neural progenitor cells investigated (Lledo, *et al.*, 2008). Further research is also required to determine the potential for adult-born neurons to integrate into the surrounding parenchyma following focal ischemic injury.

2.4 Excitotoxic brain injury

Striatal injection of the neurotoxin quinolinic acid (QA) generates the selective loss of the GABAergic medium spiny neurons in the striatum. This model has been used to investigate

the effect of excitotoxic striatal injury on SVZ-derived neurogenesis. QA lesioning results in a significant increase in progenitor cell proliferation at days 1 – 14 following injury (Tattersfield, *et al.*, 2004, Collin, *et al.*, 2005). In addition, both expansion of the RMS and aberrant migration of SVZ-derived Dcx-expressing progenitor cells into the lesioned striatum has been demonstrated following QA-induced striatum cell loss (Tattersfield, *et al.*, 2004, Collin, *et al.*, 2005, Gordon, *et al.*, 2007). In order to elucidate the temporal profile of progenitor cell migration in response to QA-induced striatal cell loss, Gordon and colleagues (Gordon, *et al.*, 2007) used retroviral tracing to label SVZ-derived progenitor cells and track their migratory profile. This study demonstrated that SVZ-derived progenitor cell migration was significantly enhanced in the RMS of QA lesioned animals immediately following, and up to 30 days following QA-induced striatal cell loss. This was in contrast to the migratory response observed in both TBI and ischemic stroke, and demonstrated that recruitment of SVZ-derived progenitor cells into the QA lesioned striatum was not at the expense of olfactory bulb migration. In addition, Gordon and colleagues (Gordon, *et al.*, 2007) identified that aberrant migration of SVZ-derived progenitor cells into the QA lesioned striatum is transient, with progenitor cell recruitment predominantly observed by cells labeled either 2 days prior or up to 3 days following QA lesioning. Interestingly, a change in the morphology of the recruited SVZ-derived progenitor cells was observed over time. SVZ-derived progenitor cells labeled either 2 days prior, or on the day of QA lesioning predominantly exhibited a bipolar morphology and expressed Dcx. In contrast, the majority of progenitor cells labeled from the day of QA lesioning up to 3 days following lesioning displayed a multipolar morphology and did not express Dcx (Gordon, *et al.*, 2007). This indicates that striatal cell loss induces an expansion of the SVZ progenitor cell population, in which a sub-population of SVZ-derived progenitor cells are responsive to recruitment into the lesioned area. In addition, the novel observation of a temporal change in the morphological profile of progenitor cells recruited into the QA lesioned striatum is of great interest, and warrants further investigation. This alteration in progenitor cell morphology may be in response to changes in environmental cues present in the lesioned striatum. Following recruitment into the QA-lesioned striatum, about 80% of adult-born neurons survive up to 6 weeks, when they express the mature neuronal marker NeuN and phenotypic markers of striatal medium spiny neurons (DARPP-32) and interneurons (parvalbumin or neuropeptide Y) (Tattersfield, *et al.*, 2004, Collin, *et al.*, 2005). However, similar to the observations made in models of ischemic stroke, relatively few adult-born neurons survive long term. The low level of adult-born cell survival in models of both focal ischemia and excitotoxic striatal cell loss indicates that further investigation is required in both injury models to determine the effect of the environment of cell fate, integration and long-term survival.

3. Factors modulating compensatory neurogenesis

The precise mechanisms underlying injury-induced compensatory neurogenesis in the adult brain are unclear. However, several potential mechanisms have been proposed. First it is believed that the release of mitogenic factors from dying neurons and reactive glia probably increases the proliferation of neural progenitor cells and the survival of newly generated neurons. Expression of mitogenic factors can also alter transcriptional signaling pathways in neural progenitor cells, redirecting neurogenic processes from a normal physiological role (Bath, *et al.*, 2010, Hodge, *et al.*, 2011, Jones, *et al.*, 2011). Increased expression of cytokines

and chemokines from reactive microglia and blood vessels are also involved in directing the migration of neural progenitor cells to areas of neuronal loss and injury, as well as controlling adult-born neuron survival (Gonzalez-Perez, *et al.*, 2010). Angiogenesis and the expression of pro-angiogenic factors appear to play an important role both in progenitor cell proliferation and survival as well as migration (Xiong, *et al.*, 2010, Yang, *et al.*, 2011). In addition, alteration in neurochemical signaling, such as GABAergic and glutamtergic transmission, and neuronal activity have been shown to modulate neurogenesis following brain injury (Deisseroth, *et al.*, 2004, Ge, *et al.*, 2007). Thus, it appears multiple mechanisms underlie the regulation of compensatory neurogenesis following brain injury; these will be summarized in the following section.

3.1 Potential mechanisms of increased hippocampal neurogenesis after seizure activity

Multiple studies have demonstrated that several factors known to promote neural progenitor cell proliferation and neuron survival such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), fibroblast growth factor-2 (FGF2), vascular endothelial growth factor (VEGF) and sonic hedgehog (Shh) are all up-regulated in the hippocampus after acute seizures (Lowenstein, *et al.*, 1993, Gall, *et al.*, 1994, Riva, *et al.*, 1994, Gómez-Pinilla, *et al.*, 1995, Shetty, *et al.*, 2003, Croll, *et al.*, 2004, Shetty, *et al.*, 2004, Banerjee, *et al.*, 2005). Increased levels of GABA in the dentate gyrus during the early post-seizure period may also positively regulate hippocampal neurogenesis, as studies show that GABA has crucial roles in regulating various steps of adult neurogenesis, including progenitor cell proliferation, migration and differentiation of neuroblasts, and synaptic integration of adult-born neurons (Ge, *et al.*, 2007). Further, increased levels of neuropeptide Y (NPY) found typically after acute seizures may enhance the proliferation of neural progenitor cells in the dentate gyrus, as studies have shown that neural progenitor cells increase neurogenesis in the presence of NPY (Howell, *et al.*, 2003, Howell, *et al.*, 2005, Howell, *et al.*, 2007, Rodrigo, *et al.*, 2010).

3.2 Abnormal migration of adult-born cells after acute seizure activity

As discussed in Section 2.1, aberrant migration of progenitor cells is observed in response to seizure-induced cell loss. The precise reason for aberrant migration of adult-born granule cells is still being examined. However, it has been shown that acute seizures do not significantly influence the proliferation of nestin-expressing neural stem cells but rather stimulate the division of Dcx-expressing transient amplifying cells and immature neurons (Jessberger, *et al.*, 2005). Based on this, it has been proposed that delayed proliferation during the process of neurogenesis interferes with migration, leading to a significant dispersion of Dcx-positive cells away from the granule cell layer into the dentate hilus and the molecular layer. In addition, recent evidence suggests that a loss of the migration guidance cue Reelin due to seizure activity may lead to aberrant chain migration of newly born dentate granule cells (Gong, *et al.*, 2007). Interneuron subsets typically lost in human and experimental TLE express Reelin, and dentate granule progenitor cells express the downstream Reelin signaling molecule Disabled 1 (Dab1). Prolonged seizure activity has been shown to decrease Reelin expression in the adult rat dentate gyrus and increase Dab1 expression in hilar ectopic neuroblasts. Further, exogenous Reelin increased detachment of chain-migrating neuroblasts in dentate gyrus explants, and blockade of Reelin signaling

increased chain migration (Gong, *et al.*, 2007). These observations suggest that Reelin modulates dentate gyrus progenitor cell migration and loss of Reelin expression in the epileptic adult hippocampus may contribute to ectopic chain migration and aberrant integration of newborn granule cells.

3.3 Potential mechanisms underlying decreased neurogenesis in chronic TLE

The precise mechanisms underlying decreased neurogenesis in chronic TLE are unknown, however several explanations have been proposed [for review see (Kuruba, *et al.*, 2009)]. While a role for chronic inflammation is an attractive hypothesis, this has been ruled out as only minimal density of activated microglia have been observed in the hippocampus during chronic epilepsy (Hattiangady, *et al.*, 2004). One potential mechanism may be a reduction in mitogenic factors such as FGF-2, BDNF and insulin-like growth factor 1 (IGF-1) in the epileptic hippocampus resulting in an unfavourable neurogenic environment (Hattiangady, *et al.*, 2004, Shetty, *et al.*, 2004). As neuronal differentiation rather than progenitor cell number and proliferation is predominantly affected in chronic TLE, the presence of an unfavourable hippocampal milieu due to reduction of mitogenic factors currently remains the most plausible mechanism (Altar, *et al.*, 2004, Chan, *et al.*, 2008).

3.4 Potential mechanisms of increased neurogenesis after TBI

Several studies have shown that neurotrophic factor expression is significantly altered after TBI. Some neurotrophic factors such as NGF and BDNF are up-regulated, while others such as neurotrophic factor-3 (NT-3) have been shown to be down-regulated (Yang, *et al.*, 1996, Hicks, *et al.*, 1997, Oyesiku, *et al.*, 1999, Truettner, *et al.*, 1999). Interestingly, BDNF levels after TBI have been reported to be increased to a greater extent in older rather than younger animals (Shah, *et al.*, 2006), despite the well known fact that older age is correlated with a worse outcome after TBI. Results from the CCI model also suggest that FGF-2 is up-regulated, potentially stimulating post-traumatic neurogenesis (Yoshimura, *et al.*, 2003). In addition, up-regulation of VEGF has been observed following both CCI (Sköld, *et al.*, 2005, Lu, *et al.*, 2011) and lateral FPI (Lee, *et al.*, 2010), and may be involved in enhancing neurogenesis and promoting migration following TBI as observed in rodent models of focal ischemia.

3.5 Potential mechanisms of increased neurogenesis after stroke

Potential mediators of stroke-induced cell proliferation and neurogenesis are beginning to be identified (Yan, *et al.*, 2006, Zhang, *et al.*, 2008, Leker, *et al.*, 2009, Luo, 2011). Through infusion studies, a range of growth factors have been identified to play a role in regulating SVZ neurogenesis following focal ischemia. Factors such as GDNF, VEGF, EGF, transforming growth factor-α (TGF-α) and IGF-1 have all been shown to increase progenitor cell proliferation in the ipsilateral SVZ following ischemic damage (Jin, *et al.*, 2002, Sun, *et al.*, 2003, Schänzer, *et al.*, 2004, Kobayashi, *et al.*, 2006, Ninomiya, *et al.*, 2006, Yan, *et al.*, 2006, Leker, *et al.*, 2009, Guerra-Crespo, *et al.*, 2010). VEGF was shown not only to increase progenitor cell proliferation, but to also increase the survival of adult-born neurons and induce neurite outgrowth in newborn cells (Wang, *et al.*, 2009, Zheng, *et al.*, 2010). Another study (Ninomiya, *et al.*, 2006) demonstrated that EGF infusion into the ischemic brain caused the number of Type C transient amplifying cells to increase and the number of neuroblasts to decrease. However, 6 weeks after the discontinuation of EGF infusion, a significant

increase in the number of neuroblasts was found, both in the ischemic striatum and SVZ. Co-administration of EGF and FGF2 into the lateral ventricle for 5 days in a rodent model of global cerebral ischemia has been shown to increase the proliferative rate and differentiation of newly generated hippocampal neurons (Nakatomi, *et al*., 2002). The newborn neurons exhibited histological markers of young and maturing neurons, appropriate connectivity and synapse formation as well as electrophysiological characteristics of young neurons. Further, memory deficits were resolved in EGF and FGF2 treated rats within 90 days. Erythropoietin (EPO) also plays a role in regulating compensatory SVZ neurogenesis following ischemic injury. EPO stimulates the maturation, differentiation and survival of hematopoietic progenitor cells and promotes angiogenesis. While EPO and its receptor are only weakly expressed in normal adult brain, expression of EPO and its receptor is greatly increased in neurons, neural progenitor cells, glia and cerebrovascular endothelial cells in response to brain injury. Infusion of EPO into the adult lateral ventricles results in a decrease in the number of neural progenitor cells in the SVZ, an increase in neural precursor cells migrating to the olfactory bulb and an increase in the generation of new olfactory bulb neurons (Shingo, *et al*., 2001). Further, delivery of EPO for 7 days following 7 days of EGF treatment has been shown to enhance SVZ neurogenesis and direct progenitor cell migration to the ischemic cortex, resulting in cortical regeneration and functional recovery (Kolb, *et al*., 2006). It is thought that EPO might affect the number of daughter cells that stay in cell cycle and promote cell cycle exit and terminal differentiation with preference towards neuronal differentiation (Shingo, *et al*., 2001). Systemic administration of BDNF has also been shown to induce neurogenesis and improve sensorimotor function in a rodent model of ischemic injury (Schabitz, *et al*., 2007). In addition, a range of signaling pathways appear to be important in regulating compensatory neurogenesis following ischemic injury. These include notch, retinoid, bone morphogenic protein, tumor necrosis factor-alpha (TNF-α) and Shh pathways (Androutsellis-Theotokis, *et al*., 2006, Chou, *et al*., 2006, Iosif, *et al*., 2008, Plane, *et al*., 2008, Zhang, *et al*., 2008, Sims, *et al*., 2009, Wang, *et al*., 2009).

3.6 The role of chemoattractants in regulating neural progenitor cell migration following brain injury

A fundamental issue concerning progenitor cell migration in the adult brain is to understand the extracellular cues and mechanisms that allow the persistence of normal migratory pathways, as well as the recruitment of progenitor cells into the areas of neural damage. Increasing evidence indicates the involvement of developmental signals that are maintained in restricted regions of the adult brain, including factors such as extracellular matrix molecules, Eph-Ephrin interactions, neuregulins, and a range of chemoattractant and chemorepulsive molecules [for review see (Cayre, *et al*., 2009)]. In addition, several mechanisms and migratory tracks have been proposed for the guidance of migrating progenitor cells towards regions of neural damage. These include migration along: 1) myelinated fiber tracks; 2) radial processes; and 3) blood vessels [for review see (Cayre, *et al*., 2009)]. Besides these mechanisms, inflammation-induced chemoattraction plays a major role in progenitor cell migration following neural cell loss. Upon insult or infection, the brain exhibits a profound innate response, characterised predominantly by robust activation of microglia (resident macrophages of the CNS). Activated microglia play a dual role, scavenging the damaged and dying neurons as well as initiating a prompt local inflammatory reaction. The inflammatory response involves production of pro-inflammatory cytokines and chemokines, as well as various reactive nitrogen and oxygen

species. Cytokines released by microglia subsequently activate resident astrocytes, which again release cytokines. Peripheral macrophages are recruited into the brain by chemotaxis in response to a superfamily of cytokines called chemokines. Chemokines are small, secreted proteins that play crucial roles in leukocyte migration under normal conditions as well as during neuroinflammatory responses. Following injury to the adult brain, a range of cytokines and chemokines have been shown to be up-regulated in the region of neural cell death, including GRO-α, IL-8, IP-10, MCP-1, MCP-2, MIP-1α , RANTES , SDF-1α , and TNF-α (Mcmanus, *et al*., 1998, Das, *et al*., 2008, Gordon, *et al*., 2009, Whitney, *et al*., 2009). In addition, chemokine receptors, including CXCR1, CXCR2, CXCR4, CXCR7, CCR1, CCR2, CCR3 and CCR5, are widely expressed on neural progenitor cells (Ji, *et al*., 2004, Tran, *et al*., 2004, Gordon, *et al*., 2009). The expression of chemokine receptors on neural progenitor cells signifies the crucial roles played by chemokines in guiding progenitor cell migration and the influence these factors have in the recovery process in the injured CNS.

While a number of cytokines and chemokines involved in the inflammatory process have been demonstrated to play a role in directing progenitor cell migration, MCP-1 and SDF-1α and their receptors have been the most widely examined and clearly regulate the directed migration of endogenous neural progenitor cells from the SVZ to the injured brain following either ischemic or excitotoxic neural cell loss (Imitola, *et al*., 2004, Belmadani, *et al*., 2006, Robin, *et al*., 2006, Yan, *et al*., 2006, Gordon, *et al*., 2009). The SDF-1α receptors CXCR4 and CXCR7 are highly expressed on neural progenitor cells. SDF-1α expression is highly up-regulated in reactive astrocytes, microglia and endothelial cells in the ischemic striatum during several weeks after focal ischemic injury (Thored, *et al*., 2006) and has been shown to induce the migration of progenitor cells *in vitro* (Peng, *et al*., 2004) and *in vivo* to areas of hypoxic-ischemic-induced inflammation via CXCR4 signalling pathways (Imitola, *et al*., 2004, Robin, *et al*., 2006). The chemokine MCP-1 is also up-regulated in response to inflammation and induces the migration of neural progenitor cells. The MCP-1 receptor CCR2 is expressed by neural progenitor cells and MCP-1 recruits progenitor cells to the site of brain inflammation by binding to CCR2 and inducing their migration (Widera, *et al*., 2004, Belmadani, *et al*., 2006, Gordon, *et al*., 2009). These studies clearly indicate that neuroinflammation and the resulting expression of cytokines and chemokines play a major role in directing the migration of progenitor cells in the injured brain. However, inflammatory cues involved in directing the migration of progenitor cells can also contribute to decreased survival of these migrating cells, creating juxtaposition between regeneration and ongoing cell loss and highlighting the complexity of the neuroinflammatory environment: on one hand it is useful for attracting progenitor cells to the appropriate region for neural replacement, but on the other hand it prevents efficient cell replacement by affecting the survival abilities of the migrating precursor cells (Whitney, *et al*., 2009). The opposing properties of neuroinflammation therefore complicates the development of therapeutic strategies involving the use of cytokines or chemokines.

4. Therapeutic strategies

4.1 Chronic TLE

The major issue associated with chronic TLE is the observed reduction in hippocampal neurogenesis and potential contribution this plays to the persistence of spontaneous seizures, learning and memory deficits, and depression prevalent in chronic TLE. Based on studies in animal models of brain disease and injury, the following strategies may provide

mechanisms by which to increase hippocampal neurogenesis in chronic epilepsy; administration of neurotrophic factors, physical exercise, exposure to an enriched environment and antidepressant therapy [for review see (Kuruba, *et al.*, 2009)]. Administration of neurotrophic factors is relevant as many factors that promote neurogenesis (e.g: BDNF, FGF-2, IGF-1) are reduced in chronic epilepsy (Hattiangady, *et al.*, 2004, Shetty, *et al.*, 2004). Supporting this, a range of studies have demonstrated that administration of neurotrophic factors to both the normal and injured adult rodent brain can enhance hippocampal neurogenesis (Lichtenwalner, *et al.*, 2001, Yoshimura, *et al.*, 2001, Jin, *et al.*, 2003, Scharfman, *et al.*, 2005, Rai, *et al.*, 2007, Paradiso, *et al.*, 2009, Paradiso, *et al.*, 2011). Performing physical exercise and environmental enrichment have also been shown to enhance hippocampal neurogenesis, potentially through increased expression of a range of mitogenic factors such as BDNF, FGF2, NGF, IGF-1 and VEGF as well as phosphorylation of cAMP-response binding protein (CREB) (Nithianantharajah, *et al.*, 2006, Van Praag, 2008, Llorens-Martín, *et al.*, 2009, 2010, Lafenetre, *et al.*, 2011) and may provide an appealing non-invasive therapeutic approach for the treatment of chronic TLE (Dhanushkodi, *et al.*, 2008, Arida, *et al.*, 2009). Antidepressant therapy in chronic TLE is another interesting approach for increasing neurogenesis and reducing cognitive impairments, as antidepressant therapy enhances hippocampal neurogenesis probably via increases in levels of serotonin, noradrenaline, BDNF, CREB and a range of other mitogenic factors (Sahay, *et al.*, 2007, Thomas, *et al.*, 2008, Lanni, *et al.*, 2009). In particular, a recent study demonstrated that repeated administration of the antidepressant agent citalopram counteracted kainic acid-induced neuronal loss and dispersion of PSA-NCAM-positive cells within the granule cell layer of the hippocampus (Jaako, *et al.*, 2011). Citalopram also counteracted the downregulation of Reelin on both mRNA and protein levels. As decreased neurogenesis, cognitive impairment and depression coexist in chronic epilepsy, prolonged antidepressant treatment may provide an effective strategy for easing these problems.

4.2 Traumatic brain injury

For TBI, it remains unclear whether compensatory neurogenesis contributes at all to functional recovery. However, several studies have examined the effect of administering neurogenic agents to rodent models of TBI to assess the effect on neuronal replacement and functional recovery. Administration of EGF or FGF-2 into the lateral ventricles following FPI has been shown to increase the rate of memory recovery in the Morris Water Maze, and produce a concomitant increase in the number of new hippocampal neurons co-labeled with BrdU and NeuN (Sun, *et al.*, 2009, Sun, *et al.*, 2010). Interestingly, intraventricular administration of the calcium-binding protein S100β following TBI has also been shown to increase the percentage of newly generated hippocampal neurons expressing NeuN and improve cognitive recovery in the Morris water Maze [for review see (Kleindienst, *et al.*, 2007)]. This is in conflict with clinical data in which an increase in CSF levels of S100β is correlated with poor prognosis in patients with TBI. Delivery of VEGF to the lateral FPI model has been shown to significantly increase the number of BrdU labeled adult-born neurons in the adult hippocampus, but does not change the number of BrdU labeled newborn cells per se (Lee&Agoston, 2010) suggesting that in the hippocampus VEGF predominantly mediates survival of adult-born neurons rather than progenitor cell proliferation. In contrast, Thau-Zuchman and colleagues (Thau-Zuchman, *et al.*, 2010) observed an increase in the number of proliferating cells in the SVZ and the perilesion cortex following infusion of VEGF into the lateral ventricles of mice after TBI. Further, while

functional outcome was significantly improved in mice treated with VEGF compared to vehicle treated animals following TBI, fate analysis demonstrated that most newborn cells differentiated into astrocytes and oligodendroglia, and only a few cells differentiated into neurons (Thau-Zuchman, *et al.*, 2010).

The effect of mitogen support on hippocampal neurogenesis following TBI has also been examined using transgenic models. FGF-2(-/-) mice subjected to CCI injury exhibit a reduction in the number of both BrdU-positive cells and BrdU-positive neurons when compared to FGF-2(+/+) mice. In contrast, over-expression of FGF-2 by intracerebral injection of herpes simplex virus-1 amplicon vectors encoding for this factor increased both the number of dividing cells and BrdU-positive neurons (Yoshimura, *et al.*, 2003). This suggests that FGF-2 up-regulates neurogenesis and protects the survival of adult-born neurons in the adult hippocampus after TBI. BDNF has also been shown to play a role in regulating the survival of adult-born immature neurons in the hippocampus following TBI, with the level of adult-born immature neuron death in the dentate gyrus significantly increased in BDNF conditional knockout mice following TBI.

A number of studies have demonstrated that the injured brain can be stimulated to promote angiogenesis and neurogenesis, which are coupled restorative processes that contribute to functional recovery in both TBI and stroke [for review see (Xiong, *et al.*, 2010)]. Studies have demonstrated that intraperitoneal administration of EPO post-TBI significantly increases BDNF expression and enhances hippocampal neurogenesis with subsequent improvement in sensorimotor and spatial learning functions (Meng, *et al.*, Lu, *et al.*, 2005, Xiong, *et al.*, 2008, Xiong, *et al.*, 2010). Statins also show neurorestorative effects in animal models of TBI through the induction of angiogenesis and neurogenesis. Simvastatin treatment provides long-lasting (3 month) functional improvement after TBI in rats. This was coupled with increased expression of VEGF and BDNF and enhanced in the dentate gyrus of rats following TBI (Lu, *et al.*, 2007, Wu, *et al.*, 2008). Clinical trials investigating the use of either EPO or CEPO, or the use of statins for the treatment of TBI are currently being undertaken [for review see (Xiong, *et al.*, 2010)].

4.3 Stroke

A range of therapeutic strategies promoting regeneration in stroke are being investigated. However, some of the most interesting approaches are based around the use of statins and the phosphodiesterase type 5 (PDE5) inhibitors such as sildenafil (Xiong, *et al.*, 2010). Statins have been shown to induce angiogenesis, neurogenesis and synaptogenesis, and to enhance functional recovery after stroke in rats (Chen, *et al.*, 2003). It is thought that expression of BDNF, VEGF and VEGFR2, and regulation of Notch signaling activity contribute to these regenerative processes (Chen, *et al.*, 2005, Chen, *et al.*, 2008). Clinical trials for both lovastatin and simvastatin in stroke patients are currently being undertaken [for review see (Xiong, *et al.*, 2010)]. Given the wide use of statins, their favourable safety profile, rare serious adverse effects and the extensive preclinical data showing neuroprotection and neurorestoration in rodent models of stroke, further clinical studies investigating the potential use of statins to promote neuroregeneration following stroke are warranted. The PDE5 inhibitor sildenafil has also been shown to promote neurogenesis and reduce functional deficits when administered to rats either 2 or 24 hours after ischemic injury (Zhang, *et al.*, 2002), or for 7 consecutive days starting 7 days following focal ischemia (Zhang, *et al.*, 2006). Further, treatment of ischemic stroke with a long-acting PDE5 inhibitor tadalafil improves functional recovery, which is associated with increases in brain cGMP levels and enhanced

angiogenesis and neurogenesis (Zhang, *et al.*, 2006). Treatment of ischemic stroke with EPO is also under investigation, with additional studies examining the use of nonhematopoietic EPO analogues such as CEPO. As discussed in Section 4.2, EPO has been shown to promote both neurogenesis and angiogenesis, resulting in functional recovery in rodent models of focal ischemic injury. Clinical trials investigating the therapeutic application of EPO or CEPO for the treatment of stroke are currently being undertaken [for review see (Xiong, *et al.*, 2010)].

5. Conclusions

The presence of both neural and glial progenitor cells in the adult central nervous system (CNS), and the capacity of these cells to migrate through this mature structure to areas of pathological damage and injury raises hope for the development of new therapeutic strategies to treat brain injury. Although at present time the compensatory neurogenesis described after various types of brain injuries appears to be modest, the development of a strategy promoting the proliferation, directed mobilization and phenotypic induction of endogenous progenitor cells to areas of neural cell loss remains of high interest. However, the development of novel neuroregenerative strategies focusing on the promotion of compensatory adult neurogenesis will only be achieved once we fully understand the mechanisms promoting the response of endogenous progenitor cells to neural injury and cell loss. An important factor that needs to be addressed when investigating therapeutic strategies by which to enhance compensatory neurogenesis following brain injury is whether adult-born cells become functional and integrate appropriately into existing circuitry and contribute to the recovery process, or whether they just enhance or restore the functionality and survival of existing dysfunctional cells. While striving to identify potential factors or the redirected use of current pharmaceuticals to promote compensatory neurogenesis for the treatment of brain injury, caution must also be taken. Post-traumatic epilepsy is a fairly common morbidity associated with both stroke and TBI and one postulated mechanism for this is that aberrant neurogenesis serves as the epileptic focus (Parent, *et al.*, 2008). Therefore, any strategy aimed at enhancing neurogenesis may inadvertently result in this and other unwanted side effects. In addition, since many strategies aimed towards enhancing neurogenesis promote cell growth, it remains a possibility that increasing proliferation may result in potentially unwanted tumour growth. Thus, while enhancing compensatory neurogenesis for the treatment of brain injury remains an exciting and potentially revolutionary therapeutic strategy, many issues regarding specificity, mechanism and potential toxicity need to be thoroughly investigated before meaningful clinical intervention can occur.

6. References

Altar, CA, Laeng, P, Jurata, LW, *et al.*, (2004). Electroconvulsive seizures regulate gene expression of distinct neurotrophic signaling pathways. *The Journal of Neuroscience*. 24, pp. (2667-2677)

Altman, J & Das, GD. (1965). Autoradiographic and histological evidence of postnatal neurogenesis in rats. *Journal of Comparative Neurology*. 124, pp. (319-335),

Androutsellis-Theotokis, A, Leker, RR, Soldner, F, *et al.*, (2006). Notch signalling regulates stem cell numbers in vitro and in vivo. *Nature*. 442, pp. (823 -826).

Arida, RM, Scorza, FA, Scorza, CA, *et al.*, (2009). Is physical activity beneficial for recovery in temporal lobe epilepsy? Evidences from animal studies. *Neuroscience & Biobehavioral Reviews*. 33, pp. (422-431).

Arvidsson, A, Collin, T, Kirik, D, *et al.*, (2002). Neuronal replacement from endogenous precursors in the adult brain after stroke. *Nature Medicine*. 8, pp. (963-970),

Banerjee, SB, Rajendran, R, Dias, BG, *et al.*, (2005). Recruitment of the sonic hedgehog signalling cascade in electroconvulsive seizure-mediated regulation of adult rat hippocampal neurogenesis. *European Journal of Neuroscience*. 22, pp. (1570-1580).

Bath, KG & Lee, FS. (2010). Neurotrophic factor control of adult svz neurogenesis. *Developmental Neurobiology*. 70, pp. (339-349).

Belmadani, A, Tran, PB, Ren, D, *et al.*, (2006). Chemokines regulate the migration of neural progenitors to sites of neuroinflammation. *J. Neurosci.* 26, pp. (3182-3191),

Bengzon, J, Kokaia, Z, Elmer, E, *et al.*, (1997). Apoptosis and proliferation of dentate gyrus neurons after single and intermittent limbic seizures. *PNAS*. 94, pp. (10432 - 10437),

Cayre, M, Canoll, P & Goldman, JE. (2009). Cell migration in the normal and pathological postnatal mammalian brain. *Progress in Neurobiology*. 88, pp. (41-63).

Chan, JP, Cordeira, J, Calderon, GA, *et al.*, (2008). Depletion of central BDNF in mice impedes terminal differentiation of new granule neurons in the adult hippocampus. *Molecular and Cellular Neuroscience*. 39, pp. (372-383).

Chen, J, Zhang, ZG, Li, Y, *et al.*, (2003). Statins induce angiogensesis, neuogenesis and synaptogenesis after stroke. *Annals of Neurology*. 53, pp. (743-751)

Chen, J, Zhang, C, Jiang, H, *et al.*, (2005). Atorvastatin induction of VEGF and BDNF promotes brain plasticity after stroke in mice. *J Cereb Blood Flow Metab*. 25, pp. (281-290).

Chen, J, Zacharek, A, Li, A, *et al.*, (2008). Atorvastatin promotes presenilin-1 expression and notch1 activity and increases neural progenitor cell proliferation after stroke. *Stroke*. 39, pp. (220-226)

Chirumamilla, S, Sun, D, Bullock, MR, *et al.*, (2002). Traumatic brain injury induced cell proliferation in the adult mammalian central nervous system. *Journal of Neurotrauma*. 19, pp. (693-703)

Chou, J, Harvey, BK, Chang, C-F, *et al.*, (2006). Neuroregenerative effects of BMP7 after stroke in rats. *Journal of the Neurological Sciences*. 240, pp. (21-29)

Clelland, CD, Choi, M, Romberg, C, *et al.*, (2009). A functional role for adult hippocampal neurogenesis in spatial pattern separation. *Science*. 325, pp. (210-213)

Collin, T, Arvidsson, A, Kokaia, Z, *et al.*, (2005). Quantitative analysis of the generation of different striatal neuronal subtypes in the adult brain following excitotoxic injury. *Experimental Neurology*. 195, pp. (71-80).

Croll, S, Goodman, J & Scharfman, H. (2004). Vascular endothelial growth factor (VEGF) in seizures: A double-edged sword. *Adv Exp Med Biol*. 548, pp. (57-68)

Curtis, MA, Kam, M, Nannmark, U, *et al.*, (2007). Human neuroblasts migrate to the olfactory bulb via a lateral ventricular extension. *Science*. 315, pp. (1243-1249)

Das, S & Basu, A. (2008). Inflammation: A new candidate in modulating adult neurogenesis. *Journal of Neuroscience Research*. 86, pp. (1199-1208).

Dash, PK, Mach, SA & Moore, AN. (2001). Enhanced neurogenesis in the rodent hippocampus following traumatic brain injury. *Journal of Neuroscience Research*. 63, pp. (313-319).

Deisseroth, K, Singla, S, Toda, H, *et al.*, (2004). Excitation-neurogenesis coupling in adult neural stem/progenitor cells. *Neuron*. 42, pp. (535-552).

Dhanushkodi, A & Shetty, AK. (2008). Is exposure to enriched environment beneficial for functional post-lesional recovery in temporal lobe epilepsy? *Neuroscience & Biobehavioral Reviews*. 32, pp. (657-674).

Doetsch, F & Alvarez-Buylla, A. (1996). Network of tangential pathways for neuronal migration in adult mammalian brain. *PNAS*. 93, pp. (14895-14900)

Doetsch, F, Garcia-Verdugo, JM & Alvarez-Buylla, A. (1997). Cellular composition and three-dimensional organization of the subventricular germinal zone in the adult mammalian brain. *Journal of Neuroscience*. 17, pp. (5046-5061)

Doetsch, F, Caille, I, Lim, DA, *et al.*, (1999). Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. *Cell*. 97, pp. (703-716)

Dupret, D, Revest, J-M, Koehl, M, *et al.*, (2008). Spatial relational memory requires hippocampal adult neurogenesis. *PLoS ONE*. 3, pp. (e1959)

Emery, DL, Fulp, CT, Saatman, KE, *et al.*, (2005). Newly born granule cells in the dentate gyrus rapidly extend axons into the hippocampal CA3 region following experimental brain injury. *Journal of Neurotrauma*. 22, pp. (978-988)

Eriksson, PS, Perfilieva, E, Bjork-Eriksson, T, *et al.*, (1998). Neurogenesis in the adult human hippocampus. *Nature Medicine*. 4, pp. (1313-1317)

Gall, C, Berschauer, R & Isackson, P. (1994). Seizures increase basic fibroblast growth factor mRNA in adult rat forebrain neurons and glia. *Brain Research Molecular Brain Research*. 21, pp. (190-205)

Ge, S, Pradhan, DA, Ming, G-L, *et al.*, (2007). GABA sets the tempo for activity-dependent adult neurogenesis. *Trends in Neurosciences*. 30, pp. (1-8)

Gheusi, G, Cremer, H, Mclean, H, *et al.*, (2000). Importance of newly generated neurons in the adult olfactory bulb for odor discrimination. *PNAS*. 97, pp. (1823-1828)

Goings, GE, Wibisono, BL & Szele, FG. (2002). Cerebral cortex lesions decrease the number of bromodeoxyuridine-positive subventricular zone cells in mice. *Neuroscience Letters*. 329, pp. (161-164)

Goings, GE, Sahni, V & Szele, FG. (2004). Migration patterns of subventricular zone cells in adult mice change after cerebral cortex injury. *Brain Research*. 996, pp. (213-226)

Goldman, S. (2005). Stem and progenitor cell-based therapy of the human central nervous system. 23, pp. (862-871).

Gómez-Pinilla, F, Van Der Wal, EA & Cotman, CW. (1995). Possible coordinated gene expressions for FGF receptor, FGF-5, and FGF-2 following seizures. *Experimental Neurology*. 133, pp. (164-174).

Gong, C, Wang, T-W, Huang, HS, *et al.*, (2007). Reelin regulates neuronal progenitor migration in intact and epileptic hippocampus. *J. Neurosci.* 27, pp. (1803-1811)

Gonzalez-Perez, O, Quiñones-Hinojosa, A & Garcia-Verdugo, J. (2010). Immunological control of adult neural stem cells. *Journal of Stem Cells*. 5, pp. (23-31)

Gordon, RJ, Tattersfield, AS, Vazey, EM, *et al.*, (2007). Temporal profile of subventricular zone progenitor cell migration following quinolinic acid-induced striatal cell loss. *Neuroscience*. 146, pp. (1704-1718)

Gordon, RJ, Mcgregor, AL & Connor, B. (2009). Chemokines direct neural progenitor cell migration following striatal cell loss. *Molecular and Cellular Neuroscience*. 41, pp. (219-232).

Gould, E, Vail, N, Wagers, M, *et al.*, (2001). Adult-generated hippocampal and neocortical neurons in macaques have a transcient existence. *PNAS*. 98, pp. (10910-10917)

Gray, WP & Sundstron, LE. (1998). Kainic acid increases the proliferation of granule cell progenitors in the dentate gyrus of the adult rat. *Brain Research*. 790, pp. (52-59)

Guerra-Crespo, M, Sistos, A, Gleason, D, *et al.*, (2010). Intranasal administration of pegylated transforming growth factor-[alpha] improves behavioral deficits in a chronic stroke model. *Journal of Stroke and Cerebrovascular Diseases*. 19, pp. (3-9).

Hattiangady, B, Rao, MS & Shetty, AK. (2004). Chronic temporal lobe epilepsy is associated with severely declined dentate neurogenesis in the adult hippocampus. *Neurobiology of Disease*. 17, pp. (473-490).

Hattiangady, B & Shetty, AK. (2008). Implications of decreased hippocampal neurogenesis in chronic temporal lobe epilepsy. *Epilepsia*. 49, pp. (26-41).

Hattiangady, B & Shetty, AK. (2010). Decreased neuronal differentiation of newly generated cells underlies reduced hippocampal neurogenesis in chronic temporal lobe epilepsy. *Hippocampus*. 20, pp. (97-112).

Hicks, RR, Numan, S, Dhillon, HS, *et al.*, (1997). Alterations in BDNF and NT-3 mRNAs in rat hippocampus after experimental brain trauma. *Molecular Brain Research*. 48, pp. (401-406).

Hodge, RD & Hevner, RF. (2011). Expression and actions of transcription factors in adult hippocampal neurogenesis. *Developmental Neurobiology*. In press.

Houser, CR. (1990). Granule cell dispersion in the dentate gyrus of humans with temporal lobe epilepsy. *Brain Research*. 535, pp. (195-204).

Howell, OW, Scharfman, HE, Herzog, H, *et al.*, (2003). Neuropeptide Y is neuroproliferative for post-natal hippocampal precursor cells. *J Neurochem*. 86, pp. (646-659)

Howell, OW, Doyle, K, Goodman, JH, *et al.*, (2005). Neuropeptide Y stimulates neuronal precursor proliferation in the post-natal and adult dentate gyrus. *Journal of Neurochemistry*. 93, pp. (560-570).

Howell, OW, Silva, S, Scharfman, HE, *et al.*, (2007). Neuropeptide Y is important for basal and seizure-induced precursor cell proliferation in the hippocampus. *Neurobiology of Disease*. 26, pp. (174-188).

Imayoshi, I, Sakamoto, M, Ohtsuka, T, *et al.*, (2008). Roles of continuous neurogenesis in the structural and functional integrity of the adult forebrain. *Nat Neurosci*. 11, pp. (1153-1161).

Imitola, J, Raddassi, K, Park, KI, *et al.*, (2004). Directed migration of neural stem cells to sites of CNS injury by the stromal cell-derived factor 1 α/CXC chemokine receptor 4 pathway. *PNAS*. 101, pp. (18117-18122),

Iosif, RE, Ahlenius, H, Ekdahl, CT, *et al.*, (2008). Suppression of stroke-induced progenitor proliferation in adult subventricular zone by tumor necrosis factor receptor 1. *Journal of Cerebral Blood Flow,* 28, pp. (1574 - 1587).

Jaako, K, Aonurm-Helm, A, Kalda, A, *et al.*, (2011). Repeated citalopram administration counteracts kainic acid-induced spreading of PSA-NCAM-immunoreactive cells and loss of Reelin in the adult mouse hippocampus. *European Journal of Pharmacology*. In Press.

Jessberger, S, Römer, B, Babu, H, *et al.*, (2005). Seizures induce proliferation and dispersion of doublecortin-positive hippocampal progenitor cells. *Experimental Neurology*. 196, pp. (342-351).

Ji, JF, He, BP, Dheen, ST, *et al.*, (2004). Expression of chemokine receptors CXCR4, CCR2, CCR5 and CXC3R1 in neural progenitor cells isolated from the subventricular zone of the adult rat brain. *Neuroscience Letters*. 355, pp. (236-240)

Jin, K, Minami, M, Lan, JQ, *et al.*, (2001). Neurogenesis in dentate subgranular zone and rostral subventricular zone after focal ischemia in the rat. *PNAS*. 98, pp. (4710-4715)

Jin, K, Zhu, Y, Sun, Y, *et al.*, (2002). Vascular endothelial growth factor (VEGF) stimulates neurogenesis in vitro and in vivo. *PNAS*. 99, pp. (11946-11950)

Jin, K, Sun, Y, Xie, L, *et al.*, (2003). Neurogenesis and aging: FGF-2 and HB-EGF restore neurogenesis in hippocampus and subventricular zone of aged mice. *Aging Cell*. 2, pp. (175-183).

Jin, K, Sun, Y, Xie, L, *et al.*, (2003). Directed migration of neuronal precursors into the ischemic cerebral cortex and striatum. *Molecular and Cellular Neuroscience*. 24, pp. (171-189)

Jin, K, Wang, X, Xie, L, *et al.*, (2006). Evidence for stroke-induced neurogenesis in the human brain. *PNAS*. pp. (0603512103)

Jones, KS & Connor, B. (2011). Proneural transcription factors Dlx2 and Pax6 are altered in adult SVZ neural precursor cells following striatal cell loss. *Molecular and Cellular Neuroscience*. 47, pp. (53-60).

Kaplan, MS & Hinds, JW. (1977). Neurogenesis in the adult rat: Electron microscopic analysis of light radioautographs. *Science*. 197, pp. (1092-1094)

Kleindienst, A, Hesse, F, Bullock, MR, *et al.*, T. W. John and I. R. M. Andrew. (2007). The neurotrophic protein s100b: Value as a marker of brain damage and possible therapeutic implications. *Progress in brain research*. Volume 161, pp. (317-325).

Kobayashi, T, Ahlenius, H, Thored, P, *et al.*, (2006). Intracerebral infusion of glial cell line-derived neurotrophic factor promotes striatal neurogenesis after stroke in adult rats. *Stroke*. 37, pp. (2361-2367)

Kolb, B, Morshead, C, Gonzalez, C, *et al.*, (2006). Growth factor-stimulated generation of new cortical tissue and functional recovery after stroke damage to the motor cortex of rats. *J Cereb Blood Flow Metab*. 27, pp. (983-997).

Kornack, DR & Rakic, P. (1999). Continuation of neurogenesis in the hippocampus of the adult macaque monkey. *PNAS*. 96, pp. (5768-5773)

Kuruba, R, Hattiangady, B & Shetty, AK. (2009). Hippocampal neurogenesis and neural stem cells in temporal lobe epilepsy. *Epilepsy & Behavior*. 14, pp. (65-73).

Lafenetre, P, Leske, O, Wahle, P, *et al.*, (2011). The beneficial effects of physical activity on impaired adult neurogenesis and cognitive performance. *Frontiers in Neuroscience*. 5, pp. 51.

Lagace, DC, Whitman, MC, Noonan, MA, *et al.*, (2007). Dynamic contribution of nestin-expressing stem cells to adult neurogenesis. *J. Neurosci.* 27, pp. (12623-12629)

Lanni, C, Govoni, S, Lucchelli, A, *et al.*, (2009). Depression and antidepressants: Molecular and cellular aspects. *Cellular and Molecular Life Sciences*. 66, pp. (2985-3008).

Lee, C & Agoston, DV. (2010). Vascular endothelial growth factor is involved in mediating increased de novo hippocampal neurogenesis in response to traumatic brain injury. *Journal of Neurotrauma*. 27, pp. (541-553)

Leker, R, Lasri, V & Chernoguz, D. (2009). Growth factors improve neurogenesis and outcome after focal cerebral ischemia. *Journal of Neural Transmission*. 116, pp. (1397-1402).

Leker, RR, Toth, ZE, Shahar, T, *et al.*, (2009). Transforming growth factor [alpha] induces angiogenesis and neurogenesis following stroke. *Neuroscience*. 163, pp. (233-243).

Lichtenwalner, RJ, Forbes, ME, Bennett, SA, *et al.*, (2001). Intracerebroventricular infusion of insulin-like growth factor-i ameliorates the age-related decline in hippocampal neurogenesis. *Neuroscience*. 107, pp. (603-613).

Lie, DC, Song, H, Colamarino, SA, *et al.*, (2004). Neurogenesis in the adult brain: New strategies for central nervous system diseases. *Annual Review of Pharmacology and Toxicology*. 44, pp. (399-421)

Liu, F, You, Y, Li, X, *et al.*, (2009). Brain injury does not alter the intrinsic differentiation potential of adult neuroblasts. *J. Neurosci.* 29, pp. (5075-5087)

Liu, YWJ, Curtis, MA, Gibbons, HM, *et al.*, (2008). Doublecortin expression in the normal and epileptic adult human brain. *European Journal of Neuroscience*. 28, pp. (2254-2265).

Lledo, P-M, Merkle, FT & Alvarez-Buylla, A. (2008). Origin and function of olfactory bulb interneuron diversity. *Trends in Neurosciences*. 31, pp. (392-400).

Llorens-Martín, M, Torres-Alemán, I & Trejo, JL. (2009). Reviews: Mechanisms mediating brain plasticity: IGF1 and adult hippocampal neurogenesis. *The Neuroscientist*. 15, pp. (134-148)

Llorens-Martín, M, Torres-Alemán, I & Trejo, JL. (2010). Exercise modulates insulin-like growth factor 1-dependent and -independent effects on adult hippocampal neurogenesis and behaviour. *Molecular and Cellular Neuroscience*. 44, pp. (109-117).

Lois, C & Alvarez-Buylla, A. (1994). Long-distance neuronal migration in the adult mammalian brain. *Science*. 264, pp. (1145-1147)

Lois, C, Garcia-Verdugo, J-M & Alvarez-Buylla, A. (1996). Chain migration of neuronal precursors. *Science*. 271, pp. (978-981)

Lowenstein, DH, Seren, MS & Longo, FM. (1993). Prolonged increases in neurotrophic activity associated with kainate-induced hippocampal synaptic reorganization. *Neuroscience*. 56, pp. (597-604).

Lu, D, Mahmood, A, Qu, C, *et al.*, (2005). Erythropoietin enhances neurogenesis and restores spatial memory in rats after traumatic brain injury. *Journal of Neurotrauma*. 22, pp. (1011-1017)

Lu, D, Qu, C, Goussev, A, *et al.*, (2007). Statins increase neurogenesis in the dentate gyrus, reduce delayed neuronal death in the hippocampal CA3 region, and improve spatial learning in rat after traumatic brain injury. *Journal of Neurotrauma*. 24, pp. (1132-1146)

Lu, K-T, Sun, C-L, Wo, PYY, *et al.*, (2011). Hippocampal neurogenesis after traumatic brain injury is mediated by vascular endothelial growth factor receptor-2 and the RAF/MEK/ERK cascade. *Journal of Neurotrauma*. 28, pp. (441-450)

Luo, Y. (2011). Cell-based therapy for stroke. *Journal of Neural Transmission*. 118, pp. (61-74).

Luskin, MB. (1993). Restricted proliferation and migration of postnatally generated neurons derived from the forebrain subventricular zone. *Neuron*. 11, pp. (173-189)

Mcmanus, C, Berman, JW, Brett, FM, *et al.*, (1998). MPC-1, MCP-2 and MCP-3 expression in multiple sclerosis lesions: An immunohistochemical and in situ hybridization study. *Journal of Neuroimmunology*. 86, pp. (20-29).

Meng, Y, Xiong, Y, Mahmood, A, *et al.*, Dose-dependent neurorestorative effects of delayed treatment of traumatic brain injury with recombinant human erythropoietin in rats. *Journal of Neurosurgery*. 0, pp. (1-11)

Miles, DK & Kernie, SG. (2008). Hypoxic-ischemic brain injury activates early hippocampal stem/progenitor cells to replace vulnerable neuroblasts. *Hippocampus*. 18, pp. (793-806).

Nakatomi, H, Kuriu, T, Okabe, S, *et al.*, (2002). Regeneration of hippocampal pyramidal neurons after ischemic brain injury by recruitment of endogenous neural progenitors. *Cell*. 110, pp. (429-441)

Ninomiya, M, Yamashita, T, Araki, N, *et al.*, (2006). Enhanced neurogenesis in the ischemic striatum following EGF-induced expansion of transit-amplifying cells in the subventricular zone. *Neuroscience Letters*. 403, pp. (63-67)

Nithianantharajah, J & Hannan, AJ. (2006). Enriched environments, experience-dependent plasticity and disorders of the nervous system. *Nat Rev Neurosci*. 7, pp. (697-709)

Ohab, JJ, Fleming, S, Blesch, A, *et al.*, (2006). A neurovascular niche for neurogenesis after stroke. *J. Neurosci.* 26, pp. (13007-13016)

Oyesiku, NM, Evans, C-O, Houston, S, *et al.*, (1999). Regional changes in the expression of neurotrophic factors and their receptors following acute traumatic brain injury in the adult rat brain. *Brain Research*. 833, pp. (161-172).

Palmer, TD, Willhoite, AR & Gage, F. (2000). Vascular niche for adult hippocampal neurogenesis. *The Journal of Comparative Neurology*. 425, pp. (479-494).

Paradiso, B, Marconi, P, Zucchini, S, *et al.*, (2009). Localized delivery of fibroblast growth factor–2 and brain-derived neurotrophic factor reduces spontaneous seizures in an epilepsy model. *PNAS* 106, pp. (7191-7196)

Paradiso, B, Zucchini, S, Su, T, *et al.*, (2011). Localized overexpression of FGF-2 and BDNF in hippocampus reduces mossy fiber sprouting and spontaneous seizures up to 4 weeks after pilocarpine-induced status epilepticus. *Epilepsia*. 52, pp. (572-578).

Parent, JM, Yu, TW, Leibowitz, RT, *et al.*, (1997). Dentate granule cell neurogenesis is increased by seizures and contributes to aberrant network reorganization in the adult rat hippocampus. *Journal of Neuroscience*. 17, pp. (3727-3738)

Parent, JM, Janumpalli, S, Mcnamara, JO, *et al.*, (1998). Increased dentate granule cell neurogenesis following amygdala kindling in the adult rat. *Neuroscience Letters*. 247, pp. (9-12)

Parent, JM, Valentin, VV & Lowenstein, DH. (2002). Prolonged seizures increase proliferating neuroblasts in the adult rat subventricular zone-olfactory bulb pathway. *J. Neurosci.* 22, pp. (3174-3188),

Parent, JM, Vexler, ZS, Gong, C, *et al.*, (2002). Rat forebrain neurogenesis and striatal neuron replacement after focal stroke. *Annals of Neurology*. 52, pp. (802-813)

Parent, JM. (2003). Injury-induced neurogenesis in the adult mammalian brain. *The Neuroscientist*. 9, pp. (261-272)

Parent, JM, Elliott, RC, Pleasure, SJ, *et al.*, (2006). Aberrant seizure-induced neurogenesis in experimental temporal lobe epilepsy. *Annals of Neurology*. 59, pp. (81-91).

Parent, JM & Murphy, GG. (2008). Mechanisms and functional significance of aberrant seizure-induced hippocampal neurogenesis. *Epilepsia*. 49, pp. (19-25).

Peng, H, Huang, Y, Rose, J, *et al.*, (2004). Stromal cell-derived factor 1-mediated CXCR4 signaling in rat and human cortical neural progenitor cells. *Journal of Neuroscience Research*. 76, pp. (35-50)

Peterson, DA. (2002). Stem cells in brain plasticity and repair. *Current Opinion in Pharmacology*. 2, pp. (34-42)

Petreanu, L & Alvarez-Buylla, A. (2002). Maturation and death of adult-born olfactory bulb granule neurons: Role of olfaction. *The Journal of Neuroscience*. 22, pp. (6106-6113)

Plane, JM, Whitney, JT, Schallert, T, *et al.*, (2008). Retinoic acid and environmental enrichment alter subventricular zone and striatal neurogenesis after stroke. *Experimental Neurology*. 214, pp. (125-134).

Rai, KS, Hattiangady, B & Shetty, AK. (2007). Enhanced production and dendritic growth of new dentate granule cells in the middle-aged hippocampus following intracerebroventricular FGF-2 infusions. *European Journal of Neuroscience*. 26, pp. (1765-1779).

Ramaswamy, S, Goings, GE, Soderstrom, KE, *et al.*, (2005). Cellular proliferation and migration following a controlled cortical impact in the mouse. *Brain Research*. 1053, pp. (38-53).

Reynolds, BA & Weiss, S. (1992). Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science*. 255, pp. (1707-1710)

Reynolds, BA & Weiss, S. (1996). Clonal and population analyses demonstrate that an EGF-responsive mammalian embryonic cns precursor is a stem cell. *Developmental Biology*. 175, pp. (1-13)

Riva, MA, Donati, E, Tascedda, F, *et al.*, (1994). Short- and long-term induction of basic fibroblast growth factor gene expression in rat central nervous system following kainate injection. *Neuroscience*. 59, pp. (55-65).

Robin, AM, Zhang, ZG, Wang, L, *et al.*, (2006). Stromal cell-derived factor 1a mediates neural progenitor cell motility after focal cerebral ischemia. *Journal of Cerebral Blood Flow & Metabolism*. 26, pp. (125-134)

Rochefort, C, Gheusi, G, Vincent, J-D, *et al.*, (2002). Enriched odor exposure increases the number of newborn neurons in the adult olfactory bulb and improves odor memory. *The Journal of Neuroscience*. 22, pp. (2679-2689)

Rodrigo, C, Zaben, M, Lawrence, T, *et al.*, (2010). NPY augments the proliferative effect of FGF2 and increases the expression of FGFR1 on nestin positive postnatal hippocampal precursor cells, via the Y1 receptor. *Journal of Neurochemistry*. 113, pp. (615-627).

Sahay, A, Drew, MR & Hen, R. E. S. Helen. (2007). Dentate gyrus neurogenesis and depression. *Progress in brain research*. Volume 163, pp. (697-722, 822).

Schabitz, W-R, Steigleder, T, Cooper-Kuhn, CM, *et al.*, (2007). Intravenous brain-derived neurotrophic factor enhances poststroke sensorimotor recovery and stimulates neurogenesis. *Stroke*. 38, pp. (2165-2172)

Schänzer, A, Wachs, F, Wilhelm, D, *et al.*, (2004). Direct stimulation of adult neural stem cells in vitro and neurogenesis in vivo by vascular endothelial growth factor. *Brain Pathology*. 14, pp. (237-248)

Scharfman, H, Goodman, J, Macleod, A, *et al.*, (2005). Increased neurogenesis and the ectopic granule cells after intrahippocampal bdnf infusion in adult rats. *Experimental Neurology*. 192, pp. (348-356).

Scharfman, HE, Goodman, JH & Sollas, AL. (2000). Granule-like neurons at the hilar/CA3 border after status epilepticus and their synchrony with area CA3 pyramidal cells: Functional implications of seizure-induced neurogenesis. *The Journal of Neuroscience*. 20, pp. (6144-6158)

Scharfman, HE, Sollas, AL, Smith, KL, *et al.*, (2002). Structural and functional asymmetry in the normal and epileptic rat dentate gyrus. *The Journal of Comparative Neurology*. 454, pp. (424-439).

Scharfman, HE, Sollas, AE, Berger, RE, *et al.*, (2003). Perforant path activation of ectopic granule cells that are born after pilocarpine-induced seizures. *Neuroscience*. 121, pp. (1017-1029).

Seri, B, Garcia-Verdugo, JM, Mcewen, BS, *et al.*, (2001). Astrocytes give rise to new neurons in the adult mammalian hippocampus. *Journal of Neuroscience*. 21, pp. (7153-7160)

Shah, SA, Prough, DS, Garcia, JM, *et al.*, (2006). Molecular correlates of age-specific responses to traumatic brain injury in mice. *Experimental Gerontology*. 41, pp. (1201-1205).

Shetty, AK, Zaman, V & Shetty, GA. (2003). Hippocampal neurotrophin levels in a kainate model of temporal lobe epilepsy: A lack of correlation between brain-derived neurotrophic factor content and progression of aberrant dentate mossy fiber sprouting. *Journal of Neurochemistry*. 87, pp. (147-159).

Shetty, AK, Rao, MS, Hattiangady, B, *et al.*, (2004). Hippocampal neurotrophin levels after injury: Relationship to the age of the hippocampus at the time of injury. *Journal of Neuroscience Research*. 78, pp. (520-532).

Shingo, T, Sorokan, ST, Shimazaki, T, *et al.*, (2001). Erythropoietin regulates the in vitro and in vivo production of neuronal progenitors by mammalian forebrain neural stem cells. *Journal of Neuroscience*. 21, pp. (9733-9743)

Sims, JR, Lee, S-W, Topalkara, K, *et al.*, (2009). Sonic hedgehog regulates ischemia/hypoxia-induced neural progenitor proliferation. *Stroke*. 40, pp. (3618-3626)

Sköld, MK, Gertten, CV, Sandbergnordqvist, A-C, *et al.*, (2005). VEGF and vEGF receptor expression after experimental brain contusion in rat. *Journal of Neurotrauma*. 22, pp. (353-367)

Song, H-J, Stevens, CF & Gage, FH. (2002). Neural stem cells from adult hippocampus develop essential properties of functional cns neurons. *Nature Neuroscience*. 5, pp. (438-445)

Sun, D, Colello, RJ, Daugherty, WP, *et al.*, (2005). Cell proliferation and neuronal differentiation in the dentate gyrus in juvenile and adult rats following traumatic brain injury. *Journal of Neurotrauma*. 22, pp. (95-105)

Sun, D, Bullock, MR, Mcginn, MJ, *et al.*, (2009). Basic fibroblast growth factor-enhanced neurogenesis contributes to cognitive recovery in rats following traumatic brain injury. *Experimental Neurology*. 216, pp. (56-65).

Sun, D, Bullock, MR, Altememi, N, *et al.*, (2010). The effect of epidermal growth factor in the injured brain after trauma in rats. *Journal of Neurotrauma*. 27, pp. (923-938)

Sun, Y, Jin, K, Xie, L, *et al.*, (2003). VEGF-induced neuroprotection, neurogenesis, and angiogenesis after focal cerebral ischemia. *The Journal of Clinical Investigation*. 111, pp. (1843-1851).

Tattersfield, AS, Croon, RJ, Liu, YW, *et al.*, (2004). Neurogenesis in the striatum of the quinolinic acid lesion model of huntington's disease. *Neuroscience*. 127, pp. (319-332)

Thau-Zuchman, O, Shohami, E, Alexandrovich, AG, *et al.*, (2010). Vascular endothelial growth factor increases neurogenesis after traumatic brain injury. *J Cereb Blood Flow Metab*. 30, pp. (1008-1016).

Thom, M, Sisodiya, SM, Beckett, A, *et al.*, (2002). Cytoarchitectural abnormalities in hippocampal sclerosis. *Journal of Neuropathology & Experimental Neurology*. 61, pp. (510-519).

Thomas, LB, Gates, MA & Steindler, DA. (1996). Young neurons from the adult subependymal zone proliferate and migrate along an astrocyte, extracellular martix-rich pathway. *Glia*. 17, pp. (1-14)

Thomas, RM & Peterson, DA. (2008). Even neural stem cells get the blues: Evidence of a molecular link between modulation of adult neurogenesis and depression. *Gene Expression*. 14, pp. (1-100)

Thored, P, Arvidsson, A, Cacci, E, *et al.*, (2006). Persistent production of neurons from adult brain stem cells during recovery after stroke. *Stem Cells*. 24, pp. (739-747)

Tran, PB, Ren, D, Veldhouse, TJ, *et al.*, (2004). Chemokine receptors are expressed widely by embryonic and adult neural progenitor cells. *Journal of Neuroscience Research*. 76, pp. (20-34)

Truettner, J, Schmidt-Kastner, R, Busto, R, *et al.*, (1999). Expression of brain-derived neurotrophic factor, nerve growth factor, and heat shock protein hsp70 following fluid percussion brain injury in rats. *Journal of Neurotrauma*. 16, pp. (471-486)

Van Praag, H, Kempermann, G & Gage, FH. (1999). Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nature Neuroscience*. 2, pp. (266 - 270)

Van Praag, H, Schinder, AF, Christie, BR, *et al.*, (2002). Functional neurogenesis in the adult hippocampus. *Nature*. 415, pp. (1030-1034)

Van Praag, H. (2008). Neurogenesis and exercise: Past and future directions. *NeuroMolecular Medicine*. 10, pp. (128-140).

Wang, H-C & Ma, Y-B. (2010). Experimental models of traumatic axonal injury. *Journal of Clinical Neuroscience*. 17, pp. (157-162).

Wang, L-P, Kempermann, G & Kettenmann, H. (2005). A subpopulation of precursor cells in the mouse dentate gyrus receives synaptic gabaergic input. *Molecular and Cellular Neuroscience*. 29, pp. (181-189).

Wang, X, Mao, X, Xie, L, *et al.*, (2009). Involvement of notch1 signaling in neurogenesis in the subventricular zone of normal and ischemic rat brain in vivo. *J Cereb Blood Flow Metab*. 29, pp. (1644-1654).

Wang, Y-Q, Cui, H-R, Yang, S-Z, *et al.*, (2009). VEGF enhance cortical newborn neurons and their neurite development in adult rat brain after cerebral ischemia. *Neurochemistry International*. 55, pp. (629-636).

Whitman, MC & Greer, CA. (2009). Adult neurogenesis and the olfactory system. *Progress in Neurobiology*. 89, pp. (162-175).

Whitney, NP, Eidem, TM, Peng, H, *et al.*, (2009). Inflammation mediates varying effects in neurogenesis: Relevance to the pathogenesis of brain injury and neurodegenerative disorders. *Journal of Neurochemistry*. 108, pp. (1343-1359)

Widera, D, Holtkamp, W, Entschladen, F, *et al.*, (2004). MCP-1 induces migration of adult neural stem cells. *European Journal of Cell Biology*. 83, pp. (381-387)

Wu, H, Lu, D, Jiang, H, *et al.*, (2008). Simvastatin-mediated upregulation of VEGF and BDNF, activation of the PI3K/AKT pathway, and increase of neurogenesis are associated with therapeutic improvement after traumatic brain injury. *Journal of Neurotrauma*. 25, pp. (130-139)

Xiong, Y, Mahmood, A, Lu, D, *et al.*, (2008). Histological and functional outcomes after traumatic brain injury in mice null for the erythropoietin receptor in the central nervous system. *Brain Research*. 1230, pp. (247-257).

Xiong, Y, Mahmood, A & Chopp, M. (2010). Angiogenesis, neurogenesis and brain recovery of function following injury. *Curr Opin Investig Drugs*. 11, pp. (298-308)

Xiong, Y, Mahmood, A, Meng, Y, *et al.*, (2010). Delayed administration of erythropoietin reducing hippocampal cell loss, enhancing angiogenesis and neurogenesis, and improving functional outcome following traumatic brain injury in rats: Comparison of treatment with single and triple dose. *Journal of Neurosurgery*. 113, pp. (598-608)

Yamashita, T, Ninomiya, M, Hernandez Acosta, P, *et al.*, (2006). Subventricular zone-derived neuroblasts migrate and differentiate into mature neurons in the post-stroke adult striatum. *J. Neurosci.* 26, pp. (6627-6636)

Yan, Y-P, Sailor, KA, Lang, BT, *et al.*, (2006). Monocyte chemoattractant protein-1 plays a critical role in neuroblast migration after focal cerebral ischemia. *J Cereb Blood Flow Metab*. 27, pp. (1213-1224).

Yan, Y-P, Sailor, KA, Vemuganti, R, *et al.*, (2006). Insulin-like growth factor-1 is an endogenous mediator of focal ischemia-induced neural progenitor proliferation. *European Journal of Neuroscience*. 24, pp. (45-54).

Yang, K, Perez-Polo, J, Mu, X, *et al.*, (1996). Increased expression of brain-derived neurotrophic factor but not neurotrophin-3 mRNA in rat brain after cortical impact injury. *Journal of Neuroscience Research.* 44, pp. (157-164)

Yang, X-T, Bi, Y-Y & Feng, D-F. (2011). From the vascular microenvironment to neurogenesis. *Brain Research Bulletin*. 84, pp. (1-7).

Yoshimura, S, Takagi, Y, Harada, J, *et al.*, (2001). FGF-2 regulation of neurogenesis in adult hippocampus after brain injury. *PNAS.* 98, pp. (5874-5879)

Yoshimura, S, Teramoto, T, Whalen, MJ, *et al.*, (2003). FGF-2 regulates neurogenesis and degeneration in the dentate gyrus after traumatic brain injury in mice. *The Journal of Clinical Investigation*. 112, pp. (1202-1210).

Yu, T-S, Zhang, G, Liebl, DJ, *et al.*, (2008). Traumatic brain injury-induced hippocampal neurogenesis requires activation of early nestin-expressing progenitors. *The Journal of Neuroscience*. 28, pp. (12901-12912)

Zhang, L, Zhang, Z, Zhang, RL, *et al.*, (2006). Tadalafil, a long-acting type 5 phosphodiesterase isoenzyme inhibitor, improves neurological functional recovery in a rat model of embolic stroke. *Brain Research*. 1118, pp. (192-198).

Zhang, R, Wang, Y, Zhang, L, *et al.*, (2002). Sildenafil (viagra) induces neurogenesis and promotes functional recovery after stroke in rats. *Stroke*. 33, pp. (2675-2680)

Zhang, RL, Zhang, ZG, Zhang, L, *et al.*, (2001). Proliferation and differentiation of progenitor cells in the cortex and the subventricular zone in the adult rat after focal cerebral ischemia. *Neuroscience*. 105, pp. (33-41).

Zhang, RL, Zhang, Z, Zhang, L, *et al*., (2006). Delayed treatment with sildenafil enhances neurogenesis and improves functional recovery in aged rats after focal cerebral ischemia. *Journal of Neuroscience Research*. 83, pp. (1213-1219).

Zhang, RL, Zhang, ZG & Chopp, M. (2008). Ischemic stroke and neurogenesis in the subventricular zone. *Neuropharmacology*. 55, pp. (345-352).

Zhang, RL, Chopp, M, Gregg, SR, *et al*., (2009). Patterns and dynamics of subventricular zone neuroblast migration in the ischemic striatum of the adult mouse. *J Cereb Blood Flow Metab*. 29, pp. (1240 - 1250).

Zheng, W, Zhuge, Q, Zhong, M, *et al*., (2011). Neurogenesis in adult human brain after traumatic brain injury. *Journal of Neurotrauma*. In press,

Zheng, X-R, Zhang, S-S, Yang, Y-J, *et al*., (2010). Adenoviral vector-mediated transduction of VEGF improves neural functional recovery after hypoxia-ischemic brain damage in neonatal rats. *Brain Research Bulletin*. 81, pp. (372-377).

5

The Effects of Melatonin on Brain Injury in Acute Organophosphate Toxicity

Aysegul Bayir
Department of Emergency Medicine, Meram Faculty of Medicine
Selçuk University Konya
Turkey

1. Introduction

Organophosphates (OP) are potent toxic substances used in agriculture as insecticide and pesticides, and in warfare. Over 200,000 cases of accidental toxic exposure to OPs are reported annually (Jyaratnam, 1999).

OPs inhibit acetylcholine esterase (ACE), an enzyme which breaks down acetylcholine in cholinergic synapses in the peripheral nervous system (PNS) and central nervous system (CNS). Thus OP intoxication is characterized by findings related to hyperstimulation of cholinergic synapses in the PNS and CNS. Hyper-stimulation of cholinergic synapses in CNS may result in rapid blackout attacks and inhibition of respiratory center in medulla oblongata (Marrs, 2007). In animal studies of OPs used as chemical warfare agents, status epilepticus occurs rapidly due to severe brain damage, which is demonstrated on both electrophysiologic and histopathologic studies (McDonough et al, 1998). Pharmacological treatment of OP intoxication includes anticholinergic agents like atropine sulfate to block postsynaptic cholinergic receptors, oximes to reactivate inhibited enzymes, and antiepileptics to control seizure activity (Marrs, 2007).

In previous studies, oxidative stress caused by OPs was demonstrated in humans and rats. Lipid peroxidation in rat brain and human erythrocytes caused by OPs was confirmed as well (Abdollahi et al, 2004). Melatonin removes the potent hydroxyl radical secreted from pineal gland. Blood can easily pass the brain barrier and provides oxidative protection in the brain. At the same time, it also removes other reactive molecules such as hydrogen peroxide, singlet oxygen, peroxynitrite, and nitric oxide. Melatonin decreases oxidative stress by increasing the production of antioxidant enzymes like melatonin superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), the most important protective substance in the brain (Hsu et al, 2002).

The aim of this study was to investigate the effects of melatonin on lipid peroxidation in erythrocytes and brain tissue in the setting of acute OP intoxication in rats and compare its effects with those of routine treatment (pralidoxime and atropine).

2. Materials and methods

2.1 Experimental methods

The study was carried out in the Experimental Medicine and Research Center at Selçuk University after being approved by the Ethical Board of the Experimental Medicine and

Research Center. Twenty (12 male, 8 female, weight range 2500-4000 g) New Zealand rabbits were used. The subjects were divided into three groups: a sham group (n=8), a pralidoxime (PAM) plus atropine group (n=6), and a melatonin plus PAM plus atropine group (n=6). Subjects were anaesthetized with 50 mg/kg IM ketamine and 15 mg/kg IM xylazine HCL. The central ear artery and marginal ear vein were catheterized. Blood was drawn in EDTA tubes to measure baseline plasma ACE, nitric oxide (NO), and plasma and erythrocyte malondialdehyde (MDA).

Orogastric feeding tubes were inserted and 50 mg/kg (LD_{50}=50 mg/kg) dichlorvos was administered. One hour later, when signs of toxicity (hypersalivation, bronchospasm, fasciculations, convulsions) appeared, venous blood samples were taken again in order to measure plasma ACE, nitric oxide (NO), and plasma and erythrocyte MDA.

In the sham group, no treatment was given. Venous blood samples were taken at 12 hours after OP administration hour to measure plasma ACE, nitric oxide (NO), and plasma and erythrocyte MDA. In the PAM+atropine group, 0.05 mg/kg IV atropine was given and this dose was administered again as needed. In addition, a 30 mg/kg IV bolus of PAM was given, then 15 mg/kg IV PAM was given every 4 hours. In the melatonin plus PAM-atropine group, 10 mg/kg IV melatonin was administered, as well as PAM and atropine as in the PAM-atropine group. Blood samples were taken from the subjects in PAM-atropine and melatonin-PAM-atropine groups at 12 and 24 hours after intoxication in order to measure plasma ACE, nitric oxide (NO), and plasma and erythrocyte MDA.

At 24 hours post-intoxication, craniotomy was performed and liver samples were taken after laparotomy to evaluate ACE, NO and MDA levels. At the end of the study, subjects were sacrificed by administering a high dose of ketamine.

2.2 Biochemical methods

2.2.1 Measurement of plasma ACE activity

Plasma was separated from erythrocytes by centrifuging for 15 minutes at 3000 rpm. The following were placed into a 10 mL test tube: 3 mL of distilled water, 0.2 mL of plasma, and 3 mL of barbital phosphate (pH 8.1). The pH (pH_1) of the mixture was measured with a glass electrode pH meter. Then, 0.1 ml of 7.5% acetylcholine iodide solution was added to the reaction mixture and incubated for 20 min at 37° C. At the end of incubation period, the pH of the reaction mixture was measured (pH_2). ACE activity was calculated using the following formula:

ACE activity (ΔpH/20 minutes) = $pH_1 - pH_2 - (\Delta$ pH of the blank)

2.2.2 Measurement of ACE activity in brain tissue

To measure brain ACE activity, a brain tissue sample was homogenized (at 25% of the maximum speed) in barbital phosphate (pH 8.1) to weigh 3 ml/100 mg when wet. Homogenization was performed in an ice bath and brain homogenate was preserved in ice before cholinesterase determination. For determining brain ACE activity, 0.2 mL of tissue homogenate was used. ACE activity was calculated using the same formula as shown above.

2.2.3 Measurement of NO in plasma and brain tissue

To measure NO in plasma and brain tissue homogenate, the Nitric Oxide Synthase Assay Kit (Colorimetric) (Merck Chemicals, Darmstadt, Germany) was used.

2.2.4 Measurement of MDA in brain tissue

A brain tissue sample (0.5 g) was taken and preserved at -80° C. Using a cold 150 mM KCL solution, the tissue sample was homogenized to make a 10% homogenate, and was then centrifuged at 10,000 rpm for 10 minutes. The following substances were mixed: 0.1 ml of the homogenate, 0.2 mL of 8.1% sodium dodecyl sulfate (SDS) solution, 1.5 mL of 20% acetic acid solution (sodium hydroxide was added to this mixture for adjust pH>3), and 1.5 ml of 0.8% thiobarbituric acid liquid; this mixture was then stirred with a vortex. The mixture was then boiled in distilled water at 95° C for 60 minutes. Then it was cooled, and the following were added to the mixture: 1 mL of distilled water, 5 mL of n-butanol and pyridine (15:l, v/v) were added, and the mixture was rinsed. The resulting mixture was spun at 4,000 rpm for 10 minutes. A sample was from the upper layer of the mixture was taken, and absorbance at 532 nm was measured spectrophotometrically. MDA concentrations were derived with the following formula:

C = Measured absorbance x 320.5 x dilution factor / microprotein of homogenate= nmol/mg tissue

2.2.4 Measurement of MDA in erythrocytes

Blood was centrifuged and the plasma was separated. After being washed with normal saline solution once, 1.5 mL was taken from the erythrocyte plug and 1.5 mL of buffered sodium azide was added. 50 mL was taken from this hemolizate and 12.5 mL of Drabkin solution was added and the Hb was measured. 5 mL was taken from this mixture and 5 mL of 35% H_2O_2 was added and this mixture was incubated for 2 hours at 37° C with tubes open. After this was cooled, 3 mL was taken and 2 mL of trichloroacetic acid-arsenide solution was added and the mixture was then centrifuged at 2,500 rpm. 3 mL was taken from this supernatant and 1 mL of thiobarbituric acid was added and then the mixture was boiled for 15 min. After it cooled, absorbance at 532 nm was measured spectrophotometrically and the results were calculated for each gram of hemoglobin.

2.3 Statistical methods

Statistical analyses were performed using SPSS for Windows 13.0 (SPSS, Inc., Chicago, USA). Between group comparisons were made by repeated measurements with variance analysis (ANOVA). For significant values, Bonferroni one-way variance analysis as a post hoc test, and then the Tukey HSD test was applied. Comparisons with a P value of less than 0.05 were regarded as statistically significant. When comparing intra-group repeated measurements, the student t test was used. Means of each group's values were calculated and reported as a table. To compare tissue ACE and tissue MDA values, one-way ANOVA and then Tukey HSD tests were performed.

3. Results

All sham group animals died before 24 hours after intoxication, therefore no blood sample was collected from those subjects at 24 hours.

No significant differences between groups in erythrocyte ACE levels were found. At 12 hours after treatment, the mean erythrocyte ACE level of the melatonin+PAM+atropine group was not significantly different from that of the PAM+atropine group, but it was significantly higher than that of the sham group (p=0.023). The mean erythrocyte ACE level

in the melatonin+PAM+atropine group was significantly higher (p=0.031) than that of the PAM+atropine group (Table 1).

Treatment group	1 hour	12 hours	24 hours
Sham	1.75±1.0	1.45±1.1	
Pralidoxime + atropine	1.89±0.8	1.79±1.7	1.85±2.0
Melatonin+pralidoxime+ atropine	2.10±1.2	2.31±1.3	2.95±1.8
p value	p>0.05	p<0.01	p<0.05

Table 1. Mean erythrocyte acetylcholine esterase levels (U/L) at various times after intoxication with dichlorvos in the three groups. Levels were compared using the Mann Whitney U test.

NO levels in the three groups were not significantly different from each other at 1 hour and 12 hours post-intoxication (p>0.05). The NO levels at 24 hours post-intoxication in the melatonin+PAM+atropine group were not significantly different from that of the PAM+atropine group (p>0.05, Table 2).

Treatment group	1 hour	12 hours	24 hours
Sham	5.36±1.89	5.92±3.65	
Pralidoxime + atropine	5.10±1.81	6.21±1.68	7.73±2.67
Melatonin+pralidoxime+atropine	5.41±2.10	5.81±1.89	7.05±2.71
p value	p>0.05	p>0.05	p>0.05

Table 2. Mean erythrocyte nitric oxide levels (mmol/gr Hb) at various times after intoxication with dichlorvos in the three groups. Levels were compared using the Mann Whitney U test.

At one hour post-intoxication, mean erythrocyte MDA levels were similar in all groups. At 12 hours, the mean erythrocyte MDA levels in the melatonin+PAM+atropine group were lower than those of both the sham group and the PAM+atropine group (p=0.001, p=0.012). At 24 hours, the mean erythrocyte MDA levels in the melatonin+PAM+atropine group were significantly lower than those of the PAM+atropine group (p=0.002, Table 3).

Mean brain tissue ACE levels in the melatonin+PAM+atropine group were significantly higher than those of the sham group and PAM+atropine group (p=0.001, p=0.041, Figure 1).

Mean brain tissue NO levels in the melatonin+PAM+atropine group were not significantly different from those of the sham group and PAM+atropine group (p=0.28, p=0.65, Figure 2).

The mean brain tissue MDA levels in the melatonin+PAM+atropine group were significantly lower than those of both the sham group and PAM+atropine group (p=0.001, p=0.002, Figure 3).

Treatment group	1 hour	12 hours	24 hours
Sham	5.40±0.45	9.05±0.66	
Pralidoxime + atropine	5.37±0.67	8.80±0.30	9.48±0.76
Melatonin+pralidoxime + atropine	5.12±0.53	5.93±0.37	6.14±0.42
p value	p>0.05	p<0.05	p<0.05

Table 3. Mean erythrocyte malondialdehyde levels (nmol/mL) at various times after intoxication with dichlorvos in the three groups. Levels were compared using the Mann Whitney U test.

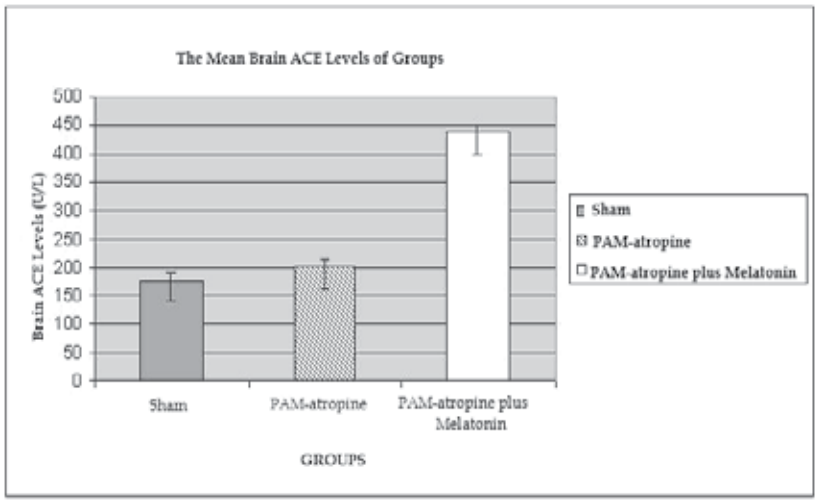


Fig. 1.

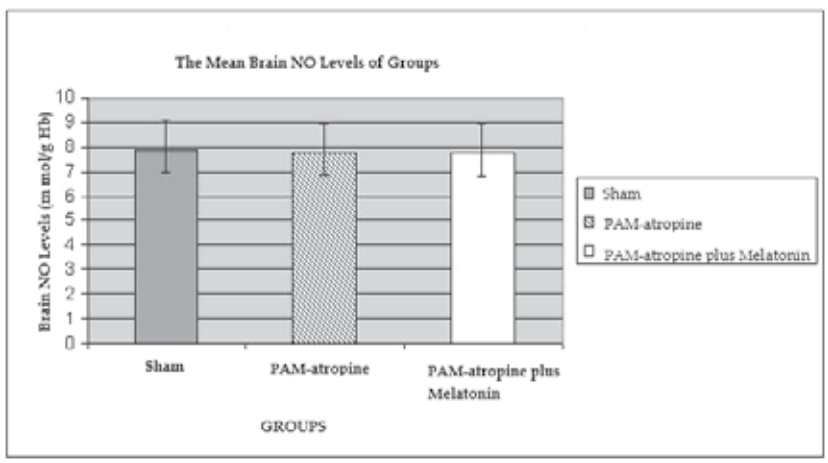


Fig. 2.

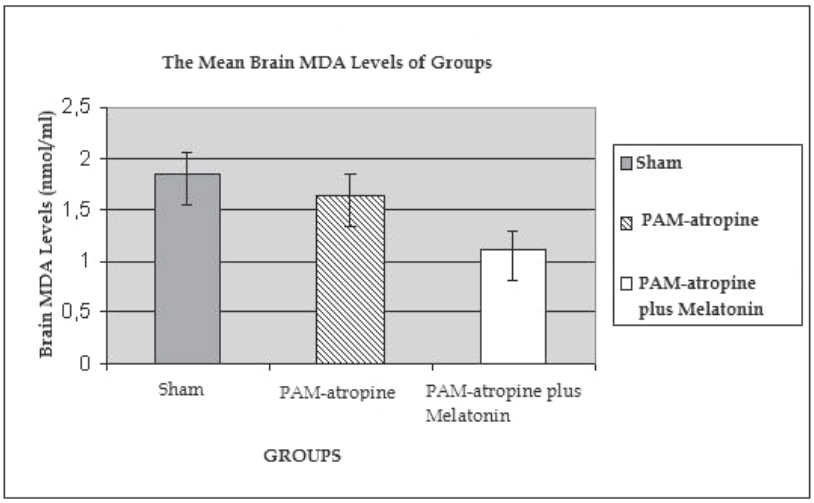


Fig. 3.

4. Discussion

Reactive oxygen species play a key role in initiating secondary brain damage (Özdemir et al, 2005; Tyurin et al, 2000). The brain is prone to oxidative damage which results from high oxygen administration. High concentrations of metals like iron can catalyze reactive radicals, which leads to intense reactive radical production. Neural membranes are also rich in polyunsaturated fatty acids which also contribute to lipid peroxidation reactions (Reiter et al, 2000). Lipid peroxidation changes cell membrane permeability, increases the rate of protein degradation, and ultimately results in the destruction of cell membranes (Tyurin et al, 2000). Non-radical substances containing alkaline and carbonyl moieties produced during the last phases of lipid peroxidation can be measured by their reaction with thiobarbituric acid. Thiobarbituric acid reactive substances (TBARS), of which MDA is the most significant, reflect lipid peroxide production. Increased erythrocyte TBARS concentrations are correlated with severity of cerebral damage (Kasprzak et al, 2001).

Toxicity after an acute intentional or accidental exposure to OP insecticides is largely a reflection of inhibition of ACE in the peripheral and central nervous systems. However, the toxic effects of OPs are not limited to ACE inhibition. In both acute and chronic OP toxictity, changes in antioxidant enzymes occur, and lipid peroxidation increases in many organs, especially the brain. In acute OP poisonings, a decrease in antioxidants occur, which upsets the critical balance between oxidants and antioxidants – thus accumulation of reactive oxygen species and cell destruction begins. In OP toxicity, oxidative stress is an important patho-physiological mechanism, especially for neurotoxicity and cerebral damage (Lukaszewicz-Hussain, 2008).

Atropine and oximes are the fundamental medicines used in the treatment of OP intoxications. Atropine blocks muscarinic receptors in the peripheral and central nervous systems, crosses the blood-brain barrier, and is widely used in OP poisonings. Pralidoxime is the most commonly used oxime in the management of OP poisonings. It reactivates ACE which has been inhibited by OPs (Eddleston et al, 2008). In OP acute poisoning, PAM's penetration into brain tissue may be enhanced by local inflammation. In sublethal OP

poisoning in rats, the group given oxime and atropine preserved cognitive functions compared to the atropine only group. The helpful effects of PAM on brain damage in OP intoxication may be partially explained by its peripheral effects which resolve any respiratory problems. Hypoxic brain damage is slight due to the peripheral effects of PAM (Shrot et al, 2009).

We found that in rabbit model of OP poisoning, melatonin added to PAM and atropine had more positive effects on erythrocytes and brain tissue than PAM and atropine alone. For example, erythrocyte ACE activity of the melatonin+PAM+atropine group was higher than the PAM+atropine group, a finding which can be attributed to lower lipid peroxidation in the group receiving melatonin. The activity of ACE localized in erythrocyte membranes is a significant indicator of OP poisoning severity (13). In previous studies (chronic and sub-chronic exposure) in rats and humans, acute OP poisoning erythrocyte ACE activity was not significantly different than levels in healthy controls (Tinoco & Halperine, 1998; Öğüt et al, 2011). On the contrary, in a rat study of subchronic OP exposure, erythrocyte ACE activity and TBARS levels were found to be significantly lower in the toxicity group compared to healthy controls (Lukaszewicz-Hussain & Moniuszko-Jakoniuk, 2005). In our acute toxicity study, we found that melatonin added to PAM-atropine was beneficial to erythrocyte and brain tissue, findings similar to those of Lukaszewicz-Hussein.

In an in vitro study by Durak D et al, the effect of C and E vitamins in human erythrocytes exposed to OPs on some anti-oxidant enzymes and MDA levels was measured (Durak et al, 2009). In their study, antioxidant enzyme levels in erythrocytes pre-treated with vitamin C and E were higher, and lipid peroxidation was slighter lower.

In our study, the addition of melatonin to 'routine' treatments for OP poisoning did not make a significant effect on erythrocyte NO levels compared to PAM+atropine. Casares et al. stated that OPs may spoil cell calcium homeostasis and change NO and NOS production, and thus decrease the effect of additional environmental negative factors. In our study, we did not find any result that supported Casares's hypothesis (Casares & Mantione, 2007).

In an in vitro OP toxicity study, levels of antioxidant enzymes like erythrocyte superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) decreased and erythrocyte MDA level increased (Karademir Çatalgöl et al, 2007). Öğüt et al. (Öğüt et al, 2011) reported that MDA levels in erythrocyte samples taken from agriculture workers who were chronically exposed to OP insecticides were significantly higher compared to levels in healthy controls. On the contrary, erythrocyte SOD, CAT and GSH-Px levels were significantly lower than healthy controls. In another in vitro study, in the group in which C and E vitamins were added before OP toxicity occurred, erythrocyte MDA levels were significantly lower compared to the group which was not treated with vitamins C and E.

In our study, erythrocyte MDA levels of the sham group were higher than those of other groups. Erythrocyte MDA levels of the melatonin+PAM+atropine group were lower than those of PAM+atropine group. This result is compatible with the results of the previous studies (Puntel et al, 2009). The intense oxidative tissue damage caused by OPs can be decreased with PAM+atropine. Puntel et al., who studied the antioxidant effects of oximes, reported that lipid peroxidation caused by various oxidizing substances was decreased with oximes. In our study, melatonin added to routine treatment decreased lipid peroxidation compared to routine PAM+atropine treatment. Thus, erythrocytes are better protected from oxidative stress when melatonin is added to the treatment regimen.

In OP poisonings, the brain is one of the most damaged organs. Sub-acute poisonings are characterized by significant brain edema and corresponding clinic symptoms. Even with a

single high dose, heavy axonal degeneration can be seen (Read et al, 2010). Major side effects of OP poisoning are rapid loss of conscious resulting from hyperstimulation in central cholinergic synapses, and inhibition of the respiratory center in the medulla oblongata. In animal models, status epilepticus with profound brain damage occurs after significant OP intoxications. Oximes pass through the blood-brain barrier insubstantially and reactivate ACE enzymes which were previously inactivated by OPs. Although the concentrations of oximes in the brain are low, they are adequate to reactivate ACE enzymes and produce positive clinical responses. However, oximes' positive effects on brain tissue are not only dependent on reactivation of ACE enzymes, because studies to date have not found a significant correlation between ACE enzyme levels reactivated in the brain (Eddleston et al, 2008). On the other hand, studies have found that even a small amount of ACE reactivation can increase the rate of survival (Shrot et al, 2009).

In our study, brain tissue ACE activity of the melatonin+PAM+atropine group was higher than that of PAM+atropine group, which were only slightly higher than that of the sham group. This result suggests that the beneficial effects of melatonin are not only related to PAM's reactivation of inactivated ACE enzyme. Hsu et al. studied the effects of melatonin on antioxidant enzymes and MDA levels in the brain tissue of rats that they exposed to OPs in vivo and in vitro (Hsu et al, 2002). OPs lead to lipid peroxidation and DNA oxidation both in vivo and in vitro mediums. However, in the melatonin-treated groups, GSH-Px activity in the brain was significantly higher than non-treated groups; MDA levels were much lover afer melatonin treatment. Our study results are compatible with theirs. Brain tissue MDA levels of the PAM+atropine group were close to those of the sham group. The MDA levels in the animals receiving melatonin were lower than the other two groups. This result indicates that a significant amount of peroxidation lipid develops in the brain after exposure to OPs. This lipid peroxidation and ensuing damage in brain tissue can be significantly decreased with melatonin.

Rats with untreated subchronic OP toxicity develop very high MDA levels in the hippocampus and low SOD levels (Chen et al, 2010). In another study, ACE activity in the hippocampus decreased after subchronic dermal exposure (Mitra et al, 2008). In chronic and subchronic exposures to OPs, the memory and learning functions of the brain are seriously affected due to damage in this area. Giving melatonin before and after acute OP poisoning in rats prevented an increase in brain tissue MDA levels. Brain tissue NO levels in groups treated with melatonin before and after toxicity were significantly higher than those of controls not given OP. In our study, lipid peroxidation in a particular region of the brain, localized ACE activity, NO levels, and oxidative damage were not researched. However, in our acute OP poisoning model, lipid peroxidation in brain tissue and oxidative damage decreased in general, and ACE activity decreased. In our study, melatonin did not show any beneficial effect on brain tissue NO levels. However, lipid peroxidation in brain tissue of the group in which melatonin was added to treatment and ACE levels were positively influenced. Our results suggest that brain damage may be decreased and memory and learning functions can be preserved with the addition of melatonin to routine OP poisoning treatment. Further studies should be performed to determine melatonin's effect in a variety of clinical processes.

Limitations of this study include its low number of subjects; but the ethics board did not allow us to use more subjects. In addition, histopathological examination of brain tissue samples was not performed.

5. Conclusion

Melatonin added to PAM and atropine in the treatment of acute OP poisoning increases ACE activity in brain tissue, and shows a beneficial effect on brain injury by decreasing lipid peroxidation and oxidative stress in brain tissue.

6. References

Abdollahi, M., Ranjbar, A., Shadnia, S., Nikfar, S., & Rezaie, A. (2004). Pesticides and oxidative stress: a review. *Med Sci Monit*;10:RA141-147, 1234-1010.

Casares, F., & Mantione, KJ. (2006). Pesticides may be altering constitutive nitric oxide release, thereby compromising health. *Med Sci Monit*; 12:RA235-240, 1234-1010.

Chen, Q., Niu, Y., Zhang, R., Guo, H., Gao, Y., Li, Y., & Liu, R. (2010). The toxic influence of paraquat on hippocampus of mice: involvement of oxidative stres. *Neurotoxicology*; 31:310-316, 0161-813X.

Durak, D., Uzun, FG., Kalender, S., Öğütçü, A., Uzunhisarcıklı, M., & Kalender, Y. (2009). Malathion-Induced oxidative stress in human erythrocytes and the protective effect of vitamins C and E in vitro. *Environ Toxicol*;24:235-242, 1520-4081.

Eddleston, M., Buckley, NA., Eyer, P., & Dawson, AH. (2008). Management of acute organophosphorus pesticide poisoning. *Lancet*;371:597-607, 0140-6736.

Hsu, C-H., Chi, B-C., & Casida, JE. (2002). Melatonin reduces phosphine-induced lipid and DNA oxidation in vitro and in vivo in rat brain. *J Pineal Res*;32:53-58, 0742-3098.

Jyaratnam, J. Acute pesticide poisoning. A major global health problem. (1990). *World Health Stat Q*; 43:139-144, 0043-8510.

Karademir Çatalgöl, B., Özden, S., & Alpertunga, B. (2007). Effects of triclorfon on malondialdehyde and antioxidant system in human erythrocytes. *Toxicol In Vitro*;21:1538-1544, 0887-2333.

Kasprzak, HA., Wozniak, A., Drewa, G., & Wozniak, B. (2001). Enhanced lipid peroxidation procesess in patients after brain contusion. *J Neurotrauma*;18:793-797. 0897-7151.

Lukaszewicz-Hussain, A., & Moniuszko-Jakoniuk, J. (2005.) A low dose of chlorfenvinphos affects hepatic enzymes in serum and antioxidant enzymes in erythrocytes and liver of the rat. *Pol J Environ Stud*;14:199-202, 1230-1485.

Lukaszewicz-Hussain, A. (2008). Subchronic intoxication with chlorfenvinphos, an organophosphate insecticide, affects rat brain antioxidative enzymes and glutathione level. *Food and Chem Tox*;46:82-86, 0278-6915.

Marrs, TC., Maynard, RL., & Sidell Frederic, R. (2007) *Chemical warefare agents toxicology and treatment*. Second Edt. John Wiley & Sons, Chichester, 978047001359.

McDonough, JH Jr., Clark, TR., Slone, TW Jr., Zoeffel, D., Brown, K., Kim, S., & Smith, CD,. (1998). Neural lesions in the rat and their relationship to EEG delta activity following seizures induced by the nevre agent soman. *Neurotoxicology*;19:381-391, 0161-813X.

Mitra, NK., Siong, HH., & Nadarajah, VD.. (2008). Evaluation of neurotoxicity of repeated dermal application of chlorpyrifos on hippocampus of adult mice. *Ann Agric Environ Med*;15:211-216, 1232-1966.

Öğüt, S., Gültekin, F., Kişioğlu, AN., & Küçüköner, E. (2011). Oxidative stress in the blood farm workers following intensive pesticide exposure. *Toxicol Ind Health*; doi:10.1177/0748233711399308, 0748-2337.

Özdemir, D., Uysal, N., Gönenç, S., Açıkgöz, O., Sönmez, A., Topçu, A., Özdemir, N., Duman, M., Semin, I., & Özkan, H.. (2005). Effect of melatonin on brain oxidative damage induced by traumatic brain injury in immature rats. *Physiol Res*;54:631-637, 0862-8408.

Punte,l GO., Carvalho, NR., Gubert, P., Palma, AS., Dalla Corte, C.L, Avila, DS., Pereira, ME., Carratu, VS., Bresolin, L., daRocha, JB., & Soares, FA.. (2009). Butane-2,3-dionethiosemicarbazone: an oxime with antioxidant properties. *Chem Biol Interact*;177:153-160, 0009-2797.

Read, DJ., L,i Y., Chao, MV., Cavanagh, JB., & Glyn, P. (2010). Organophosphates induce distal axonal damage, but not brain oedema, by inactivating neuropathy target esterase. *Toxicol Appl Pharmacol*;245:108-115,

Reiter, RJ., Tan, DX., Qi, W., Manchester, LC., Karbownik, M., & Calvo, JR. (2000). Pharmacology and physiology of melatonin in the reduction of oxidative stress in vivo. *Biol Signals Recept*;9:160-169,

Shro,t S., Markel, G., Dushnitsky, T., & Krivoy, A. (2009). The possible use of oximes as antidotal therapy in organophosphate-induced brain damage. *Neurotoxicology*; 30:167-173, 0161-813X.

Tinoco, RI., & Halperine, D. (1998). Poverty production and health: inhibition of erythrocyte cholinesterase via occupational exposure to organophosphate insecticides in Chipas, Mexico. *Arch Env Health*;53:29-35, 0003-9896.

Tyurin, VA., Tyurina, YY., Borisenko, GG., Sokolova, TV., Ritov, VB., Quinn, PJ., Rose, M., Kochanek, M., Graham, SH., & Kagan, VE. (2000). Oxidative stres following traumatic brain injury in rat: quantitation of biomarkers and detection of free radical intermediates. *J Neurochem*;75:2178-2189, 1471-4159.

6

Alzheimer's Factors in Ischemic Brain Injury

Ryszard Pluta[1] and Mirosław Jabłoński[2]
[1]*Mossakowski Medical Research Centre, Polish Academy of Sciences, Warsaw*
[2]*Lublin Medical University, Lublin*
Poland

1. Introduction

Aging nations are growing worldwide and now one in four of us may expect to experience an ischemic brain injury by the age 85. Stroke is the third most common cause of death and the second most common cause of dementia in industrialized societies with a mortality rate of circa 30% and an incidence of about 250–400 in 100,000. Stroke affects circa 700,000 people each year in the US alone, and about 50% of these individuals will experience lasting functional dysfunctions including sensory problems and cognitive deficits (Hillis 2006). It is estimated that ischemic stroke is responsible for approximately half of all patients hospitalized for acute neurological disorders. As outlined earlier, it can cause neurological dysfunctions in a number of neurological functions most commonly in the motor activity, cognitive decline, and dementia. Postischemic dementia is characterized by progressive cognitive deterioration including language, reasoning and memory. Of those individuals suffering from ischemic brain injury less than 50% will return to independent living during the following year. Even among those who regain functional independence, many stroke patients continue to manifest significant deficits, limitations and changes in their cognitive functioning and behavior. As such, stroke is one of the leading causes of disability and experiencing a stroke results in two-fold increase in risk for dementia. Other data showed that 1-in-10 developing dementia soon after first stroke, and over 1-in-3 being demented after recurrent stroke. The brain has limited responses to different kind of neuropathogens. Similar neuropathological features are observed in different cerebrovascular diseases and Alzheimer's disease (Kalaria 2000; Pluta 2004a; Pluta 2004b; De la Torre 2005; Pluta 2006a; Benarroch 2007; Niedermeyer 2007; Pluta 2007c; Bell, Zlokovic 2009). Brain stroke is the leading cause of cognitive impairment worldwide. These data are supported by observations in clinical as well as in experimental studies, which suggest that ischemic brain injury is a major risk factor of dementia ranking only second to age (Gorelick 1997; Pluta 2006a; Pluta 2007c). Dementia, which is observed following different brain ischemic injuries, is associated with intellectual impairment and finally brain atrophy (Hossmann et al., 1987; Loeb et al., 1988; Tatemichi et al., 1990; Pluta 2002b; Kiryk et al., 2011). Amyloid plaques, which are the main pathological hallmarks of Alzheimer's disease, account for about 90% of dementias including ischemic-type dementia (Jendroska et al., 1995; Wisniewski, Maslinska 1996; Shi et al., 1998; Pluta, 2007a; Qi et al., 2007). The relationship between brain ischemic injury dementia and Alzheimer's disease type dementia is recently much debated. The mechanisms of the progressive cognitive decline after ischemic brain injury are not yet clear

but animal investigations have demonstrated an increase in expression and processing of amyloid precursor protein to β-amyloid peptide (Pluta et al., 1994b; Pluta et al., 1997b; Pluta et al., 1997c; Pluta et al., 1998b; Lin et al., 1999; Shi et al., 2000; Lin et al., 2001; Badan et al., 2004; Pluta et al., 2009) and an increase in the phosphorylation of tau protein (Dewar, Dawson 1995; Wen et al., 2004b; Wen et al., 2004c, Wen et al., 2007). Moreover, the common mechanism that links progressive cognitive decline after ischemic brain injury and during Alzheimer's disease is neuroinflammation (Koistinaho et al., 2002), which can cause gradual neurodegeneration during prolonged face of injury. However, the link between ischemic brain injury and delayed progressive cognitive decline opens a new area for potential treatment in that the onset of the progressive cognitive decline after ischemia is delayed. The above data raise the question whether Alzheimer's related proteins affect ischemic brain tissue. The details of Alzheimer's protein-related mechanisms, which probably mediate ischemic brain cell damage and neurotoxicity (Mattson et al., 2000; Malm, Koistinaho 2007) and involvement of these proteins in brain accumulation will be reviewed. This chapter summarizes some of the findings, which suggest that ischemic overexpression of amyloid precursor protein renders the brain more vulnerable to ischemic episodes (Koistinaho et al., 2002) and describes the factors that are involved in increased neuronal susceptibility to ischemic injury (Mattson et al., 2000; Malm, Koistinaho 2007).

1.1 Consequences of ischemic brain injury

Brain ischemic injury is the most common chronic cause of disability around world and has generally a negative influence on the individuals it affects, caregivers and society as a whole (Flynn et al., 2008). Ischemic stroke survivors suffer from chronic progressing neurological disabilities that significantly influence their ability to return to society. A more insidious consequence of brain ischemia is a post-stroke dementia (Jellinger 2007) that is also associated with severe disability. Worldwide brain vascular disorders like ischemia are responsible for 5.4 million deaths every year (Flynn et al., 2008). Circa 3% of total healthcare finances are attributable to brain ischemia. Cost of ischemic stroke to the EU economy is estimated at 21 billion euro and to USA economy at 2.2 trillion dollars (Fillit, Hill 2002, Flynn et al., 2008). The global scale of the problem and the enormous associated costs it is clear that there is an urgent need for advances in the prevention of ischemic brain injury and its consequences like postischemic dementia. Dementia is the worst consequence for survivors following brain ischemia and being responsible for approximately 20% of all confirmed dementias (Fillit, Hill 2002). Globally cerebrovascular diseases dementia varies from 10 to 50% depending on the diagnostic criteria, geographic location and population demographic (Leys et al., 2002). Recently it is becoming clear, that cerebrovascular diseases dementia in fact shares many risk factors in common with Alzheimer's disease. Indeed ischemic brain injuries may precede the onset of this form of dementia strongly suggesting that brain ischemic episodes may trigger neurodegenerative dementias. Postischemic dementia connected with chronic delayed secondary injury occurs in individuals suffering from focal or global brain ischemia in a progressive manner (Jellinger 2007). The chronic postischemic injury including dementia has received far less attention in clinical and experimental stroke investigations. Vascular dementia incorporates cognitive dysfunction with cerebrovascular diseases.

1.2 Epidemiology of postischemic dementia

Epidemiological studies have shown that the prevalence of dementia in ischemic brain injury patients is nine-fold higher than controls at 3 months (Madureira et al., 2001;

Pohjasvaara et al., 1998; Tatemichi et al., 1992) and 4-12 times higher than in controls 4 years after a lacunar infarct (Loeb et al., 1992). Different patterns of cognitive decline as effect of ischemia brain injury have been shown by longitudinal epidemiological, studies which have suggested a progressive course of dementia following ischemic stroke. Tatemichi et al., (1990) presented that the incidence of dementia was 6.7% among patients directly after 1 year of survival in a group of 610 subjects who were initially free of dementia following stroke. Bornstein et al., (1996) reported that 32% individuals who were initially free of dementia directly after stroke developed incidental dementia during 5 years of survival following first ischemic episode. Henon et al., (2001) observed a sample of 169 patients who had been free of dementia before stroke and reported that the cumulative proportion of individuals with incidental dementia was 21.3% after 3 years of survival. Altieri et al., (2004) examined 191 free of dementia stroke patients for a 4 years, and noted that the incidence of dementia increasing gradually with 21.5% subjects had developed dementia by the end of the follow-up time. In population-based investigations of stroke and dementia subjects, Kokmen et al., (1996) checked the medical records of 971 patients who were nondemented before first stroke. The incidence of dementia was 7% at 1 y, 10% at 3 y, 15% at 5 y and 23% at 10 y. Desmond et al., (2002) performed functional assessments annually on 334 ischemic brain injury patients and 241 ischemia free control individuals, all of whom were free of dementia in baseline examinations, and noted a progressive course of dementia with the incidence rate of 8.94/100 person/year in the ischemic group and 1.37/100 person/year in the control group. In two studies based on subjects presenting with a lacunar infarction as their first ischemic stroke, Samuelsson et al., (1996) found that 4.9% and 9.9% of 81 patients had dementia after 1 and 3 years of observation, respectively, and Loeb et al., (1992) reported that 23.2% individuals had dementia during an average of 4 years of survival.

Removal of the above deficits/abnormalities is a topic to which a neurologist and scientists devotes little time. In different patients, some spontaneous functional restoration is noted during weeks/months after ischemic brain injury. However, in general, this spontaneous recovery is incomplete. Moreover, ischemic brain injury often leaves its victims functionally devastated and as such is the leading cause of permanent disability requiring long-term institutional care in our nations. The loss of life quality years and health care resources are staggering. The situation is even aggravated by the fact that unlike many other neurological diseases, no safe, effective therapy is available for the majority of patients with acute ischemic brain injury. The burden after ischemic brain injury on our societies is dramatically increasing. Thus, an understanding of the underlying progressing pathological processes/cascades is urgently needed. This chapter tends to summarize the neuropathological changes of chronic postischemic brain injury and reveal the convinced mechanisms.

2. Amyloid precursor protein and β-amyloid peptide after ischemia

After brain ischemia, amyloid precursor protein mRNA had enhanced till 200% in the brain during the seventh day of reperfusion. The above data suggest that local ischemic brain injury increases amyloid precursor protein mRNA level, which may contribute to the progression of cognitive impairment in ischemic brain injury (Abe et al., 1991; Koistinaho et al., 1996; Shi et al., 1998; Shi et al., 2000). Above studies also show that focal ischemic brain injury alters Kunitz protease inhibitor amyloid precursor protein/amyloid precursor protein 695 ratios in brain and this shift in precursor isoforms could be related to degeneration and activation of astrocyte following the ischemic injury (Kim et al., 1998). In permanent local

brain ischemia injury, amyloid precursor protein mRNA species, which contain a Kunitz-type protease inhibitor domain, were expressed in the cortex by day 21 of survival but the net amount of precursor mRNA did not change. This investigation suggests a selective role of amyloid precursor protein species that contain the Kunitz protease inhibitor domain in cascade of focal brain ischemia (Abe et al., 1991). After local ischemia amyloid precursor protein 770 and amyloid precursor protein 751 mRNAs were increased during 7 days in the brain (Koistinaho et al., 1996).

Animals after focal and global ischemic brain injury with a survival time up to 1 year presented increased brain immunoreactivity to the β-amyloid peptide and as well as to the N- and C-terminal of amyloid precursor protein. The staining was observed extracellularly and intracellularly (Pluta et al., 1994b; Hall et al., 1995; Tomimoto et al., 1995; Horsburgh, Nicoll, 1996a; Ishimaru et al., 1996a; Yokota et al., 1996; Pluta et al., 1997b; Pluta et al., 1998b; Lin et al., 1999; Pluta 2000; Lin et al., 2001; Sinigaglia-Coimbra et al., 2002; Fujioka et al., 2003; Yang, Simpkins 2007). Different fragments of amyloid precursor protein were noted in astrocytes, neurons, oligodendrocytes, and microglia (Banati et al., 1995; Palacios et al., 1995; Pluta et al., 1997b; Nihashi et al., 2001; Pluta, 2002a; Pluta2002b; Badan et al., 2003; Badan et al., 2004). Animals with long survival after ischemic brain injury from 0.5 to 1 year showed pathological brain staining only to the β-amyloid peptide and to the C-terminal of amyloid precursor protein (Pluta et al., 1998b; Pluta 2000). The reactive astrocytes with deposition of different fragments of amyloid precursor protein might be involved in the development of glial scar (Nihashi et al., 2001; Pluta 2002a; Pluta 2002b; Badan et al., 2003; Badan et al., 2004). Reactive astrocytic cells with pathological level of β-amyloid peptide deposition might be involved in pathological repair of host tissue after ischemic brain injury including astrocytes death (Pluta et al., 1994b; Pluta 2002b; Wyss-Coray et al., 2003; Takuma et al., 2004).

Pathological amyloid precursor protein staining especially for β-amyloid peptide and C-terminal has been observed in periventricular and subcortical white matter after ischemic brain injury (Pluta et al., 2006, Pluta et al., 2008). The more intense postischemic brain injury of white matter is, the more extensive is the staining of different parts of amyloid precursor protein in this region (Yam et al., 1997). In contrast, in our unpublished studies, the data are opposite. We noted ischemic time-independent intensity of immunostaining, shorter ischemic brain injury stronger reactivity. Probably, this kind of abnormalities is responsible for leukoaraiosis formation after ischemic brain injury (Pluta et al., 2008). Extracellular accumulation of different fragments of amyloid precursor protein ranged from multifocal widespread very small dots to regular amyloid plaques (Pluta et al., 1994b; Pluta et al., 1998b; Pluta 2000; Pluta 2002b; Pluta 2003). Multifocal and widespread different kinds of amyloid plaques were observed mainly in the ischemic hippocampus, brain and entorhinal cortex, and corpus callosum, and subventriculary (Pluta et al., 1994b; Pluta et al., 1997b; Pluta et al., 1998b; Pluta 2000; Pluta 2003; Pluta 2005; Pluta et al., 2006; Pluta et al., 2008; Pluta et al., 2009; Pluta et al., 2010).

The accumulation of the β-amyloid peptide in astrocytes and the C-terminal of amyloid precursor protein in ischemic neurons underline the likely importance of these two proteins in ischemic brain injury cascade of degeneration (Pluta et al., 1994b; Yokota et al., 1996; Pluta 2002b; Badan et al., 2003; Badan et al., 2004). Moreover, the above parts of precursor deposits suggest that these fragments of precursor may initiate synaptic pathology and finally promote retrograde neuronal death after ischemic injury (Oster-Granite et al., 1996). The

aforementioned observations indicate that the late neurotoxic β-amyloid peptide and C-terminal of amyloid precursor protein deposition after ischemic brain injury may represent a secondary injury process that could deteriorate the ischemic brain outcome by unexpected additional neurons death (Pluta et al., 1997c, Pluta et al., 1998b). Following ischemia β-amyloid peptide is produced as a result of neurons injury (Ishimaru et al., 1996a) and probably appears its effects, influencing ischemic neurons and glia as dementia. It is generally received that β-amyloid peptide takes part in neurons death (Cotter et al., 1999). The β-amyloid peptide is a toxic protein and entangles within an ischemic process in astrocytes, oligodendrocytes, and microglia that lead neurons and glia finally to death (Giulian et al., 1995).

3. Amyloid precursor protein secretases after ischemia

The amyloid precursor protein is cleaved by α-secretase and it is not pathological pathway in brain. Ischemic brain injury results in the downregulation of α-secretase mRNA and decreases its net activity (Nalivaeva et al., 2004; Yan et al., 2007). In the pathological pathway called amyloidogenic precursor is cleaved by β-secretase and γ-secretase to form β-amyloid peptide. The formation of β-amyloid peptide in the brain after ischemic injury increases and impairs the memory (Yan et al., 2007). Current investigations have shown that brain ischemia stimulates the formation and activity of β-secretase in brain tissue (Wen et al., 2004a; Chuang et al., 2008). Presenilin, which is overexpressed after ischemic brain injury (Tanimukai et al., 1998; Pennypacker et al., 1999), is involved in ischemic β-amyloid peptide synthesis by γ-secretase (Polavarapu et al., 2008).

4. Amyloid precursor protein intracellular domain after ischemia

Important brain trigger, which initiates amyloid precursor protein cleavage, is ischemic episode. The main proteolysis is performed by α- or β-secretase that produce large soluble N-terminal parts called respectively soluble N-terminal domain of amyloid precursor protein α (sAPPα) or soluble N-terminal domain of amyloid precursor protein β (sAPPβ). These fragments are release into the extracellular space. Remaining C-terminal domains are bind with membrane and called respectively C-terminal fragment 83 or 99 (CTF83/CTFα or CTF99/CTFβ). The second cleavage occurs in the intramembrane area by γ-secretase, which depending on where the first proteolysis was made and finally releases either the β-amyloid peptide or p3 fragment. This phenomenon seems to be largely nonselective occurring in at least 3 different sites of the amyloid precursor protein like V636, A638 and L645 (ε-cleavage site) (Sastre et al., 2001; Yu et al., 2001). The final products are β-amyloid peptide 40/42 and an intracellular 50 aa C-terminal of amyloid precursor protein domain (5kDa) (Pinnix et al., 2001). Amyloid intracellular domain is very labile and can be further disintegrated by the insulin degrading enzyme or proteasome. Amyloid intracellular domain with specific binding proteins initiating a signal cascade, which subsequently migrates to the cell nucleus to become a component of a transcriptional process but the adaptor protein FE65 rescues the amyloid intracellular domain from rapid proteolysis.

5. Tau protein after ischemia

Tau protein overexpression in neurons was observed in the hippocampus (Geddes et al., 1994) and the brain cortex (Dewar et al., 1993; Dewar et al., 1994) after ischemic brain injury

(Sinigaglia-Coimbra et al., 2002). Moreover, an increase of tau immunostaining was noted in glia and oligodendrocytes following local brain ischemia (Dewar, Dawson 1995; Irving et al., 1997). Additionally pathological tau protein was found in microglia around the ischemic core (Uchihara et al., 2004). The above data indicate that only some neurons display pathologically changed tau protein following ischemic brain injury (Dewar, Dawson 1995), which may reflect an early alterations state of the degenerative processes in these cells (Irving et al., 1997). Another study noted a complete dephosphorylation of tau protein after ischemic brain injury (Mailliot et al., 2000). The dephosphorylation of tau protein may influence its transportation between axon and cell body and affects its susceptibility to proteolysis (Shackelford, Yeh 1998). Some other study noted that tau protein itself blocks transport of amyloid precursor protein from the neuron body into axon and dendrites causing amyloid precursor accumulation in the neuron body (Stamer et al., 2002). The recent studies show that after ischemic brain injury, hyperphosphorylated tau protein accumulates in cortical neurons and colocalizes with signs of apoptosis. This process may be important element in the etiology in ischemic brain degeneration. The above observations indicate that neuron ischemic apoptosis is connected with tau protein hyperphosphorylation (Wen et al., 2004b; Wen et al., 2007). Wen et al. (2004c, 2007) noted that reversible brain ischemia is associated with neurofibrillary tangle-like tauopathy formation in the brain. These data provide groundwork for the cause of dementia after ischemic brain injury (Wen et al., 2004c).

6. Presenilins after ischemia

Ischemic brain injury overexpression of presenilin 1 gene in neurons of the CA3 sector and dentate gyrus was noted (Tanimukai et al., 1998). In above study, increased expression of presenilin 1 mRNA was the highest at day 3 of reperfusion in affected regions. The above data suggest that the overexpression of presenilin 1 mRNA may be associated with some responses of neurons injured by ischemic pathology. In another study, the increased expression of presenilin mRNA was found in the hippocampus, striatum, cortex, and cerebellum following local ischemic brain injury (Pennypacker et al., 1999). Generally presenilin mRNA exhibited the highest expression in the hippocampus and brain cortex. The expressions were higher on the contralateral side to the local ischemic brain injury. This difference may reflect a loss in brain cells e.g. neurons expressing presenilin genes on the ipsilateral side. Staining of presenilin was more expressed in glia than in neurons and in a trace of the pyramidal neurons of hippocampus after ischemic brain injury (Pluta 2001). Presenilin 1 increases neuron vulnerability to ischemia by increasing intracellular calcium (Mattson et al., 2000; Pluta et al., 2009). A current investigation presented that presenilin 1 and intracellular calcium regulates neuron glutamate uptake (Yang et al., 2004). Taken together, above data indicate that presenilins and intracellular calcium may play an important role in regulating glutamate uptake, and therefore they may influence glutamate toxicity in the ischemic brain injury.

7. Apolipoproteins after ischemia

Astrocytic apolipoprotein E mRNA overexpression with the highest level at day 7 after ischemic brain injury was found, which suggests that ischemic neuron injury results in the induction of certain genes in the brain within reactive astrocytes and this induction may

contribute to amyloidogenesis following brain ischemia (Ali et al., 1996). Apolipoprotein E mRNA overexpression in glia but not in neurons was noted in ischemic penumbra with a peak on 21st day. In ischemic core apolipoprotein E mRNA overexpression was observed in macrophages (Kamada et al., 2003). Overexpression of clusterin mRNA was shown in the penumbra in permanent focal brain ischemia. In these studies, reactive astrocytes in the cortex were stained abnormally for apolipoprotein J. It was suggested that local expression of clusterin mRNA might contribute to the neuroinflammation, which representing a main factor in secondary injury processes after focal ischemic brain episodes (Van Beek et al., 2000). After moderate ischemic brain injury a time-dependent deposition of clusterin was noted in pyramidal neurons of the CA1 and the CA2 sector in the hippocampus undergoing delayed neuronal death. Overexpression of apolipoprotein J mRNA in contrast to neuronal protein staining appeared to be glial in origin with increases in mRNA the hippocampus fissure and only a very weak signal over the CA1 and the CA2 pyramidal neuron layer. The above results support the idea that clusterin is synthesized in the astrocytes, secreted outside and next taken up by dying neurons (Nishio et al., 2003). Clusterin deposition was observed in neurons destined to die by apoptosis. Moreover, pathological overexpression of clusterin suggests that the synthesis of this protein was a result of selective delayed neuronal death rather than involvement in the pathological cascade of events that cause it (Walton et al., 1996).
The pathological immunostaining for apolipoproteins A1, E, and J was shown extracellularly and intracellularly (Hall et al., 1995; Kida et al., 1995; Pluta et al., 1995a; Horsburgh, Nicoll 1996a, Horsburgh, Nicoll 1996b; Ishimaru et al., 1996b; Pluta 2000; Kamada et al., 2003). Intracellular staining was noted in damaged neurons exhibiting features of ischemic injury (Pluta 2000). Less often immunostaining for above proteins was observed in glia (Kamada et al., 2003). Extracellular accumulations of apolipoproteins were irregular and well delineated and mainly diffuse. Strong staining was noted also in acellular, necrotic, irregular and spider-like foci (Kida et al., 1995; Pluta et al., 1995a; Ishimaru et al., 1996a). It is important to notice that accumulations of apolipoproteins colocalize with aggregates of different parts of amyloid precursor protein (Kida et al., 1995; Pluta et al., 1995b). Apolipoprotein E promotes the deposition of β-amyloid peptide into the oligomeric and the fibrillar form. Clusterin is engaged in transport of β-amyloid peptide through the blood-brain barrier. The main activity of apolipoproteins A1, E and J is controlling the level of soluble β-amyloid peptide in the intracellular and the extracellular space of brain tissue as well as their influence on fibrillar β-amyloid peptide conversion. Apolipoprotein E induces β-amyloid peptide increased lysosomal leakage and finally apoptosis in neuronal cells (Ji et al., 2002). Apolipoproteins A1, E, and J influence the deposition, structure and neurotoxicity of the β-amyloid peptide in brain ischemia. Additionally, in β-amyloid peptide production apolipoproteins E and J are involved prior to its accumulation. The above studies show principal roles of apolipoproteins E and J in β-amyloid peptide accumulation and that they play an important role in it extracellular β-amyloid peptide metabolism independent of β-amyloid peptide synthesis. These observations indicate that apolipoproteins A1, E and J deposition following ischemic brain injury may be a secondary damaging phenomenon, which could deteriorate healing of ischemic neurons indirectly influences development of ischemic-type dementia.

8. α-synuclein after ischemia

Brain ischemia provoked changes in a presynaptic protein α-synuclein in the ischemic hippocampus (Ishimaru et al., 1998; Kitamura et al., 2001). Intense α-synuclein

immunostaining was found in the perivascular neighborhood of the CA1 sector in experiments with long-term survival following ischemic brain injury (Kitamura et al., 2001). In degenerating regions after brain ischemia glia presented intense reactivity for α-synuclein (Ishimaru et al., 1998). The above results suggest that α-synuclein may be essential protein in the neuropathological ischemic cascade (Goedert 2001). Abnormal α-synuclein accumulation might disrupt synaptic function, resulting in cognitive deficits (Hashimoto, Masliah 1999). The pathology of α-synuclein disturbs the synaptic activity that finally causes retrograde neurons loss in the ischemic brain injury (Goedert 2001).

9. Platelets after ischemia

Pluta et al., (1994c) for the first time directly presented the involvement of platelets in pathological processes after ischemic brain injury. They documented a key role of platelets during repeated vessels occlusion following ischemic brain injury (Pluta et al., 2009). These authors observed augmented thrombocytes aggregations and adhesiveness to vessel endothelium, which very well correlated with ischemic brain disease progression. Other study presented increased platelet microparticles and membrane remnants during reperfusion after ischemic brain damage (Mossakowski et al., 1993; Horstman et al., 2009). Next some study reported circulating platelets complexes and platelets-leukocytes aggregates in systemic circulation following brain ischemia injury (Ritter et al., 2005). Thus chronic abnormal platelets activity following brain ischemia injury now is established as an important pathological phenomenon. It may be suggested that platelets activity after ischemic insult is directly connected with development of general inflammation reply. However, the founding of platelets outside brain vessels after ischemic brain injury (Pluta et al., 1994c; Pluta 2003; Pluta 2006b; Pluta 2007a; Pluta 2007b) comes to evidence of platelets involvement in complex processes of neuroinflammtion and neurodegeneration. Different elements of coagulation system have been noted in brain ischemia episodes including collagen in perivascular space (Pluta et al., 1994c). Above findings, together with other direct evidences suggest that platelets interaction with white blood cells and next with the blood-brain barrier vessels is responsible for leukocyte passage through ischemic blood-brain barrier. Platelets are capable of directly activating lymphocytes and are responsible for synthesis of immunoglobulins (Cognasse et al., 2007). In addition it is suggested involvement of platelet-activating factor in disruption endothelial tight junctions what means opening of the blood-brain barrier (Callea et al., 1999; Brkovic, Sirois 2007; Adamson et al., 2008; Knezevic et al., 2009). We feel that above observations are important in understanding the etiology of ischemic brain neurodegeneration with dementia and Alzheimer's disease etiology.

10. Neuropathology after ischemia

Most of the experiments with reference to ischemic brain injury were conducted on small rodents. The reproduction of overlapping pathological mechanisms in small rodent models is a suitable approach to unravel of causal relationships. Studies were conducted to support the hypothesis that the anatomy of the brain vasculature in small rodents is not different from that of humans. The preference to perform brain ischemia studies on rodents are also supported by pragmatic reasons including a high homogeneity due to inbreeding, accessibility and lower costs. For several reasons, the favored brain region for the study of

ischemic injury is the hippocampus. First, the hippocampus is the part of brain, which displays the same pathology as human ischemic brain. Second, the hippocampus is implicated in spatial learning and memory. Third, the hippocampus, especially its area CA1 is one of the brain sectors very sensitive to ischemic injury like in humans. Finally, the distinct laminar organization of the hippocampus and its final mapped synaptic connections allow exact layer-type or cell-type investigations. With respect to the above observations and metabolism, cerebral blood flow and pathology few models of brain ischemia, which mimicked human condition have been presented (Kirino 1982; Pulsinelli et al., 1982; Smith et al., 1984; Pluta et al., 1991). In these models selective ischemic pyramidal neurons death was noted in the CA1 sector of the hippocampus (Kirino 1982; Pulsinelli et al., 1982; Pluta 2000; Pluta 2002b). Loss of neurons develops during 7 days after ischemia and is called delayed neuronal death (Kirino 1982). Three min of ischemic brain injury in gerbils and 10 min in rats are sufficient to start this characteristic hippocampal pathology (Kirino 1982; Pulsinelli et al., 1982; Pluta 2000; Pluta 2002b). Prolongation of ischemic brain injury in rats to 10-20 min results in complete neurons death in the CA1 sector of the hippocampus and neuronal injury in the brain cortex and striatum (Pulsinelli et al., 1982; Kiryk et al., 2011).
Prolongation of recirculation time ends in neuronal alterations in hippocampal regions of nonselective vulnerability (Pluta et al., 2009). Striatal pathology is mainly noted in the dorsolateral area and influence medium-sized neurons (Pluta 2002b). In the brain cortex, the layers 3, 5 and 6 presented neuronal changes (Pulsinelli et al., 1982; Pluta 2000; Pluta 2002b). Within these regions of selective neurons pathology strong activation of astrocytes and microglia were showed (Petito et al., 1990; Schmidt-Kastner et al., 1990; Gehrmann et al., 1992; Morioka et al., 1992; Orzyłowska et al., 1999; Pluta 2000; Pluta 2002b). In brain areas with neuronal disappearance and neuronal cobweb interruption brain ischemic atrophy finally develops (Hossmann et al., 1987; Pluta 2002b; Pluta 2004b; Pluta, Ulamek 2006) with all neurodegenerative consequences.

11. Neuroinflammation after ischemia

Ischemic brain injury is associated with both acute and chronic neuroinflammatory reactions, involving activation, hypertrophy and proliferation of astrocytes and microglia. Ischemically activated astrocytes in the CA1 area of the hippocampus overexpress cytokines (Orzylowska et al., 1999). These data show that upregulation of neuroinflammatory mediators by astrocytes are directly connected with selective vulnerability of neuronal cells in ischemic brain injury (Orzylowska et al., 1999; Touzani et al., 2002). The above data suggest that neurons in vulnerable sectors in ischemic brain are targets of astrocytes interleukin-1β. This idea is supported by overexpression of neuronal interleukin-1 receptor (Touzani et al., 2002). In addition, it was confirmed that interleukin-1β is the important factor in brain ischemia cells damage and edema formation (Yamasaki et al., 1995). Chronic synthesis by ischemic brain neuroinflammatory factors may start a self-sufficient cycle that shifts ischemic pathology into hallmarks typical for Alzheimer's disease. In ischemic brain interleukin-1 is a key factor, which motivates neurons to pathological cleavage of amyloid precursor (Griffin et al., 1998) and emits inflammatory mediators. All these events result in neuronal abnormal function and finally their death. Neuronal loss arises from neuroinflammatory factors, which induce neuronal damages that trigger microglia activity with further self-propagation of the neuroinflammatory events. Additionally, evidence has been showed that β-amyloid promotes the release of neuroinflammatory pathogens by microglia (Giulian et al., 1995). In the

hippocampus glia activity precedes neurons alternations and persists for long time after ischemic brain injury. Initially, this activity was combined with repair responses at the site of the brain injury, but currently it has been shown that neuroinflammatory reaction is a key play in the evolution processes of ischemic brain pathology (Stoll et al., 1998).

Considerable evidence indicates that neuroinflammatory cascade modulates both the synthesis factors and proliferation reactions of activated astrocytes (Smith, Hale 1997), which exert both beneficial and harmful effects during repair mechanisms in the injured brain (Stoll et al., 1998). The reactive glia produce cytokines, which next stimulate glia, cytokine production and gliosis in a self-propagating, cycle (Barone, Feuerstein 1999). Neuroinflammatory genes overexpression peaks 24 h in the damage area, then decrease (Schroeter et al., 2003). Additionally, ischemic brain injury not only causes tissue cell injury, but also engages neuroinflammatory reactions that include the movement and depositions of leukocytes, macrophages, monocytes and different serum proteins due to open of the blood brain barrier (Danton, Dietrich 2003). In addition to the core ischemic injury neuroinflammatory reactions in the remote region to the primary ischemic injury have also been observed. Using the focal model of brain ischemia degeneration was noted in thalamus and substantia nigra in areas which are supplied by opened cerebral arteries and these areas showed no sign of ischemia (Danton, Dietrich 2003). Neurodegeneration in thalamus and substantia nigra were preceded by TNFα overexpression, supporting the role of neuroinflammation in the remote region to the ischemic brain lesion (Danton, Dietrich 2003). Other authors additionally reported a transient overexpression of IL-6 in the substantia nigra following focal brain ischemia (Dihne, Block 2001). Increased number of neuronal progenitor cells has been noted in the hippocampus following focal (Takasawa et al., 2002) and global (Jin et al., 2001) brain ischemia and in subventricular zone after *cardiac arrest* in rats (Andjus et al., 2010), with a considerable number of cells differentiating into astrocytes that support the neuroinflammatory reaction in the remote area distal to the primary deadly injury. Finally focal or global brain ischemic can induce a general inflammatory reaction both in the brain and peripheral body system. Inflammatory markers such as interleukin-6 and matrix metalloproteinases-9 are significantly elevated in blood plasma following brain ischemia (Castillo, Rodriguez 2004).

Neuroinflammation has also been implicated in the neuropathogenesis of dementia. In dementia patients neuroinflammation is often combined with β-amyloid peptide accumulation and neurofibrillary tangles development (Moore, O'Banion 2002). Neuronal cells in the hippocampus are peculiarly vulnerable to the influence of chronic inflammation (Hauss-Wengrzyniak et al., 2000; Wenk, Barnes 2000). In addition, hippocampus, the center involved in learning and memory, which demonstrates the greatest early activation of microglia in the different diseases, finally shows the highest degree of neuropathology and atrophy (Hossmann et al., 1987; Cagnin et al., 2001; Pluta 2002b; Pluta 2004b; Pluta, Ulamek 2006). A large number of different experimental and clinical treatment studies presented that reactive microglia and proinflammatory factors are present at areas of β-amyloid plaques accumulation and anti-inflammatory therapy decreases the progression of the diseases connected with amyloid pathology in own etiology (McGeer et al., 1996; Kalaria 1999; Akiyama et al., 2000).

12. Ischemic brain cells and β-amyloid peptide

In the ischemic brain, the main pathological focus is concentrated on pyramidal neurons in hippocampus because this region of the brain is selectively vulnerable to ischemia. In

generally, complete loss of vulnerable neurons in the CA1 sector was noted during 7 days following brain ischemia (Butler et al., 2002). Moreover, one third of subjects with ischemic brain injury did not present full loss of neurons in CA1 sector following ischemia with long-term survival (Sadowski et al., 1999; Pluta 2000). In some cases, complete disappearance of all neurons of CA1 area was observed in very late stages after ischemia (Pluta 2000; Pluta 2002a; Pluta 2002b; Pluta et al., 2009). Some investigations presented marked neuropathological alterations in pyramidal neurons considered to be completely resistant to ischemic injury such as: in areas CA2, CA3, and CA4 of hippocampus and dentate gyrus (Pluta 2000; Pluta et al., 2009). These regions presented unexpectedly acute ischemic changes in neuronal cells from 1 to 24 months after ischemic brain injury (Pluta 2000; Pluta et al., 2009). Currently was noted that neuropathological processes in ischemic neurons continue well beyond the acute stage of insult (Pluta 2000; Pluta 2002a; Pluta et al., 2009; Kiryk et al., 2011). In these situations, enduring ischemic blood–brain barrier opening (Pluta 2003; Pluta 2005; Pluta 2006b; Pluta 2007b, Pluta et al., 2010) probably leads to enhanced ischemic neurons vulnerability to β-amyloid peptide (Koistinaho et al., 2002).

Some investigations reported that astrocytic apoptosis may contribute to the neuropathogenesis of different diseases such as ischemic brain injury (Koistinaho et al., 2004; Takuma et al., 2004; Pluta 2006a). Astrocytic abnormal activities observed in the ischemic brain injuries: swelling, astrogliosis, and astrocytosis (Bernaudin et al., 1998; Stoltzner et al., 2000). In ischemic brain some animal studies showed the early import of different parts of amyloid precursor protein from brain tissue and systemic circulatory to the astrocytes and in the late stages the export of the toxic β-amyloid peptide and C-terminal of amyloid precursor protein from dead astrocytes to the brain parenchyma (Pluta et al 1994b; Pluta 2002b; Koistinaho et al., 2004; Pluta 2004a; Pluta 2004b).

At early stages of ischemia-reperfusion brain injury, the N-terminal of amyloid precursor protein (Pluta et al., 1994b) may be produced by vascular endothelium that became damaged following injury (Badan et al., 2004). This hypothesis is supported by the overexpression and synthesis of β-secretase after brain ischemia (Wen et al., 2004a; Sun et al., 2006; Zhang et al., 2007). Furthermore presenilin overexpressed in postischemic brain (Tanimukai et al., 1998; Pennypacker et al., 1999; Pluta 2001) is involved in the cleavage of amyloid precursor protein to synthesize β-amyloid peptide through the γ-secretase complex (Wolfe et al., 1999). This secretase is involved in amyloidogenic cleavage of amyloid precursor protein. At the first step, amyloid precursor protein is cut at the N-terminal of the β-amyloid peptide fragment by protease called β-secretase. In the second step, the metabolite of β-secretase is cut by γ-secretase to form soluble β-amyloid peptide. Endothelial cells change structural features like: shape and size during time to become incorporated into the amyloid plaques in a close spatial relationship with damaged and dead astrocytes. The same investigations show that C-terminal of amyloid precursor protein aggregates in neurons following local brain ischemia and as the infarct increases, the C-terminal of amyloid precursor protein staining become increasingly larger in the core even though the neurons are dying and the core becomes largely acellular (Badan et al., 2004). The same and other studies reported that β-amyloid peptide and C-terminal of amyloid precursor protein noted in microglia could be due to the phagocytosis of dead neurons remnants containing β-amyloid peptide and C-terminal of amyloid precursor protein by microglia (Badan et al., 2004; D'Andrea et al., 2004). Moreover, there are data demonstrating that astrocytes but not microglia can swallow up β-amyloid peptide (Matsunaga et al., 2003; Wyss-Coray et al., 2003; Pluta 2006a). Other studies show that C-terminal of amyloid

precursor protein triggers the loss of astrocytes whereas the death of neurons is a secondary and result of the neuronal dependency on astrocytes for antineurotoxic amyloid guard (Abramov et al., 2003; Pluta 2006a). The accumulation of some parts of amyloid precursor protein in astrocytes may be important in promotion of amyloidosis in ischemic brain tissue in which chronic astrocytosis is probably play a key role in the occurrence of different kinds of amyloid plaques.

13. Blood–brain barrier after ischemia

Ischemic brain injury provoked a number of vessel abnormalities, which are open tight junctions and blood–brain barrier, diffuse leakage through necrotic vessels and vasospasm (Petito et al., 1982; Mossakowski et al., 1993; Mossakowski et al., 1994; Pluta et al 1994a; Wisniewski et al., 1995; Gartshore et al., 1997; Shinnou et al., 1998; Lippoldt et al., 2000; Ueno et al., 2002; Pluta 2003; Pluta 2005; Pluta et al., 2006b). Till one year after ischemic brain injury brain white and gray regions contained many diffuse and focal sites of horseradish peroxidase and gadolinium extravasations (Mossakowski et al., 1994; Pluta et al., 1994a; Pluta 2003; Pluta 2005; Pluta et al., 2006; Andjus 2010). Horseradish peroxidase leakage involved capillaries, venules, veins and arterioles. The above leakage was observed in hippocampus, cortex, thalamus and basal ganglia, and cerebellum. In summary in ischemic brains were chronic blood–brain barrier abnormalities.

Short-term survival after ischemic brain injury, animals presented within gray and white matter around blood-brain barrier vessels staining for all parts of amyloid precursor protein (Pluta et al., 1994b). On the contrary, after long-term survival immunostaining only for the neurotoxic β-amyloid peptide and to the C-terminal of amyloid precursor protein was noted (Pluta et al., 1997b; Pluta 2000; Pluta 2003; Pluta 2005; Pluta et al., 2010). Multiple and abundant β-amyloid peptide and C-terminal of amyloid precursor protein staining embraced or adjoined the blood-brain barrier vessels. Diffuse deposits of β-amyloid peptide and C-terminal of amyloid precursor protein like "puff of smoke" were also noted. Immunostaining inside capillaries with a halo of β-amyloid peptide and C-terminal of amyloid precursor protein staining around vessels (Pluta 2005; Pluta et al., 2009) indicated diffusion of this part of amyloid precursor and β-amyloid peptide across the blood-brain barrier vessels. Above deposits were observed mainly in the hippocampus, entorhinal and brain cortex.

The above observations were supported by *i.v.* injection of human β-amyloid peptide 42 into animals with *cardiac arrest*, which accumulated amyloid in the ischemic brain white and gray matter in perivascular space (Pluta et al., 1996; Pluta et al., 1997a; Pluta et al., 1999). β-amyloid peptide 42 can be moved by the blood–brain barrier receptor mediated system (Deane et al., 2003; Deane et al., 2004a; Deane et al., 2004b) and by blood–brain barrier leakage caused by ischemic brain injury (Pluta et al., 1996; Pluta et al., 1997a; Pluta et al., 1999; Pluta et al., 2000) or β-amyloid peptide toxicity on blood–brain barrier after ischemia (Thomas et al., 1996; Fiala et al., 1998; Farkas et al., 2003; Paris et al., 2004a; Paris et al., 2004b).

The pathological immunostaining for apolipoproteins A1, E and J was observed mainly around vessels (Kida et al., 1995; Pluta 2000). Perivascular deposits of above proteins were well delineated and irregular. Diffuse, broad, but faint areas were also seen. Extracellular apolipoproteins E and J staining were strongly labeled by antibody to apolipoprotein A1, stronger than by apolipoprotein E antibody (Kida et al., 1995; Pluta et al., 1995a). They were

immunostained stronger by antibody to apolipoprotein E than apolipoprotein J (Kida et al., 1995; Pluta et al., 1995a). It is important to notice that deposits around vessels of apolipoproteins colocalize with deposits of different parts of amyloid precursor protein (Kida et al., 1995; Pluta et al., 1995a). Apolipoprotein E can promote the aggregation of β-amyloid peptide into the fibrillar formation. Clusterin is involved in transport of β-amyloid peptide through the blood–brain barrier. The general role of apolipoproteins is controlling the content of β-amyloid peptide in the extracellular space of brain tissue as well as their control on amyloid plaques development. These data demonstrate significant additive effects of apolipoproteins on controlling β-amyloid peptide accumulation around blood-brain barrier vessels and that they play a main role in influencing extracellular brain β-amyloid peptide metabolism/clearance independent of β-amyloid peptide formation. Another activity, for apolipoprotein E in ischemic brain tissue is the proposed extracellular clearance of ischemic brain parenchyma by reverse movement of amyloid into blood (Pluta et al., 2000). Delayed clearance may exacerbate healing of the ischemic blood–brain barrier. Above data point out that around vessels apolipoproteins deposition following ischemic brain injury represents a secondary injury processes that could hamper healing and outcome of ischemic brain.

After brain ischemia injury thrombocytes are forming aggregates, which adhere to the endothelium lining of blood–brain barrier vessels (Pluta et al., 1994c; Pluta 2003; Pluta 2005). As an effect of this pathology the "no-reflow phenomenon" is developing (Mossakowski et al., 1993; Pluta et al., 1994c; Pluta 2003). Moreover, trombocytes were noted on the abluminal side of vessels following ischemic brain injury (Pluta et al., 1994c; Pluta 2003; Pluta 2005). This kind of pathology was observed in capillaries, venules, veins and arterioles independently of time after ischemic brain injury. Some study suggests that brain ischemia results in development platelet-leukocytes aggregates (Ishikawa et al., 2004) in the peripheral circulatory system (Ritter et al., 2005). Another study showed strong platelet-leukocyte-endothelium reactions following focal ischemic brain injury (Ishikawa et al., 2004). An increasing body of evidence has supported the idea that white cells can play an additional pathological function in brain ischemia injury (Caceres et al., 1995; Gidday et al., 2005). White blood cells matrix metalloproteinase-9 recruited to the brain ischemic tissue next white blood cells to the same brain regions in a positive feedback manner and influence chronic opening of blood–brain barrier following a primary ischemic injury (Gidday et al., 2005). Investigation by electron microscopy of ischemic blood–brain barrier presented leukocytes adhering to the endothelial cells of capillaries and venules (Caceres et al., 1995). This observation is suggested probable movement of leukocytes across blood-brain barrier vessels. Endothelial cells alterations and white blood cells aggregation and finally their adherence to vessel wall also reflect a "no-reflow phenomenon".

14. Ischemic blood–brain barrier and β-amyloid peptide

In ischemic blood-brain barrier vessels damaged endothelium presented ruptures of endothelial membranes (Caceres et al., 1995). Other studies of ischemic endothelium presented an increased number of endothelium microvilli and deep crater-like pits, and enlarged junctional ridging with undulations of basement membrane (Pluta et al., 1991). As an effect of presented alterations, platelets developed microthrombi, which attached to the vessel wall and caused a permanent supply of neurotoxic constrictors such as β-amyloid peptide (Chen et al., 1995; Thomas et al., 1996). Final effect of above phenomenon is

pathological vasoconstriction during reperfusion (Wisniewski et al., 1995; Ohtake et al., 2004). Recent data suggest that thrombocytes are the main ischemic factor in recirculation injury, not only through thrombus formation but also as cause of inflammation in cooperation with leukocytes (Nishijima et al., 2004). Due to the fact that β-amyloid peptide causes vasoconstriction (Thomas et al., 1996; Niwa et al., 2000) and endothelium damage (Thomas et al., 1996), a role for β-amyloid peptide in vasoconstriction and blood–brain barrier pathology has been proved. During reperfusion after brain ischemia, islets of necrotic endothelial cells in blood-brain barrier were noted (Petito et al., 1982; Mossakowski et al., 1994; Pluta et al., 1994a). Necrotic blood–brain barrier characterizes diffuse leakage of blood elements (Petito et al., 1982; Mossakowski et al., 1994; Pluta et al., 1994a; Pluta et al., 1994c) and different parts of amyloid precursor protein from blood serum (Pluta et al., 1994b; Pluta et al., 1996). This process is probably due to senescent endothelium and this phenomenon is increased during recirculation and is augmented by β-amyloid peptide toxicity (Mossakowski et al., 1994). Senescent endothelium is a common feature of vessel aging (Erusalimsky, Kurz 2005) and is also influenced by ischemic episodes (Mossakowski et al., 1994). Another problem during reperfusion with senescent endothelium is covering it with β-amyloid peptide where β-amyloid peptide acts as antiangiogenic factor (Paris et al., 2004a; Paris et al., 2004b). Ischemic brain insults together with β-amyloid peptide have harmful effects on astrocytes and pericytes (Lupo et al., 2001; Anfuso et al., 2004) and can influence blood–brain barrier vessel angiogenesis and finally can regulate the blood–brain barrier activity (Ramsauer et al., 2002).

15. Disabilities after ischemia

In addition to pathological and pathophysiological effects, cognitive abnormalities have been showed after ischemic brain injury (Block 1999; Kiryk et al., 2011). The cognitive abnormalities were found in regions of selective vulnerability to ischemic injury and they come before neuronal death. In addition, other brain areas, which are devoid of ischemic primary neurons lesions, display some functional changes. These abnormalities mainly seem to be due synaptic damage. Ischemic brain injury does not result in long-lasting neurological deficits in ischemic animals (Block 1999; Kiryk et al., 2011). Some spontaneous recovery of sensorimotor function has been demonstrated after brain ischemia (Yang, Simpkins 2007). Following ischemic brain injury a locomotor hyperactivity has been noted for 7 days (Kuroiwa et al., 1991; Karasawa et al., 1994). Hyperactivity was directly connected with neuronal alterations in the ischemic hippocampus (Kuroiwa et al., 1991; Kiryk et al., 2011). Longer ischemia and longer locomotor hyperactivity is significantly associated with increased hippocampal neurons changes (Block 1999). After ischemic brain injury, impairment in habituation up to 6 months as revealed by longer exploration time was noted (Mileson, Schwartz 1991; Colbourne, Corbett, 1995). Brain ischemia results in reference and working memory deficits (Davis et al., 1986; Kiyota et al., 1991; Kiryk et al., 2011). In addition, brain ischemia in animals leads to deterioration of spatial memory for up to 1.5 year (Block, Schwarz 1998; Karhunen et al., 2003; Kiryk et al., 2011). Deterioration of cognitive impairment has been observed consistently during reperfusion (Roof et al., 2001; Karhunen et al., 2003; Kiryk et al., 2011). Besides, data on repetitive ischemic brain injury have shown persistent locomotor hyperactivity, reduced anxiety, and severe cognitive deficits (Ishibashi et al., 2006). Above pathology was associated with brain atrophy, which connected with diffuse neurons loss in the CA1 sector of the hippocampus and in the brain

cortex (Ishibashi et al., 2006). Alertness and sensorimotor capacities are affected for 2 days, whereas deficits in learning and memory seem to be rather long lasting (Kiryk et al., 2011). Taken together strong evidence from both basic research and epidemiological studies indicated that the deterioration of cognitive activities could not be explained only by direct primary ischemic brain injury, but rather by a progressive consequence of the additive effects of the ischemic episodes, aging and Alzheimer's factors (Pasquier, Leys 1997; Popa-Wagner 2007).

16. New guarding of ischemic brain injury

16.1 Anti-amyloid therapy

1998 is a turning point in the new history of novel strategies in ischemic brain injury and Alzheimer's disease treatment (Pluta et al., 1998a). At first the full success against human β-amyloid peptide 42 *i.v.* immunization in rats with brain ischemic injury (Pluta et al., 1998a; Pluta et al., 1999) and second moderate effect by intraperitoneal immunization in transgenic mouse overexpressing amyloid precursor (Schenk et al., 1999) and third peripheral administration antibodies against β-amyloid peptide (Bard et al., 2000) led to the fast development of new therapies against amyloid pathology.

Human β-amyloid peptide removal/treatment has remarkable effects in ischemic brain injury (Pluta et al., 1998a; Pluta et al., 1999; Pluta, Ulamek 2008) and less effect in mice with overexpressed amyloid pathology (Schenk et al., 1999). Experience in patient's vaccination was less convincing (Nicoll et al., 2003; Lemere et al., 2006; Hawkes, McLaurin 2007). Trials in cases with amyloid pathology were stopped when 6% of immunized patients developed meningoencephalitis (Nicoll et al., 2003; Orgogozo et al., 2003; Gilman et al., 2005). Moreover, only 20% of patients synthesized antibody against amyloid (Gilman et al., 2005). Patients treated with autovaccine in *post mortem* examination had less amyloid plaques in brain, as well as occurrence of T-cell lymphocytes. Recently, it has been proved that antibodies against β-amyloid are in normal human immunoglobulin that in particular recognize and inhibit the toxic hallmarks of β-amyloid peptide (Dodel et al., 2004). Within the past decade treatments have been concerned on inhibitors of γ- and β-secretases responsible for cleavage β-amyloid peptide from amyloid precursor protein (Dovey et al., 2001; Selkoe 2001; Roberts 2002). Reduction of β-amyloid peptide in the brain parenchyma of aged rats after oral administration of the γ-secretase inhibitors has been noted result in decrease levels of β-amyloid in both cerebrospinal fluid and brain tissue (Best et al., 2006; El Mouedden et al., 2006). Another recent study was used antibody anti-β-secretase in which decrease of amyloid was shown in transgenic model of amyloid pathology (Rakover et al., 2007). This decrease correlated very well with improvement of cognitive function. Two single-chain antibodies have been shown to possess α-secretase activity supplying a novel use of vaccine (Rangan et al., 2003; McCarty 2006). Another group of scientists have used small particle libraries to screen for substances that either interfere with assembly of β-amyloid particles into fibrils (Lashuel et al., 2002; De Felice et al., 2004) or disaggregate them (Soto 2001; Gong et al., 2003; Blanchard et al., 2004).

Neprilysin is β-amyloid peptide degrading enzyme in the brain (Kanemitsu et al., 2003). Human neprilysin gene transfer into brain leads to a remarkable decrease of β-amyloid deposits in transgenic mice with amyloid pathology (Marr et al., 2003). These observations proved that the deficient metabolism of β-amyloid caused by decrease level of neprilysin might contribute to pathological amyloidogenic cascades including ischemic brain injury.

Ischemic brain injury results in the downregulation of α-secretase mRNA and decreases its net activity (Nalivaeva et al., 2004; Yan et al., 2007). Insulin degrading enzyme is another enzyme for β-amyloid clearance in the brain (McCarty 2006). Overexpression of above enzyme reduces β-amyloid levels and retards or completely prevents amyloid plaques development in the brain (Leissring et al., 2003). Some other enzymes like endothelin converting enzyme and angiotensin converting enzyme degraded/metabolized β-amyloid peptide, too (Eckman et al., 2003, Hemming, Selkoe 2005).
Treatment by gelsolin a molecule that has high affinity for β-amyloid reduced the level of β-amyloid in the brain intra- and extracellular space by peripheral action (Matsuoka et al., 2003). Other β-amyloid drug curcumin can moved across blood-brain barrier and reduce amyloid level and amyloid plaque burden in transgenic mice with amyloid pathology (Yang et al., 2005). The enoxaparin β-amyloid drug significantly reduced β-amyloid aggregates in cortex and the total amyloid cortical concentration by combining the blood serum β-amyloid peptide in systemic circulatory (Bergamaschini et al., 2004). In compliance with the sink hypothesis molecules, which are combining β-amyloid peptide in inactive complexes in the blood serum decreases the level of blood β-amyloid peptide, which then increase a net efflux of β-amyloid peptide from the brain into blood plasma (DeMattos et al., 2001; DeMattos et al., 2002).
Recently endogenous receptor for advanced glycation-end-products peptides and β-amyloid peptide antibodies has been found in sick and healthy subjects (Mruthinti et al., 2004). These observations suggest that naturally occurring antibodies for β-amyloid peptide and receptor for advanced glycation-end-products control β-amyloid peptide level in brain and peripheral blood.

16.2 Anti-tauopathy therapy

A novel therapy has been directed against hyperphosphorylated tau protein either by inhibiting various protein kinases or promoting phosphatase activities (Lau et al., 2002; Iqbal, Grudke-Iqbal 2004; Klafki et al., 2006). Recent *in vitro* studies shown particles, which inhibited tau protein fibrillization making these molecules a promising candidate to test them in experimental conditions (Chirita et al., 2004). A new interesting data concerning therapy against amyloid have been presented lastly in animals in which triple transgenic mice were injected with β-amyloid peptide antibodies (Oddo et al., 2004). β-amyloid peptide antibodies inducted *i.v.* lead to clearance of early hyperphosphorylated tau protein deposits (Oddo et al., 2004).
Some study showed that memantine reversed hyperphosphorylation of tau protein in hippocampal slices (Li et al., 2004) and this effect of memantine occurred by disinhibition of the activity of protein phosphatase 2A (Chohan et al., 2006) that earlier was noted to be downregulated in brains with amyloid pathology (Gong et al., 1993). Based on above data it was shown in humans that treatment amyloid pathology by memantine during one year significantly decreases hyperphosphorylated tau in cerebrospinal fluid (Gunnarsson et al., 2006).

16.3 Suppressing neuroinflammation

In ischemic brains the microglia are presented as neuroinflammatory invaders, which adding additional events *via* synthesis of cytokines designed to answer to primary neuropathology. This activity may lead to significant progression of brain ischemia cases

through neurons loss. Epidemiological studies suggest that long use of anti-inflammatory treatment in amyloid and neuroinflammatory disease like Alzheimer's disease can prevent its development (Moore, O'Banion 2002; Szekely et al., 2004). From these observations considerable studies were undertaken to investigate the influence of anti-inflammation treatment in ischemic and amyloid brain diseases. These studies include nonsteroidal anti-inflammatory therapy (Morihara et al., 2005), cannabinoids (Ramirez et al., 2005) and peroxisome proliferator-activated receptor-γ agonists (Sastre et al., 2003; Echeverria et al., 2005; Heneka et al., 2005; Sastre et al., 2006). Current results from transgenic model of amyloid pathology was presented data that therapy against β-secretase decreases reaction of neuroinflammation in brain (Rakover et al., 2007).
Delivery umbilical cord blood cells 48 h after ischemic brain injury are developing neuroprotection by blocking the neuroinflammatory reactions (Willing et al., 2007). Above cells show protective activities *via*: modulating the neuroinflammatory response, stopping the apoptotic events and enhancing neurogenesis and angiogenesis. Activation of sigma-1 and -2 receptors *via* 1,3-di-*0*-tolylguanidin injection 24 h after ischemic brain injury is impressive in reducing ischemic damage (Willing et al., 2007). Above substance is protective by reducing inflammatory reaction and decreasing intracellular calcium in neurons and by stopping the synthesis of cytokines. In ischemic brain informations from the damaging neuronal cells trigger immune cells for an inflammatory activity, with overproduction of cytokines. Whether the cause is known or not, neurological disorders present similar cellular neuronal abnormalities and inflammation. These treatments approaches may not only be beneficial for therapy of ischemic brain injury (Willing et al., 2007) but also other neurological disorders. Above presented treatments act in a similar manner by increasing neurons survival and inhibiting the activity of general immune system (Willing et al., 2007).

16.4 Protecting blood-brain barrier

The natural activity of the brain is associated with the coupling between cerebral blood flow and transport *via* the blood-brain barrier and neurons activity. Cerebral blood flow controls the neuronal physiological environment not only by regulation of local blood flow but also by regulating focal transport through blood-brain barrier. The blood-brain barrier is an energetic system with two sites of transport by its blood- and brain-facing sites. Structure and function of the blood facing side allows entry of nutrients products but opposite brain facing eliminate metabolites such as β-amyloid peptide from brain (Pluta et al., 2000; Deane et al., 2004a; Deane et al., 2004b; Zlokovic 2005). A main role of the blood-brain barrier is control of the brain pool of pathological β-amyloid peptide. The aim of this part of chapter is to analyze knowledge of the association of the ischemic blood-brain barrier with final ischemic brain injury, especially with regards to the formation different amyloid plaques (Pluta 2006a; Pluta 2006b; Pluta 2007a; Pluta 2007b) and to develop a consensus on whether blood-brain barrier changes are a valid target for brain ischemia treatment (Pluta 2006a; Pluta 2006b; Pluta 2007a; Pluta 2007b; Pluta, Ulamek 2008).
According to the new ischemic blood-brain barrier maturation idea of ischemic brain injury (Pluta 2006a) all parts of blood-brain barrier such as endothelium, basal lamina, pericyte and astrocyte cells are main targets for treatment of above disorder (Sohrabji 2007). The current idea states that pathological blood-brain barrier activity caused by ischemic injury at its abluminal and luminal sides for β-amyloid with damaged neurons by ischemic insult are responsible for full-blown late onset ischemic-type dementia (Pluta 2004b; Pluta 2006a; Pluta 2006b; Pluta 2007a; Pluta 2007b). In this way a novel and more effective therapy approaches

can be formulated and more data on different kind amyloidosis can be gathered. Aforementioned data suggest that reducing movement of β-amyloid peptide from blood to brain tissue (Dickstein et al., 2006) and significantly improving reverse transport from brain into blood plasma (Pluta et al., 2000; Bell et al., 2009) and preventing ischemic events in neurons (Pluta 2007c see for references) are principal main future points in treatment of ischemic brain injury (Iwata et al., 2001; Moore, O'Banion 2002; Cheng et al., 2003; Deane et al., 2003; Borlongan et al., 2004; Deane et al., 2004a; Deane et al., 2004b; Guo et al., 2004; Kalback et al., 2004; Koistinaho et al., 2004; Tanzi et al., 2004; Pluta, Ulamek 2008]. Current data provide new information that injection with β-amyloid peptide reduces blood-brain barrier leakage, amyloid burden and microgliosis in transgenic model of amyloid pathology (Dickstein et al., 2006). It was presented that the blood-brain barrier is damaged in amyloid diseases and after β-amyloid peptide delivery the immune system clears amyloid from the brain as it would in peripheral organs lacking barriers. Once β-amyloid is cleared the activity of the blood-brain barrier is restored (Dickstein et al., 2006). This study directly proves that the blood-brain barrier is disrupted in amyloid brain diseases (Bowman et al., 2007; Sohrabji 2007; Zipser et al., 2007; Pluta et al., 2009) and that vaccination with β-amyloid peptide heals the sides of damage blood-brain barrier in transgenic mice with amyloid pathology (Dickstein et al., 2006) and ischemic brain injury (Pluta et al., 2000). Earlier my laboratory has proved that *i.v.* immunization with human β-amyloid peptide 42 in brain ischemia heals blood-brain barrier leakage for β-amyloid peptide 42 (Pluta et al., 2000) and prevent disease neuroprogression (Pluta et al., 1998a; Pluta et al., 1999). Possible explanation of the reparation of the blood-brain barrier is that the vaccination leads to the decrease in the level of circulating β-amyloid peptide (Dickstein et al., 2006), which could directly and/or indirectly damage the blood-brain barrier (Farkas et al., 2003; Marco, Skaper 2006; Bell et al., 2009). For example inflammatory factors (Boutin et al., 2001) that stimulate angiogenesis (Grammas, Ovase 2001) and β-amyloid peptide have been shown to influence an increase of some angiogenic factors like VEGF and TGF-β (Tarkowski et al., 2002; Pogue, Lukiw 2004). It can be proposed that with the removal of information provided by β-amyloid peptide the endothelial cells behave normally and tight junctions are closed, thereby restoring a natural blood-brain barrier function. Increased concentration in plasma β-amyloid peptide has been observed in a transgenic mice with amyloid pathology after active amyloid vaccination and *i.v.* delivery of molecules with an affinity to β-amyloid peptide (DeMattos et al., 2002; Matsuoka et al., 2003) and after active immunization (Pluta et al., 1998a; Pluta et al., 1999) of non-human primates (Lemere et al., 2004). It is proposed that molecules that sequester blood serum β-amyloid peptide may decrease or prevent brain amyloidosis (Matsuoka et al., 2003). In addition studies with antibodies anti-intercellular adhesion molecule-1 (Zhang et al., 1994) or platelet-endothelial cell adhesion molecule-1 (Rosenblum et al., 1994) have presented that blockage of adhesion molecules and leukocyte adhesion or platelets (>90% of β-amyloid peptide is stored in blood platelets) attachment respectively reduces ischemic brain damage after effects.

Several different ways have been suggested to remove β-amyloid peptide by blood-brain barrier including: receptor-mediated β-amyloid peptide transport by blood-brain barrier, enzyme mediated β-amyloid peptide metabolism and β-amyloid peptide bindable molecules that mediated β-amyloid peptide clearance. Receptor mediated transport of β-amyloid peptide by blood-brain barrier is responsible for both influx and efflux of β-amyloid peptide. Lipoprotein receptor-related protein mediates efflux of β-amyloid peptide from brain tissue into blood (Deane et al., 2004a; Deane et al. 2004b; Bell et al., 2009).

The interaction between lipoprotein receptor-related protein and β-amyloid peptide mediates amyloid blood-brain barrier vessels binding, endocytosis and transcytosis through blood-brain barrier into circulatory system (Herz 2003). Moreover, p-glycoprotein has been proposed to be engaged in amyloid movement by blood-brain barrier (Lam et al., 2001). Currently some data noted that the neonatal Fc receptor at the blood-brain barrier has an important role in IgG-assisted β-amyloid peptide removal from the brain (Deane et al., 2005). Receptor for advanced glycation-end-products mediates influx of β-amyloid peptide from blood into brain tissue (Deane et al., 2003; Deane et al., 2004b). Decrease of receptor for advanced glycation-end-products can reduce influx of β-amyloid peptide into brain (Deane et al., 2003). Glycoprotein 330/megalin probably is involved in receptor-mediated transport of apolipoprotein J alone and in a complex with amyloid at the blood-brain barrier (Zlokovic et al., 1996). Lipoprotein receptor-related protein and receptor for advanced glycation-end-products play opposing roles in amyloid transport through blood-brain barrier (Deane et al., 2004b). For now the most important way would be to look for new drugs, which influence the function or overexpression of β-amyloid peptide transport receptors by blood-brain barrier. The reduced function of receptor for advanced glycation-end-products and increased activity of lipoprotein receptor-related protein in ischemic blood-brain barrier might readjust the movement equilibrium for β-amyloid peptide by increasing its net efflux from brain into blood plasma. Statins, which increased lipoprotein receptor-related protein in blood-brain barrier, might facilitate the movement of β-amyloid peptide from brain tissue (Deane et al., 2004a). It is worth noting that receptor for advanced glycation-end-products blockades using receptor for advanced glycation-end-products specific IgG (Mruthinti et al., 2004) can also increase the expression of lipoprotein receptor-related protein (Deane et al., 2004b).

16.5 Therapy by estrogens

The incidence of ischemic brain injury is gender related (Pluta 2006a) and the risk of ischemic brain injury in aged women is greater than in men. The cumulative risk for ischemic brain insults in women is higher because of a lack of estrogen after menopause (Pluta 2006a). Estrogen treatment has been noted as blood–brain barrier function control through intercellular junction proteins (Kang et al., 2006) and/or intracellular transport elements and through protective effects on the cell elements of barrier such as endothelium, pericyte and astrocyte cells (Yang et al., 2005), cells which are vulnerable to influence of ischemia and aging together in the context of natural blood–brain barrier action (Sohrabji 2007; Zipser et al., 2007). Pathological opening of the blood–brain barrier can expose ischemic brain tissue to different cellular and plasma elements from blood that indirectly or directly impair neurons and press other pathological cascades. Age-related events in different sectors of the brain can have far reaching consequences for cognitive deficits e.g. after ischemic brain injury (Kiryk et al., 2011). Most scientists have taken the approach of studying estrogen effects on pathology related to ageing disorders (Simpkins et al., 1997; Dubal et al., 1998; Shi et al., 1998; Chi et al., 2002; Chi et al., 2005). Estrogens exert protective activity in animals ischemic brain injury (Simpkins et al., 1997; Dubal et al., 1998; Shi et al., 1998; Chen et al., 2001; Chi et al., 2002; Yang et al., 2005), but the mechanisms of their protection are not understood. These hormones may guard neuronal integrity (Chen et al., 2001) by readjusting the physiological activity of the blood–brain barrier (Chi et al., 2002; Chi et al., 2005). Another probable mechanism is that estrogens decreases overexpression of amyloid precursor protein messenger RNA in ischemic brain injury (Shi et al., 1998). The

protective effects of estrogens are observed in all of the neurovascular elements like: endothelial, pericyte, astrocyte and neuron cells and microglia (Chen et al., 2001; Yang et al., 2005). In addition after ischemic brain injury estrogens increase cerebral blood flow and decrease secondary ischemic episodes (McCullough et al., 2001). Prevention of ischemic brain injury and treatment of repeated ischemic episodes after primary ischemic insult may have important implications for delayed postischemic pathology like dementia. In view of the earlier data that cognitive deficits are progressing after ischemic brain injury (Kiryk et al., 2011), there is the distinct possibility that we can stop this decline by targeting the gradually progressing degenerative events, which follows ischemic brain injury by aiming at molecular processes now shown to change after brain ischemia.

17. Conclusion

The complex of overlapping events, which potentially lead to neurons death and finally dementia in ischemic brain injury, start with neuronal energy shortages due to the stopped delivery of nutrients products during ischemic episodes. The energy failure during ischemic brain injury is reflected by a fast and rapid depletion of ATP. Ischemic loss of ATP is followed by the dysfunction of ion pumps and depolarization of the neural cells, and the production of high level of reactive oxygen species, which are dangerous for neurons. Reactive oxygen species initiate lipid peroxidation and generation of lipid peroxides, which are metabolized to pathological products (Muralikrishna Adibhatla, Hatcher 2006). Parallel to these events, the activities of antioxidants decrease following ischemic brain injury (Nita et al., 2001). During recirculation a marked increase in the neurotoxic β-amyloid peptide, C-terminal of amyloid precursor protein and neuroinflammatory factors were noted. The β-amyloid peptide and C-terminal of amyloid precursor protein can act as glia stimulants. The presence of large numbers of astrocytes associated with the β-amyloid peptide and C-terminal of amyloid precursor protein deposits, in particular around vessels of the hippocampus, suggest that these amyloid deposits generate chemotactic mediators, which stimulate recruitment of next astrocytes. This suggests that astrocytes gradually accumulate β-amyloid peptide and the amount of accumulation correlates very well with the extent of pathology in the hippocampus and the survival time after ischemic brain injury. β-amyloid peptide within astrocytes appears to be of blood origin, possibly deposited by phagocytosis of locally opened blood-brain barrier (Pluta et al., 1996). In contrast, current results suggest that astrocytes could also act as a source for β-amyloid peptide, because they overexpress β-secretase in response to long-term pathology (Rossner et al., 2005). Although it remains unclear to which degree astrocytes contribute to β-amyloid peptide synthesis or its clearance, it seems apparent that astrocytes contribute to neuroinflammation cascades. In addition, microglia has been associated with amyloid plaques, which indicates that plaques development and the degree of microglia activation are interrelated. β-amyloid peptide stimulates a nuclear factor kappa B dependent pathway that is important for cytokine gene transcription, reactive astrocytes and activated microglia. Moreover, other Alzheimer proteins involved in the ischemic metabolism of amyloid precursor protein have also been implicated in neuroinflammatory reactions. Evidence suggests that neurons themselves are capable of synthesizing neuroinflammatory factors. Thus, neurons can serve as a source of cytokines including tumor necrosis factor-α and interleukin-1β (Orzyłowska et al., 1999; Heneka, O'Banion 2007). It is possible that the neurons proper may exacerbate local neuroinflammatory activities and thus contribute to own progressive distraction in ischemic

brain injury. A study with a transgenic model of amyloid pathology supports the notion that cytokines influence brain deposition of serum amyloid (Guo et al., 2002). It has been suggested that neuroinflammatory factors either raise the susceptibility for β-amyloid peptide deposition or directly influence amyloid precursor protein cleavage. Additionally, cytokines are able to transcriptionally upregulate β-secretase mRNA and its enzymatic activity (Sastre et al., 2003). β-secretase and γ-secretase are key enzymes for β-amyloid peptide synthesis. The above data can be linked to the increased overexpression and activity of β-secretase and γ-secretase noted in animal ischemic brain (Wen et al., 2004a; Polavarapu et al., 2008). Finally, interleukin-1β has been shown to significantly increase amyloid precursor protein production in astrocytes (Rogers et al., 1999). Moreover, cytokines may be involved in neurofibrillary tangle formation. The idea that interleukin-1 is the common link between β-amyloid peptide production, microglia activation and tau phosphorylation has recently been supported by a study in a triple transgenic animal model of amyloid pathology (Oddo et al., 2006). In this study, tau phosphorylation precedes β-amyloid peptide accumulation. Because the β-amyloid peptide and C-terminals of amyloid precursor protein and cytokines, such as interleukin-1β and tumor necrosis factor-α, directly impair neuronal activity, focal neuroinflammatory events may contribute to neurons dysfunction before neurons death. Neuroinflammatory mediators may directly contribute to ischemic brain degeneration like in Alzheimer's disease. β-amyloid peptide is formed by β- and γ-secretases from the amyloid precursor protein. Above reaction is called amyloidogenic cascade. In nonamyloidogenic process mediated by α- and γ-secretases amyloid precursor protein is cleaved within the β-amyloid peptide fragment. In both pathways is the synthesis of a 5 kDa fragment called the amyloid precursor protein intercellular domain that is proposed to contribute the progressing neuropathology in degenerative diseases including ischemic brain episodes (Muller et al., 2008). Scientists during the last 15 y have suggested the important role of amyloid precursor protein and its enzymatic processing in the neuropathology of ischemic brain injury. Above is also highlighted by the fact of noted overexpression of mRNA amyloid precursor protein (Shi et al., 1998; Shi et al., 2000), mRNA presenilins (Tanimukai et al., 1998; Pennypacker et al., 1999) and mRNA β-secretase following brain ischemia (Wen et al., 2004a; Chuang et al., 2007). The amyloid precursor protein intercellular domain is synthesized intracellular following enzymatic processing from the plasma membrane-derived amyloid precursor protein and probably might have a more direct influence on ischemic pathology than extracellular β-amyloid peptide. Over the past decade the participation of amyloid precursor protein intercellular domain in cell events have been presented including modulation in gene expression, apoptosis, cytoskeletal dynamics and suppression of neurogenesis (Muller at al., 2008). All these processes initiate and contribute to a *vicious cycle* of the disease process, resulting in progressive synaptic and neuronal dysfunction and loss in ischemia with dementia (Pluta et al., 2009; Kiryk et al., 2011).

Primary brain ischemia creates secondary repeated, transient and silent focal ischemic episodes, which are sufficient to sustain chronic/gradual oxidative stress and other events that could be the reason for the creepy and progressive neurons damage and death (Pluta et al., 2009; Pluta et al., 2010). Evidence derived from mice overexpressing the C-terminal of amyloid precursor protein, indicates that this part of the amyloid precursor protein may promote synaptic degeneration and retrograde neurons death (Oster-Granite et al., 1996), and finally dementia (Nalbantoglu et al., 1997). Moreover, ischemic brain injury is age-dependent (Oster-Granite et al., 1996; Popa-Wagner 2007).

18. Acknowledgment

This study was supported by founds from Mossakowski Medical Research Centre (T5).

19. References

Abe K, Tanzi RE, Kogure K. (1991). Selective induction of Kunitz type protease inhibitor domain-containing amyloid precursor protein mRNA after persistent focal ischemia in rat cerebral cortex. *Neurosci Lett* 125:172–174.

Abramov AY, Canevari L, Duchen MR. (2003). Changes in intracellular calcium and glutathione in astrocytes as the primary mechanism of amyloid neurotoxicity. *J Neurosci* 23:5088–5095.

Adamson RH, Ly JC, Sarai RK, Lenz JF, Altangerel A, Drenckhahn D, Curry FE. (2008) Epac/Rap1 pathway regulates microvascular hyperpermeability induced by PAF in rat mesentery. *Am J Physiol Heart Circ Physiol* 294:H1188-H1196.

Akiyama H, Barger S, Barnum S, Bradt B, Bauer J, Cole GM, Cooper NR, Eikelenboom P, Emmerling M, Fiebich BL, Finch CE, Frautschy S, Griffin WS, Hampel H, Hull M, Landreth G, Lue L, Mrak R, Mackenzie IR, McGeer PL, O'Banion MK, Pachter J, Pasinetti G, Plata-Salaman C, Rogers J, Rydel R, Shen Y, Streit W, Strohmeyer R, Tooyoma I, Van Muiswinkel FL, Veerhuis R, Walker D, Webster S, Wegrzyniak B, Wenk G, Wyss-Coray T. (2000). Inflammation and Alzheimer's disease. *Neurobiol Aging* 21:383-421.

Ali SM, Dunn E, Oostveen JA, Hall ED, Carter DB. (1996). Induction of apolipoprotein E mRNA in the hippocampus of the gerbil after transient global ischemia. *Mol Brain Res* 38:37-44.

Altieri M, Di Piero V, Pasquini M, Gasparini M, Vanacore N, Vicenzini E, Lenzi GL. (2004). Delayed poststroke dementia: a 4-year follow-up study. *Neurology* 62:2193-2197.

Andjus PR, Michetti F, Pluta R, Bacic G. (2010) Imaging the breakdown of the BBB and neuroinflammation in models of neurodegeneration. Conference Abstract: Pharmacology and Toxicology of the Blood-Brain Barrier: State of the Art, Needs for Future Research and Expected Benefits for the EU. Frontiers Conferences. Doi:10.3389/conf.fphar.2010.02.00023 (1-3).

Anfuso CD, Assero G, Lupo G, Nicota A, Cannavo G, Strosznajder RP, Rapisarda P, Pluta R, Alberghima M. (2004). Amyloid beta (1–42) and its beta (25–35) fragment induce activation and membrane translocation of cytosolic phospholipase A (2) in bovine retina capillary pericytes. *Biochem Biophys Acta* 1686:125–138.

Badan I, Platt D, Kessler C, Popa-Wagner A. (2003). Temporal dynamics of degenerative and regenerative events associated with cerebral ischemia in aged rats. *Gerontology* 49:356–365.

Badan I, Dinca I, Buchhold B, Suofu Y, Walker L, Gratz M, Platt D, Kessler CH, Popa Wagner A. (2004). Accelerated accumulation of N- and C-terminal beta APP fragments and delayed recovery of microtubule-associated protein 1B expression following stroke in aged rats. *Eur J Neurosci* 19:2270–2280.

Banati RB, Gehrmann J, Wiesner C, Hossmann KA, Kreutzberg GW. (1995). Glial expression of the β-amyloid precursor protein (APP) in global ischemia. *J Cereb Blood Flow Metab* 15:647–654.

Bard F, Cannon C, Barbour R, Burke RL, Games D, Grajeda H, Guido T, Hu K, Huang J, Johnson-Wood K, Khan K, Kholodenko D, Lee M, Lieberburg I, Motter R, Nguyen M, Soriano F, Vasquez N, Weiss K, Welch B, Seubert P, Schenk D, Yednock. (2000). Peripherally administered antibodies against amyloid beta-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. *Nat Med* 6:916-919.

Barone FC, Feuerstein GZ. (1999). Inflammatory mediators and stroke: new opportunities for novel therapeutics, *J Cereb Blood Flow Metab* 19:819-834.

Bell RD, Deane R, Chow N, Long X, Sagare A, Singh I, Streb JW, Guo H, Rubio A, Van Nostrand W, Miano JM, Zlokovic BV. (2009) SRF and myocardin regulate LRP-mediated amyloid-beta clearance in brain vascular cells. *Nat Cell Biol* 11:143-153.

Bell RD, Zlokovic BV. (2009). Neurovascular mechanisms and blood-brain barrier disorder in Alzheimer's disease. *Acta Neuropathol* 118:103-113.

Benarroch E. (2007). Neurovascular unit dysfunction: A vascular component of Alzheimer disease? *Neurology* 68:1730-1732.

Bergamaschini L, Rossi E, Storini C, Pizzimenti S, Distaso M, Pergo C, De Luigi A, Vergani C, De Simoni MG. (2004). Peripheral treatment with enoxaparin, a low molecular weight heparin, reduces plaques and beta-amyloid accumulation in a mouse model of Alzheimer's disease. *J Neurosci* 24:4181-4186.

Bernaudin M, Nouvelot A, MacKensie ET, Petit E. (1998). Selective neuronal vulnerability and specific glial reactions in hippocampal and neocortical organotypic cultures submitted to ischemia. *Exp Neurol* 150:30–39.

Best JD, Jay MT, Out F, Churcher I, Reilly M, Morentin-Gutierrez P, Pattison C, Harrison T, Shearman MS, Atack JR. (2006). In vivo characterization of Aβ(40) changes in brain and cerebrospinal fluid using the novel γ-secretase inhibitor N-[cis-4-[(4-chlorophenyl)sulfonyl]-4-(2,5-difluorophenyl)cyclohexyl]-1,1,1-trifluoromethane-sulfonami-de (MRK-560) in the rat. *J Pharmacol Exp Ther* 317:786-790.

Blanchard BJ, Chen A, Rozenboom LM, Stafford KA, Weigele P, Ingram VM. (2004). Efficient reversal of Alzheimer's disease fibril formation and elimination of neurotoxicity by a small molecule. *Proc Natl Acad Sci USA* 101:14326-14332.

Block F, Schwarz M. (1998). Global ischemic neuronal damage relates to behavioural deficits: a pharmacological approach. *Neuroscience* 82:791-803.

Block F. (1999). Global ischemia and behavioural deficits. *Prog Neurobiol* 58:279-295.

Borlongan CV, Lind JG, Dillon-Carter O, Yu G, Hadman M, Cheng C, Carroll J, Hess DC. (2004). Bone marrow grafts restore cerebral blood flow and blood-brain barrier in stroke rats. *Brain Res* 1010:108-116.

Bornstein NM, Gur AY, Treves TA, Reider-Groswasser I, Aronovich BD, Klimovitzky SS, Varssano D, Korczyn AD. (1996). Do silent brain infarctions predict the development of dementia after first ischemic stroke? *Stroke* 27:904-905.

Boutin H, LeFeuvre RA, Horai R, Asano M, Iwakura Y, Rothwell NJ. (2001). Role of IL-1α and IL-1β in ischemic brain damage. *J Neurosci* 21:5528-5534.

Bowman GL, Kaye JA, Moore M, Waichunas D, Carlson NE, Quinn JF. (2007) Blood-brain barrier impairment in Alzheimer disease: stability and functional significance. *Neurology* 68:1809-1814.

Brkovic A, Sirois MS. (2007) Vascular permeability induced by VEGF family members in vivo: role of endogenous PAF and NO synthesis. *J Cell Biochem* 100:727-737.

Butler TL, Kassed CA, Sanberg PR, Willing AE, Pennypacker KR. (2002). Neurodegeneration in the rat hippocampus and striatum after middle cerebral artery occlusion. *Brain Res* 929:252–260.

Caceres MJ, Schleien CL, Kuluz JW, Gelman B, Dietrich WD. (1995). Early endothelial damage and leukocyte accumulation in piglet brains following cardiac arrest. *Acta Neuropathol* 90:582–591.

Cagnin A, Brooks DJ, Kennedy AM, Gunn RN, Myers R, Turkheimer FE, Jones T, Banati RB. (2001). In-vivo measurement of activated microglia in dementia. *Lancet* 358:461-467.

Callea L, Arese M, Orlandini A, Bargnani C, Priori A, Bussolino F. (1999). Platelet activating factor is elevated in cerebral spinal fluid and plasma of patients with relapsing-remitting multiple sclerosis. *J Neuroimmunol* 94:212-221.

Castillo J, Rodriguez I. (2004). Biochemical changes and inflammatory response as markers for brain ischaemia: molecular markers of diagnostic utility and prognosis in human clinical practice, *Cerebrovasc Dis* 17:Suppl 1:7-18.

Chen M, Inestrosa NC, Ross GS, Fernandez HL. (1995). Platelets are the primary source of amyloid-β peptide in human blood. *Biochem Biophys Res Commun* 213:96–103.

Chen J, Xu W, Jiang H. (2001). 17beta-estradiol protects neurons from ischemic damage and attenuates accumulation of extracellular excitatory amino acids. *Anesth Analg* 92:1520–1523.

Cheng T, Liu D, Griffin JH, Fernandez JA, Castellio F, Rosen ED, Fukudome K, Zlokovic BV. (2003). Activated protein C blocks p53-mediated apoptosis in ischemic human brain endothelium and is neuroprotective. *Nat Med* 9:338-342.

Chi OZ, Liu X, Weiss HR. (2002). Effects of 17beta-estradiol on blood-brain barrier disruption during focal ischemia in rats. *Horm Metab Res* 34:530–534.

Chi OZ, Hunter C, Liu X, Weiss HR. (2005). Effects of 17beta-estradiol on blood-brain barrier disruption in focal ischemia during GABA (A) receptor inhibition. *Horm Metab Res* 37:209–213.

Chirita C, Necula M, Kuret J. (2004). Ligand-dependent inhibition and reversal of tau filament formation. *Biochemistry* 43:2879-2887.

Chohan MO, Khatoon S, Grundke-Iqbal IG, Iqbal K. (2006). Involvement of I2PP2A in the abnormal hyperphosphorylation of tau and its reversal by memantine. *FEBS Lett* 580:3973-3979.

Chuang CM, Hsieh CL, Lin HY, Lin JG. (2008). Panax Notoginseng Burk attenuates impairment of learning and memory functions and increases ED1, BDNF and beta-secretase immunoreactive cells in chronic stage ischemia-reperfusion injured rats. *Am J Chin Med* 36:685-693.

Cognasse F, Hamzeh-Cognasse H, Lafarge S, Chavarin P, Cogne M, Richard Y, Garraud O. (2007). Human platelets can activate peripheral blood B cells and increase production of immunoglobulins. *Exp Hematol* 35:1376-1387.

Colbourne F, Corbett D. (1995). Delayed postischemic hypothermia: a six month survival study using behavioral and histological assessments of neuroprotection. *J Neurosci* 15:7250-7260.

Cotter RL, Burke WJ, Thomas VS, Potter JF, Zheng J, Gendelman HE. (1999). Insights into the neurodegenerative process of Alzheimer's disease: a role for mononuclear phagocyte-associated inflammation and neurotoxicity. *J Leukocyte Biol* 65:416–427.

D'Andrea MR, Cole GM, Ard MD. (2004). The microglial phagocytic role with specific plaque types in the Alzheimer disease brain. *Neurobiol Aging* 25:675–683.

Danton GH, Dietrich WD. (2003). Inflammatory mechanisms after ischemia and stroke. *J Neuropathol Exp Neurol* 62:127-136.

Davis HP, Tribuna J, Pulsinelli WA, Volpe BT. (1986). Reference and working memory of rats following hippocampal damage induced by transient forebrain ischemia. *Physiol Behav* 37:387-392.

Deane R, Du Yan S, Submamaryan RK, LaRue B, Jovanovic S, Hogg E, Welch D, Manness L, Lin C, Yu J, Zhu H, Ghiso J, Frangione B, Stern A, Schmidt AM, Armstrong DL, Arnold B, Liliensick B, Nawroth P, Hofman F, Kind M, Stern D, Zlokovic B. (2003). RAGE mediates amyloid-β peptide transport across the blood-brain barrier and accumulation in brain. *Nat Med* 9:907–913.

Deane R, Wu Z, Sagare A, Davis J, Du Yan S, Hamm K, Xu F, Parisi M, LaRue B, Hu HW, Spijkers P, Guo H, Song X, Lenting PJ, Van Nostrand WE, Zlokovic BV. (2004). LRP/amyloid β-peptide interaction mediates differential brain efflux of Aβ isoforms. *Neuron* 43:333-344.

Deane R, Wu Z, Zlokovic BV. (2004). RAGE (yin) versus LRP (yang) balance regulates alzheimer amyloid β-peptide clearance through transport across the blood-brain barrier. *Stroke* 35:Suppl 1:2628-2631.

Deane R, Sagare A, Hamm K, Parisi M, LaRue B, Guo H, Wu Z, Holtzman DM, Zlokovic BV. (2005). IgG-assisted age-dependent clearance of Alzheimer's amyloid beta peptide by the blood-brain barrier neonatal Fc receptor. *J Neurosci* 25:11495-11503.

De Felice FG, Vieira MN, Saraiva LM, Figueroa-Villar JD, Garcia-Abreu J, Liu R, Chang L, Klein WL, Ferreira ST. (2004). Targeting the neurotoxic species in Alzheimer's disease: inhibitors of Abeta oligomerization. *FASEB J* 18:1366-1372.

De la Torre JC. (2005). Is Alzheimer's disease preceded by neurodegeneration or cerebral hypoperfusion? *Ann Neurol* 57:783-784.

DeMattos RB, Bales KR, Cummins DJ, Dodart JC, Paul SM, Holtzman DM. (2001). Peripheral anti-A beta antibody alters CNS and plasma A beta clearance and decrease brain A beta burden in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci USA* 98:8850-8855.

DeMattos RB, Bales KR, Cummins DJ, Paul SM, Holtzman DM. (2002). Brain to plasma amyloid-β efflux: a measure of brain amyloid burden in a mouse model of Alzheimer's disease. *Science* 295:2264-2267.

DeMattos RB, Bales KR, Cummins DJ, Dodart JC, Paul SM, Holtzman DM. (2001). Peripheral anti-A beta antibody alters CNS and plasma A beta clearance and decrease brain A beta burden in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci USA* 98:8850-8855.

DeMattos RB, Bales KR, Cummins DJ, Paul SM, Holtzman DM. (2002). Brain to plasma amyloid-β efflux: a measure of brain amyloid burden in a mouse model of Alzheimer's disease. *Science* 295:2264-2267.

Desmond DW, Moroney JT, Sano M, Stern Y. (2002). Incidence of dementia after ischemic stroke: results of a longitudinal study. *Stroke* 33:2254-2260.

Dewar D, Graham DI, Teasdale GM, McCulloch J. (1993). Alz-50 and ubiquitin immunoreactivity is induced by permanent focal cerebral ischaemia in the cat. *Acta Neuropathol* 86:623-629.

Dewar D, Graham DI, Teasdale GM, McCulloch J. (1994). Cerebral ischemia induces alterations in tau and ubiquitin proteins. *Dementia* 5:168-173.

Dewar D, Dawson D. (1995). Tau protein is altered by focal cerebral ischaemia in the rat: an immunohistochemical and immunoblotting study. *Brain Res* 684:70-78.

Dickstein DL, Biron KE, Ujiie M, Pfeifer CG, Jeffries AR, Jefferies WA. (2006). Aβ peptide immunization restores blood-brain barrier integrity in Alzheimer disease. *FASEB J* 20:426-433.

Dihne M, Block F. (2001). Focal ischemia induces transient expression of IL-6 in the substantia nigra pars reticulata, *Brain Res* 889:165-173.

Dodel RC, Du Y, Depboylu C, Hampel H, Frolich L, Haag A, Hemmeter U, Paulsen S, Teipel SJ, Brettschneider S, Spottke A, Nolker C, Moller HJ, Wie X, Farlow M, Sommer N, Oertel WH. (2004). Intravenous immunoglobulins containing antibodies against β-amyloid for the treatment of Alzheimer's disease. *J Neurol Neurosurg Psychiatry* 75:1472-1474.

Dovey HF, John V, Anderson JP, Chen LZ, de Saint Andrieu P, Fang LY, Freedman SB, Folmer B, Goldbach E, Holsztynska EJ, Hu KL, Johnson-Wood KL, Kennedy SL, Kholodenko D, Knops JE, Latimer LH, Lee M, Liao Z, Lieberburg IM, Motter RN, Mutter LC, Nietz J, Quinn KP, Sacchi KL, Seubert PA, Shopp GM, Thorsett ED, Tung JS, Wu J, Yang S, Yin CT, Schenk DB, May PC, Altstiel LD, Bender MH, Boggs LN, Britton TC, Clemens JC, Czilli DL, Dieckman-McGinty DK, Droste JJ, Fuson KS, Gitter BD, Hyslop PA, Johnstone EM, Li WY, Little SP, Mabry TE, Miller FD, Audia JE. (2001). Functional gamma-secretase inhibitors reduce beta-amyloid peptide levels in the brain. *J Neurochem* 76:173-181.

Dubal DB, Kashon ML, Pettigrew LC, Ren JM, Finklestein SP, Rau SW, Wise PM. (1998). Estradiol protects against ischemic injury. *J Cereb Blood Flow Metab* 18:1253–1258.

Echeverria V, Clerman A, Dore S. (2005). Stimulation of PGE2 receptors EP2 and EP4 protects cultured neurons against oxidative stress and cell death following β-amyloid exposure. *Eur J Neurosci* 22:2199-2206.

Eckman EA, Watson M, Marlow L, Sambamurti K, Eckman CB. (2003). Alzheimer's disease beta-amyloid peptide is increased in mice deficient in endothelin-converting enzyme. *J Biol Chem* 278:2081-2084.

El Mouedden M, Vandermeeren M, Meet T, Mercken M. (2006). Reduction of Aβ levels in the Sprague Dawley rat after oral administration of the functional γ-secretase inhibitor, DAPT: a novel non-transgenic model for Aβ production inhibitors. *Curr Pharm Des* 12:671-676.

Erusalimsky JD, Kurz DJ. (2005). Cellular senescence in vivo: its relevance in aging and cardiovascular disease. *Exp Gerontol* 40:634–642.

Farkas IG, Czigner A, Farkas E, Dobo E, Soos K, Penke B, Eudresz V, Mihaly A. (2003). Beta-amyloid peptide-induced blood-brain barrier disruption facilitates T-cell entry into the rat brain. *Acta Histochem* 105:115–125.

Fiala M, Zhang L, Gan X, Sherry B, Tanb D, Graves MC, Hama S, Way D, Weinand M, Witte M, Lorton D, Kuo YM, Roher AE. (1998). Amyloid-beta induces chemokine secretion and monocyte migration across a human blood-brain barrier model. *Mol Med* 4:480–489.

Fillit H, Hill J. (2002). The costs of vascular dementia: a comparison with Alzheimer's disease. *J Neurol Sci* 203-204:35-39.

Flynn RWV, MacWalter RSM, Doney ASF. (2008). The cost of cerebral ischemia. *Neuropharmacology* 55:250-256.

Fujioka M, Taoka T, Matsuo Y, Mishima K, Ogoshi K, Kondo Y, Isuda M, Fujiwara M, Asano T, Sakaki T, Miyasaki A, Park D, Siesjo BK. (2003). Magnetic resonance imaging shows delayed ischemic striatal neurodegeneration. *Ann Neurol* 54:732–747.

Gartshore G, Patterson J, Macrae IM. (1997). Influence of ischemia and reperfusion on the course of brain tissue swelling and blood-brain barrier permeability in a rodent model of transient focal cerebral ischemia. *Exp Neurol* 147:353–360.

Geddes JW, Schwab C, Craddock S, Wilson JL, Pettigrew LC. (1994). Alterations in tau immunostaining in the rat hippocampus following transient cerebral ischemia. *J Cereb Blood Flow Metab* 14:554-564.

Gehrmann J, Bonnekoh P, Miyazawa T, Hossmann KA, Kreutzberg G. (1992). Immunocytochemical study of early microglial activation in ischemia. *J Cereb Blood Flow Metab* 12:257-269.

Gidday JM, Gasche YG, Copin JC, Shah AR, Perez RS, Shapiro SD, Chan PH, Park TS. (2005). Leukocyte-derived matrix metalloproteinase-9 mediates blood-brain barrier breakdown and is proinflammatory after transient focal cerebral ischemia. *Am J Physiol Heart Circ Physiol* 289:H558–H568.

Gilman S, Koller M, Black RS, Jenkins L, Griffith SG, Fox NC, Eisner L, Kirby L, Rovira MB, Forette F, Orgogozo JM, AN1792 (QS-21)-201 Study Team. (2005). Clinical effects of Abeta immunization (AN1792) in patients with Alzheimer's disease in an interrupted trial. *Neurology* 64:1553-1562.

Giulian D, Haverkamp LJ, Li J, Karshin WL, Yu J, Tom D, Li X, Kirkpatrick JB. (1995). Senile plaques stimulate microglia to release a neurotoxin found in Alzheimer brain. *Neurochem Int* 27:119–137.

Goedert M. (2001). Alpha-synuclein and neurodegenerative diseases. *Nat Rev Neurosci* 2:492-501.

Gong CX, Singh TJ, Grundke-Iqbal I, Iqbal K. (1993). Phosphoprotein phosphatase activities in Alzheimer disease. *J Neurochem* 61:921-927.

Gong Y, Chang L, Viola KL, Loacor PN, Lambert MP, Finch CE, Krafft GA, Klein WL. (2003). Alzheimer's disease-affected brain: presence of oligomeric A beta ligands (ADDLs) suggests a molecular basis for reversible memory loss. *Proc Natl Acad Sci USA* 100:10417-10422.

Gorelick PB. (1997). Status of risk factors for dementia associated with stroke. *Stroke* 28:459-463.

Grammas P, Ovase R. (2001). Inflammatory factors are elevated in brain microvessels in Alzheimer's disease. *Neurobiol Aging* 22:837-842.

Griffin WS, Sheng JG, Royston MC, Gentleman SM, McKenzie JE, Graham DI, Roberts GW, Mrak RE. (1998). Glial-neuronal interactions in Alzheimer's disease: the potential role of a „Cytokine Cycle" in disease progression. *Brain Pathol* 8:65-72.

Gunnarsson MD, Kilander L, Sudelof J, Basun H, Lannfelt L. (2006). Reduction of hyperphosphorylated tau during memantine treatment of Alzheimer's disease. *Alzheimer's & Dementia* Suppl:2:S63-S64.

Guo JT, Yu J, Grass D, De Beer FC, Kindy MS. (2002). Inflammation-dependent cerebral deposition of serum amyloid a protein in a mouse model of amyloidosis. *J Neurosci* 22:5900-5909.

Guo H, Liu D, Gelbard H, Cheng T, Insalaco R, Fernandez JA, Griffin JH, Zlokovic BV. (2004). Activated protein C prevents neuronal apoptosis via protease-activated receptors 1 and 3. *Neuron* 41:563-572.

Hall ED, Oostveen JA, Dunn E, Carter DB. (1995). Increased amyloid protein precursor and apolipoprotein E immunoreactivity in the selectively vulnerable hippocampus following transient forebrain ischemia in gerbils. *Exp Neurol* 135:17–27.

Hashimoto M, Masliah E. (1999). Alpha-synuclein in Lewy body disease and Alzheimer's disease. *Brain Pathol* 9:707-720.

Hauss-Wegrzyniak B, Galons JP, Wenk GL. (2000). Quantitative volumetric analyses of brain magnetic resonance imaging from rat with chronic neuroinflammation. *Exp Neurol* 165:347-354.

Hawkes ChA, McLaurin J. (2007). Immunotherapy as treatment for Alzheimer's disease. *Expert Rev Neurotherapeutics* 7:1535-1548.

Hemming ML, Selkoe DJ. (2005). Amyloid beta-protein is degraded by cellular angiotensin-converting enzyme (ACE) and elevated by an ACE inhibitor. *J Biol Chem* 280:37644-37650.

Heneka MT, Sastre M, Dumitrescu-Ozimek L, Hanke A, Dewachter I, Kuiperi C, O'Banion K, Klockgether T, Van Leuven F, Landreth GE. (2005). Acute treatment with the PPARγ agonist pioglitazone and ibuprofen reduces glial inflammation and Aβ1-42 levels in APPV7171 transgenic mice. *Brain* 128:1442-1453.

Heneka MT, O'Banion MK. (2007). Inflammatory processes in Alzheimer's disease. *J Neuroimmunol* 184:69-91.

Henon H, Durieu I, Guerouaou D, Lebert F, Pasquier F, Leys D. (2001). Poststroke dementia: incidence and relationship to prestroke cognitive decline. *Neurology* 57:1216-1222.

Herz J. (2003). LRP: a bright beacon at the blood-brain barrier. *J Clin Invest* 112:1483-1485.

Hillis AE. (2006). Neurobiology of unilateral spatial neglect. *Neuroscientist* 12:119-126.

Horsburgh K, Nicoll JAR. (1996a). Selective cellular alterations in amyloid precursor protein and apolipoprotein E following transient cerebral ischaemia in the rat. *Alzheimer Res* 2:37–42.

Horsburgh K, Nicoll JAR. (1996b). Selective alterations in the cellular distribution of apolipoprotein E immunoreactivity following transient cerebral ischemia in the rat. *Neuropathol Appl Neurobiol* 22:342-349.

Horstman LL, Jy W, Bidot C, Nordberg ML, Minagar A, Alexander JS, Kelley RE, Ahn YS. (2009). Potential roles of cell-derived microparticles in ischemic brain disease. *Neurol Res* 31:799-806.

Hossmann KA, Schmidt-Kastner R, Grosse Ophoff B. (1987). Recovery of integrative central nervous function after one hour global cerebro-circulatory arrest in normothermic cat. *J Neurol Sci* 77:305-320.

Irving EA, Yatsushiro K, McCulloch J, Dewar D. (1997). Rapid alteration of tau in oligodendrocytes after focal ischemic injury in the rat: involvement of free radicals. *J Cereb Blood Flow Metab* 17:612-622.

Iqbal K, Grudke-Iqbal I. (2004). Inhibition of neurofibrillary degeneration: a promising approach to Alzheimer's disease and other tauopathies. *Curr Drug Targets* 5:495-502.

Ishibashi S, Kuroiwa T, LiYuan S, Katsumata N, Li S, Endo S, Mizusawa H. (2006). Long-term cognitive and neuropsychological symptoms after global cerebral ischemia in Mongolian gerbils. *Acta Neurochir* Suppl:96:299-302.

Ishikawa M, Cooper D, Arumugam TV, Zhang JH, Nanda A, Granger DN. (2004). Platelet-leukocyte-endothelial cell interactions after middle cerebral artery occlusion and reperfusion. *J Cereb Blood Flow Metab* 24:907–915.

Ishimaru H, Ishikawa K, Haga S, Shoji M, Ohe Y, Haga C, Sasaki A, Takahashi A, Maruyama Y. (1996a). Accumulation of apolipoprotein E and β-amyloid-like protein in a trace of the hippocampal CA1 pyramidal cell layer after ischaemic delayed neuronal death. *NeuroReport* 7:3063–3067.

Ishimaru H, Ishikawa K, Ohe Y, Takahashi A, Maruyama Y. (1996b). Cystatin C and apolipoprotein E immunoreactivities in CA1 neurons in ischemic gerbil hippocampus. *Brain Res* 709:155-162.

Ishimaru H, Ueda K, Takahashi A, Maruyama Y. (1998). Changes in presynaptic protein NACP/alpha-synuclein in an ischemic gerbil hippocampus. *Brain Res* 788:311-314.

Iwata N, Tsubuki S, Takaki Y, Shirotani K, Lu B, Gerard NP, Gerard C, Hama E, Lee HJ, Saido TC. (2001). Metabolic regulation of brain Aβ by neprilysin. *Science* 292:1550-1552.

Jellinger KA. (2007). The enigma of vascular cognitive disorder and vascular dementia. *Acta Neuropathol* 113:349-388.

Jendroska K, Poewe W, Daniel SE, Pluess J, Iwerssen-Schmidt H, Paulsen J, Barthel S, Schelosky L, Cervos-Navarro J, DeArmond SJ. (1995). Ischemic stress induces deposition of amyloid beta immunoreactivity in human brain. *Acta Neuropathol* 90:461-466.

Ji ZS, Miranda RD, Newhouse YM, Weisgraber KH, Huang Y, Mahley RW. (2002). Apolipoprotein E4 potentates amyloid β peptide-induced lysosomal leakage and apoptosis in neuronal cells. *J Biol Chem* 277:21821-21828.

Jin K, Mao XO, Eshoo MW, Nagayama T, Minami M, Simon RP, Greenberg DA. (2001). Microarray analysis of hippocampal gene expression in global cerebral ischemia, *Ann Neurol* 50:93-103.

Kalaria RN. (1999). Microglia and Alzheimer's disease. *Curr Opin Hematol* 6:15-24.

Kalaria RN. (2000). The role of cerebral ischemia in Alzheimer's disease. *Neurobiol Aging* 21:321-330.

Kalback W, Esh C, Castano EM, Rahman A, Kokjohn T, Luehrs DC, Sue L, Cisneros R, Gerber F, Richardson C, Bohrmann B, Walker DG, Beach TG, Roher AE. (2004). Atherosclerosis, vascular amyloidosis and brain hypoperfusion in the pathogenesis of sporadic Alzheimer's disease. *Neurol Res* 26:525-539.

Kamada H, Sato K, Zhang WR, Omori N, Nagano I, Shoji M, Abe K. (2003). Spatiotemporal changes of apolipoprotein E immunoreactivity and apolipoprotein E mRNA expression after transient middle cerebral artery occlusion in rat brain. *J Neurosci Res* 73:545-556.

Kanemitsu H, Tomiyama T, Mori H. (2003). Human neprilysin is capable of degrading amyloid beta peptide not only in the monomeric form but also the pathological oligomeric form. *Neurosci Lett* 350:113-116.

Kang HS, Ahn HS, Kang HJ, Gye MC. (2006). Effect of estrogen on the expression of occludin in ovariectomized mouse brain. *Neurosci Lett* 402:30–34.

Karasawa Y, Araki H, Otomo S. (1994). Changes in locomotor activity and passive avoidance task performance induced by cerebral ischemia in mongolian gerbils. *Stroke* 25:645-650.

Karhunen H, Pitkanen A, Virtanen T, Gureviciene I, Pussinen R, Ylinen A, Sivenius J, Nissinen J, Jolkkonen J. (2003). Long-term functional consequences of transient occlusion of the middle cerebral artery in rats: a 1-year follow-up of the development of epileptogenesis and memory impairment in relation to sensorimotor deficits. *Epilepsy Res* 54:1-10.

Kida E, Pluta R, Lossinsky AS, Golabek AA, Choi-Miura NH, Wisniewski HM, Mossakowski MJ. (1995). Complete cerebral ischemia with short-term survival in rat induced by cardiac arrest. II. Extracellular and intracellular accumulation of apolipoproteins E and J in the brain. *Brain Res* 674:341-346.

Kim HS, Lee SH, Kim SS, Kim YK, Jeong SJ, Ma J, Han DH, Cho BK, Suh YH. (1998). Post-ischemic changes in the expression of Alzheimer's APP isoforms in rat cerebral cortex. *NeuroReport* 9:533–537.

Kirino T. (1982). Delayed neuronal death in the gerbil hippocampus following ischemia. *Brain Res* 239:57-69.

Kiryk A, Pluta R, Figiel I, Mikosz M, Ułamek M, Niewiadomska G, Jabłoński M, Kaczmarek L. (2011). Transient brain ischemia due to cardiac arrest causes irreversible long-lasting cognitive injury. *Behav Brain Res* 219:1-7.

Kitamura Y, Ishida Y, Takata K, Kakimura J, Mizutani H, Shimohama S, Akaike A, Taniguchi T. (2001). Alpha-synuclein protein is not scavenged in neuronal loss induced by kainic acid or focal ischemia. *Brain Res* 898:181-185.

Kiyota Y, Miyamoto M, Nagaoka A. (1991). Relationship between brain damage and memory impairment in rats exposed to transient forebrain ischemia. *Brain Res* 538:295-302.

Klafki HW, Staufenbiel M, Kornhuber J, Wiltfang J. (2006). Therapeutic approaches to Alzheimer's disease. *Brain* 129:2840-2855.

Knezevic II, Predescu SA, Neamu RF, Gorovoy MS, Knezevic NM, Easington C, Malik AB, Predescu DN. (2009). Tiam1 and Rac1 are required for platelet-activating factor-induced endothelial junctional disassembly and increase in vascular permeability. *J Biol Chem* 284:5381-5394.

Koistinaho J, Pyykonen I, Keinanen R, Hokfelt T. (1996). Expression of β-amyloid precursor protein mRNAs following transient focal ischaemia. *NeuroReport* 7:2727–2731.

Koistinaho M, Kettunen MI, Goldsteins G, Keinanen R, Salminen A, Ort M, Bures J, Liu D, Kauppinen RA, Higgins LS, Koistinaho J. (2002). Beta-amyloid precursor protein transgenic mice that harbor diffuse A beta deposits but do not form plaques show increased ischemic vulnerability: role of inflammation. *Proc Natl Acad Sci USA* 99:1610-1615.

Koistinaho M, Lin S, Wu X, Esterman M, Koger D, Hanson J, Higgs R, Liu F, Malkani S, Bales KR, Paul SM. (2004). Apolipoprotein E promotes astrocyte colocalization and degradation of deposited amyloid-β peptides. *Nat Med* 10:719-726.

Kokmen E, Whisnant JP, O'Fallon WM, Chu CP, Beard CM. (1996). Dementia after ischemic stroke: a population-based study in Rochester, Minnesota (1960-1984). *Neurology* 46:154-159.

Kuroiwa T, Bonnekoh P, Hossmann KA. (1991). Locomotor hyperactivity and hippocampal CA1 injury after transient forebrain ischemia in gerbils. *Neurosci Lett* 122:141-144.

Lam FC, Liu R, Lu P, Shapiro AB, Renoir JM, Sharoni FJ, Reiner PB. (2001). Beta-amyloid efflux mediated by p-glycoprotein. *J Neurochem* 76:1121-1128.

Lashuel HA, Hartley DM, Balakhaneh D, Aggarwal A, Teichberg S, Callaway DJ. (2002). New class of inhibitors of amyloid-beta fibril formation. Implications for the mechanism of pathogenesis in Alzheimer's disease. *J Biol Chem* 277:42881-42890.

Lau LF, Schachter JB, Seymour PA, Sanner MA. (2002). Tau protein phosphorylation as a therapeutic target in Alzheimer's disease. *Curr Top Med Chem* 2:395-415.

Leissring MA, Farris W, Chang AY, Walsh DM, Wu X, Sun X, Frosch MP, Selkoe DJ. (2003). Enhanced proteolysis of beta-amyloid in APP transgenic mice prevents plaque formation, secondary pathology, and premature death. *Neuron* 40:1087-1093.

Lemere CA, Beierschmitt A, Iglesias M, Spooner ET, Bloom JK, Leverone JF, Zheng JB, Seabrook TJ, Louard D, Li D, Selkoe DJ, Palmour RM, Ervin FR. (2004). Alzheimer's disease Aβ vaccine reduces central nervous system Aβ levels in a non-human primate, the Caribbean vervet. *Am J Pathol* 165:283-297.

Lemere CA, Maier M, Jiang L, Peng Y, Seabrook TJ. (2006). Amyloid-beta immunotherapy for the prevention and treatment of Alzheimer disease: Lessons from mice, monkeys, and humans. *Rejuvenation Res* 9:77-84.

Leys D, Englund E, Erkinjuntti T. (2002). Vascular dementia. In: Qizilbash N, Schneider LS, Chui H. (Eds). *Evidence-based dementia practice.* Blakwell, Oxford.

Li L, Sengupta A, Haque N, Grundke-Iqbal I, Iqbal K. (2004). Memantine inhibits and reverses the Alzheimer type abnormal hyperphosphorylation of tau and associated neurodegeneration. *FEBS Lett* 566:261-269.

Lin B, Schmidt-Kastner R, Busto R, Ginsberg MD. (1999). Progressive parenchymal deposition of β-amyloid precursor protein in rat brain following global cerebral ischemia. *Acta Neuropathol* 97:359-368.

Lin B, Ginsberg MD, Busto R. (2001). Hyperglycemic but not normoglycemic global ischemia induces marked early intraneuronal expression of β-amyloid precursor protein. *Brain Res* 888:107–116.

Lippoldt A, Kniesel U, Liebner, S, Kalbacher H, Kirsch T, Walburg H, Haller H. (2000). Structural alterations of tight junctions are associated with loss of polarity in stroke-prone spontaneously hypertensive rat blood-brain barrier endothelial cells. *Brain Res* 885:251–261.

Loeb C, Gandolfo C, Bino G. (1988). Intellectual impairment and cerebral lesions in multiple cerebral infarcts: a clinical-computed tomography study. *Stroke* 19:560-565.

Loeb C, Gandolfo C, Croce R, Conti M. (1992). Dementia associated with lacunar infarction. *Stroke* 23:1225-1229.

Lupo G, Anfuso CD, Assero G, Strosznajder RP, Walski M, Pluta R, Alberghina M. (2001). Amyloid β (1–42) and its β (25–35) fragment induce in vitro phosphatidylcholine hydrolysis in bovine retina capillary pericytes. *Neurosci Lett* 303:185–188.

Madureira S, Guerreiro M, Ferro JM. (2001). Dementia and cognitive impairment three months after stroke. *Eur J Neurol* 8:621-627.

Mailliot C, Podevin-Dimster V, Rosenthal RE, Sergeant N, Delacourte A, Fiskum G, Buee L. (2000). Rapid tau protein dephosphorylation and differential rephosphorylation during cardiac arrest-induced cerebral ischemia and reperfusion. *J Cereb Blood Flow Metab* 20:543-549.

Malm T, Koistinaho M. (2007). Relationships between brain ischemia and Alzheimer's disease – Insights derived from preclinical animal models. In: Pluta R, editor. *Ischemia-reperfusion pathways in Alzheimer's disease*. New York: Nova Science Publishers. p 17-39.

Marco S, Skaper SD. (2006). Amyloid beta-peptide 1-42 alters tight junction, protein distribution and expression in brain microvessels endothelial cells. *Neurosci Lett* 401:219-224.

Marr RA, Rockenstein E, Mukherjee A, Kindy MS, Hersh LB, Gage FH, Verma IM, Masliah E. (2003). Neprilysin gene transfer reduces human amyloid pathology in transgenic mice. *J Neurosci* 23:1992-1996.

Matsuoka Y, Saito M, LaFrancois J, Saito M, Gaynor K, Olm V, Wangl L, Casey E, Lu Y, Shiratori C, Lemere C, Duff K. (2003). Novel therapeutic approach for the treatment of Alzheimer's disease by peripheral administration of agents with an affinity to β-amyloid. *J Neurosci* 23:29-33.

Mattson MP, Zhu HY, Yu J, Kindy MS. (2000). Presenilin-1 mutation increases neuronal vulnerability to focal ischemia in vivo and to hypoxia and glucose deprivation in cell culture: Involvement of perturbed calcium homeostasis. *J Neurosci* 20:1358-1364.

Matsunaga W, Shirokawa T, Isobe K. (2003). Specific uptake of Abeta 1–40 in rat brain occurs in astrocyte, but not in microglia. *Neurosci Lett* 342:129–131.

Matsuoka Y, Saito M, LaFrancois J, Saito M, Gaynor K, Olm V, Wangl L, Casey E, Lu Y, Shiratori C, Lemere C, Duff K. (2003). Novel therapeutic approach for the treatment of Alzheimer's disease by peripheral administration of agents with an affinity to β-amyloid. *J Neurosci* 23:29-33.

McCarty MF. (2006). Toward prevention of Alzheimers disease – Potential nutraceutical strategies for suppressing the production of amyloid beta peptides. *Med Hypotheses* 67:682-697.

McCullough LD, Alkayed NJ, Traystman RJ, Williams MJ, Hurn PD. (2001). Postischemic estrogen reduces hypoperfusion and secondary ischemia after experimental stroke. *Stroke* 32:796–802.

McGeer PL, Schulzer M, McGeer EG. (1996). Arthritis and anti-inflammatory agents as possible protective factors for Alzheimer's disease: a review of 17 epidemiologic studies. *Neurology* 47:425-432.

Mileson BE, Schwartz RD. (1991). The use of locomotor activity as a behavioral screen for neuronal damage following transient forebrain ischemia in gerbils. *Neurosci Lett* 128:71-76.

Moore AH, O'Banion MK. (2002). Neuroinflammation and anti-inflammatory therapy for Alzheimer's disease. *Adv Drug Deliv Rev* 54:1627-1656.

Morihara T, Teter B, Yang F, Lim GP, Boudinot S, Boudinot FD, Frautschy SA, Cole GM. (2005). Ibuprofen suppresses interleukin-1β induction of pro-amyloidogenic α1-antichymotrypsin to ameliorate β-amyloid pathology in Alzheimer's models. *Neuropsychopharmacology* 30:1111-1120.

Morioka T, Kalehua AN, Streit WJ. (1992). Progressive expression of immunomolecules on microglial cells in rat dorsal hippocampus following transient forebrain ischemia. *Acta Neuropathol* 83:149-157.

Mossakowski MJ, Lossinsky AS, Pluta R, Wisniewski HM. (1993). Changes in cerebral microcirculation system following experimentally induced cardiac arrest: a SEM and TEM study. In: Tomita M (ed). *Microcirculatory stasis in the brain*. Amsterdam: Elsevier Science Publishers BV, pp. 99–106.

Mossakowski MJ, Lossinsky AS, Pluta R, Wisniewski HM. (1994). Abnormalities of the blood-brain barrier in global cerebral ischemia in rats due to experimental cardiac arrest. *Acta Neurochir* Suppl:60:274–276.

Mruthinti S, Buccafusco JJ, Hill WD, Waller JL, Jackson TW, Zamrini EY, Schade RF. (2004). Autoimmunity in Alzheimer's disease: increased levels of circulating IgGs binding Abeta and RAGE peptides. *Neurobiol Aging* 25:1023-1032.

Muller T, Meyer HE, Egensperger R, Marcus K. (2008). The amyloid precursor protein intracellular domain (AICD) as modulator of gene expression, apoptosis, and cytoskeletal dynamics – Relevance for Alzheimer's disease. *Pro Neurobiol* 85:393-406.

Muralikrishna Adibhatla R, Hatcher JF. (2006). Phospholipase A2 reactive oxygen species, and lipid peroxidation in cerebral ischemia. *Free Radic Biol Med* 40:376-387.

Nalbantoglu J, Tirado-Santiago G, Lahsaini A, Poirier J, Goncalves O, Verge G, Momoli F, Welner SA, Massicotte G, Julien JP, Shapiro ML. (1997). Impaired learning and LTP in mice expressing the carboxyl terminus of the Alzheimer amyloid precursor protein. *Nature* 387:500-505.

Nalivaeva NN, Fisk L, Kochkina EG, Plesneva SA, Zhuravin IA, Babusikova E, Dobrota D, Turner AJ. (2004). Effect of hypoxia/ischemia and hypoxic preconditioning/reperfusion on expression of some amyloid-degrading enzymes. *Ann NY Acad Sci* 1035:21-33.

Nicoll JA, Wilkinson D, Holmes C, Steart P, Markham H, Weller RO. (2003). Neuropathology of human Alzheimer disease after immunization with amyloid-β peptide: a case report. *Nat Med* 9:448-452.

Niedermeyer E. (2007). Consideration of the ischemic basis and therapy of Alzheimer disease. *Clin EEG Neurosci* 38:55-56.

Nihashi T, Inao S, Kajita Y, Kawai T, Sugimoto T, Niwa M, Kabeya R, Hata N, Hayashi S, Yoshida J. (2001). Expression and distribution of beta amyloid precursor protein and beta amyloid peptide in reactive astrocytes after transient middle cerebral artery occlusion. *Acta Neurochir* 143:287–295.

Nishijima K, Kiryu J, Tsujikawa A, Miyamoto K, Honjo M, Tanikara H, Nonaka A, Yamashiro K, Katsuta H, Miyahara S, Honda Y, Ogura Y. (2004). Platelets adhering to the vascular wall mediate postischemic leukocyte-endothelial cell interactions in retinal microcirculation. *Invest Ophthalmol Vis Sci* 45:977–984.

Nishio M, Kohmura E, Yuguchi T, Nakajima Y, Fujinaka T, Akiyama C, Iwata A, Yoshimine T. (2003). Neuronal apolipoprotein E is not synthesized in neuron after focal ischemia in rat brain. *Neurol Res* 25:390-394.

Nita DA, Nita V, Spulber S, Moldovan M, Popa DP, Zagrean AM, Zagrean L. (2001). Oxidative damage following cerebral ischemia depends on reperfusion – a biochemical study in rat. *J Cell Mol Med* 5:163-170.

Niwa K, Younkin L, Ebeling C, Turner SK, Westaway D, Younkin S, Ashe KH, Carlson GA, Iadecola C. (2000). Abeta 1–40-related reduction in functional hyperemia in mouse neocortex during somatosensory activation. *Proc Natl Acad Sci USA* 97:9735–9740.

Oddo S, Billings L, Kesslak JP, Cribbs DH, LaFerla FM. (2004). Abeta immunotherapy leads to clearance of early, but not late, hyperphosphorylated tau aggregates via the proteasome. *Neuron* 43:321-332.

Oddo S, Caccamo A, Tran L, Lambert MP, Glabe CG, Klein WL, Laferla FM. (2006). Temporal profile of amyloid-beta A beta oligomerization in an in vivo model of Alzheimer disease – a link between A beta and tau pathology. *J Biol Chem* 281:1599-1604.

Ohtake M, Morino S, Kaidoh T, Inoue T. (2004). Three-dimensional structural changes in cerebral microvessels after transient focal cerebral ischemia in rats: Scanning electron microscopic study of corrosion casts. *Neuropathology* 24:219–227.

Orgogozo JM, Gilman S, Dartigues JF, Laurent B, Puel M, Kirby LC, Jouanny P, Dubois B, Eisner L, Flitman S, Michel BF, Boada M, Frank A, Hock C. (2003). Subacute meningoencephalitis in a subset of patients with Alzheimer's disease after Abeta42 immunization. *Neurology* 61:46-54.

Orzyłowska O, Oderfeld-Nowak B, Zaremba M, Januszewski S, Mossakowski MJ. (1999). Prolonged and concomitant induction of astroglial immunoreactivity of interleukin-1 beta and interleukin-6 in the rat hippocampus after transient global ischemia. *Neurosci Lett* 263:72-76.

Oster-Granite ML, McPhie DL, Greenan J, Neve RL. (1996). Age-dependent neuronal and synaptic degeneration in mice transgenic for the C terminus of the amyloid precursor protein. *J Neurosci* 16:6732–6741.

Palacios G, Mengod G, Tortosa A, Ferrer I, Palacios JM. (1995). Increased β-amyloid precursor protein expression in astrocytes in the gerbil hippocampus following ischaemia: association with proliferation of astrocytes. *Eur J Neurosci* 7:501–510.

Paris D, Patel N, DelleDonne A, Quadros A, Smeed R, Mullan M. (2004a). Impaired angiogenesis in a transgenic mouse model of cerebral amyloidosis. *Neurosci Lett* 366:80–85.

Paris D, Townsend K, Quadros A, Hunphrey J, Sun J, Brem S, Wotoczek-Obadia A, Patel N, Obregan DF, Crescentini R, Abdullah L, Coppola D, Rojiani AM, Crawford F, Segri SM, Mullan M. (2004b). Inhibition of angiogenesis by Aβ peptides. *Angiogenesis* 7:75–85.

Pasquier F, Leys D. (1997). Why are stroke patients prone to develop dementia? *J Neurol* 244:135-142.

Pennypacker KR, Hernandez H, Benkovic S, Morgan DG, Willing AE, Sanberg PR. (1999). Induction of presenilins in the rat brain after middle cerebral arterial occlusion. *Brain Res Bull* 48:539-543.

Petito CK, Pulsinelli WA, Jacobson G, Plum F. (1982). Edema and vascular permeability in cerebral ischemia: comparison between ischemic neuronal damage and infarction. *J Neuropathol Exp Neurol* 41:423–436.

Petito C, Morgello S, Felix JC, Lesser ML. (1990). The two patterns of reactive astrocytosis in postischemic rat brain. *J. Cereb Blood Flow Metab* 10:850-859.

Pinnix I, Musunuru U, Tun H, Sridharan A, Golde T, Eckman C, Ziani-Cherif C, Onstead L, Sambamurti K. (2001). A novel γ-secretase assay based on detection of the putative C-terminal fragment-γ of amyloid β protein precursor. *J Biol Chem* 276:481-487.

Pluta R, Lossinsky AS, Mossakowski MJ, Faso L, Wisniewski HM. (1991). Reassessment of new model of complete cerebral ischemia in rats. Method of induction of clinical death, pathophysiology and cerebrovascular pathology. *Acta Neuropathol* 83:1–11.

Pluta R, Lossinsky AS, Wisniewski HM, Mossakowski MJ. (1994a). Early blood-brain barrier changes in the rat following transient complete cerebral ischemia induced by cardiac arrest. *Brain Res* 633:41-52.

Pluta R, Kida E, Lossinsky AS, Golabek AA, Mossakowski MJ, Wisniewski HM. (1994b). Complete cerebral ischemia with short-term survival in rats induced by cardiac arrest. I. Extracellular accumulation of Alzheimer's β-amyloid protein precursor in the brain. *Brain Res* 649:323–328.

Pluta R, Lossinsky AS, Walski M, Wisniewski HM, Mossakowski MJ. (1994c). Platelet occlusion phenomenon after short- and long-term survival following complete cerebral ischemia in rats produced by cardiac arrest. *J Brain Res* 35:463-471.

Pluta R, Kida E, Golabek AA, Mossakowski MJ. (1995a). Accumulation of Alzheimer's β-amyloid associated proteins: apolipoproteins E, J and A-1 in rat brain after global cerebral ischemia. *J Cereb Blood Flow Metab* 15:Suppl:1:S227.

Pluta R, Kida E, Golabek AA, Mossakowski MJ. (1995b). Apolipoproteins E and J in rat ischemic brain: colocalization with amyloid protein precursor. *Eur J Neurosci* Suppl:8:170.

Pluta R, Barcikowska M, Januszewski S, Misicka A, Lipkowski AW. (1996). Evidence of blood-brain barrier permeability/leakage for circulating human Alzheimer's β-amyloid- (1–42)-peptide. *NeuroReport* 7:1261–1265.

Pluta R, Misicka A, Januszewski S, Barcikowska M, Lipkowski AW. (1997a). Transport of human β-amyloid peptide through the rat blood-brain barrier after global cerebral ischemia. *Acta Neurochir* Suppl:70:247–249.

Pluta R, Barcikowska M, Debicki G, Ryba M, Januszewski S. (1997b). Changes in amyloid precursor protein and apolipoprotein E immunoreactivity following ischemic brain injury in rat with long-term survival: influence of idebenone treatment. *Neurosci Lett* 232:95–98.

Pluta R, Barcikowska M, Mossakowski MJ, Zelman I. (1997c). Beta-amyloid peptide and C-terminal of beta-amyloid precursor protein persist for over 1 year in rat brain after global ischemia. *J Cereb Blood Flow Metab* 17(Suppl 1):S775.

Pluta R, Barcikowska M, Misicka A, Januszewski S, Lipkowski AW. (1998a). Disappearing diffuse amyloid plaques. *Neurobiol Aging* 19:S131.

Pluta R, Barcikowska M, Mossakowski MJ, Zelman I. (1998b). Cerebral accumulation of beta-amyloid following ischemic brain injury with long-term survival. *Acta Neurochir* Suppl:71:206-208.

Pluta R, Barcikowska M, Misicka A, Lipkowski AW, Spisacka S, Januszewski S. (1999). Ischemic rats as a model in the study of the neurobiological role of human β-amyloid peptide. Time-dependent disappearing diffuse amyloid plaques in brain. *NeuroReport* 10:3615-3619.

Pluta R. (2000). The role of apolipoprotein E in the deposition of β-amyloid peptide during ischemia-reperfusion brain injury. A model of early Alzheimer's disease. *Ann NY Acad Sci* 903:324–334.

Pluta R, Misicka A, Barcikowska M, Spisacka S, Lipkowski AW, Januszewski S. (2000). Possible reverse transport of β-amyloid peptide across the blood-brain barrier. *Acta Neurochir* Suppl:76:73–77.

Pluta R. (2001). Proteins associated with Alzheimer's disease in conditions predisposing to Alzheimer's-type neurodegeneration. *J Cereb Blood Flow Metab* 21:Suppl. 1:S424.

Pluta R. (2002a). Glial expression of the β-amyloid peptide in cardiac arrest. *J Neurol Sci* 203–204:277–280.

Pluta R. (2002b). Astroglial expression of the beta-amyloid in ischemia-reperfusion brain injury. *Ann NY Acad Sci* 977:102-108.

Pluta R. (2003). Blood-brain barrier dysfunction and amyloid precursor protein accumulation in microvascular compartment following ischemia-reperfusion brain injury with 1-year survival. *Acta Neurochir* Suppl:86:117–122.

Pluta R. (2004a). Alzheimer lesions after ischemia-reperfusion brain injury. *Folia Neuropathol* 42:181–186.

Pluta R. (2004b). From brain ischemia-reperfusion injury to possible sporadic Alzheimer's disease. *Curr Neurovasc Res* 1:441-453.

Pluta R. (2005). Pathological opening of the blood-brain barrier to horseradish peroxidase and amyloid precursor protein following ischemia-reperfusion brain injury. *Chemotherapy* 51:223–226.

Pluta R. (2006a). Ischemia-reperfusion factors in sporadic Alzheimer's disease. In: Welsh EM, editor. *New Research on Alzheimer's disease.* New York: Nova Science Publishers. p 183-234.

Pluta R. (2006b). Is the ischemic blood-brain barrier insufficiency responsible for full-blown Alzheimer's disease? *Neurol Res* 28:266–271.

Pluta R, Ułamek M. (2006). Brain amyloidosis following ischemia-reperfusion injury. *Curr Trends Neurol* 2:41-46.

Pluta R, Ulamek M, Januszewski S. (2006). Micro-blood-brain barrier openings and cytotoxic fragments of amyloid precursor protein accumulation in white matter after ischemic brain injury in long-lived rats. *Acta Neurochir* Suppl:96:267–271.

Pluta R. (2007a). Role of ischemic blood-brain barrier on amyloid plaques development in Alzheimer's disease brain. *Curr Neurovasc Res* 4:121-129.

Pluta R. (2007b). Is the ischemic blood-brain barrier a Trojan horse in Alzheimer's disease brain? In: Pluta R (ed). *Ischemia-reperfusion pathways in Alzheimer's disease.* New York: Nova Science Publishers, Inc., pp. 139-184.

Pluta R. (2007c). *Ischemia-reperfusion pathways in Alzheimer's disease.* New York: Nova Science Publishers.

Pluta R, Ułamek M. (2008). New proposals for treatment sporadic Alzheimer's disease. *Cent Nerv Syst Agents Med Chem* 8:286-296.

Pluta R, Januszewski S, Ulamek M. (2008). Ischemic blood-brain barrier and amyloid in white matter as etiological factors in leukoaraiosis. *Acta Neurochir* Suppl:102:353–356.

Pluta R, Ułamek M, Jabłoński M. (2009). Alzheimer's mechanisms in ischemic brain degeneration. *Anat Rec* 292:1863-1881.

Pluta R, Januszewski S, Jabłoński M, Ułamek M. (2010). Factors in creepy delayed neuronal death in hippocampus following brain ischemia-reperfusion injury with long-term survival. *Acta Neurochir* Suppl:106:37-41.

Pogue AI, Lukiw WJ. (2004). Angiogenic signaling in Alzheimer's disease. *NeuroReport* 15:1507-1510.

Pohjasvaara T, Erkinjuntti T, Ylikoski R, Hietanen M, Vataja R, Kaste M. (1998). Clinical determinants of poststroke dementia. *Stroke* 29:75-81.

Polavarapu R, An J, Zhang C, Yepes M. (2008). Regulated intramembrane proteolysis of the low-density lipoprotein receptor-related protein mediates ischemic cell death. *Am J Pathol* 172:1355-1362.

Popa-Wagner A. (2007). Alzheimer's disease pathological factors in ischemic aged brain. In: Pluta R, editor. *Ischemia-reperfusion pathways in Alzheimer's disease.* New York: Nova Science Publishers. pp. 51-84.

Pulsinelli WA, Brierley JB, Plum F. (1982). Temporal profile of neuronal damage in a model of transient forebrain ischemia. *Ann Neurol* 11:491-498.

Qi J, Wu H, Yang Y, Wand D, Chen Y, Gu Y, Liu T. (2007). Cerebral ischemia and Alzheimer's disease: The expression of amyloid-β and apolipoprotein E in human hippocampus. *J Alzheimer's Dis* 12:335-341.

Rakover I, Arbel M, Solomon B. (2007). Immunotherapy against APP β-secretase cleavage site improves cognitive function and reduces neuroinflammation in Tg2576 mice without a significant effect on brain Aβ levels. *Neurodegener Dis* 4:392-402.

Ramirez BG, Blazquez C, Gomez del Pulgar T, Guzmain M, de Ceballos ML. (2005). Prevention of Alzheimer's disease pathology by cannabinoids: neuroprotection mediated by blockade of microglial activation. *J Neurosci* 25:1904-1913.

Ramsauer M, Krause D, Dermietzel R. (2002). Angiogenesis of the blood-brain barrier in vitro and the function of cerebral pericytes. *FASEB J* 16:1274–1276.

Rangan SK, Liu R, Brune D, Planque S, Paul S, Sierks MR. (2003). Degradation of beta-amyloid by proteolytic antibody light chains. *Biochemistry* 42:14328-14334.

Ritter LS, Stempel KM, Coull BM, McDonagh PF. (2005). Leukocyte-platelet aggregates in rat peripheral blood after ischemic stroke and reperfusion. *Biol Res Nurs* 6:281-288.

Roberts SB. (2002). γ-secretase inhibitors and Alzheimer's disease. *Adv Drug Deliv Rev* 54:1579-1588.

Rogers JT, Leiter LM, McPhee J, Cahill CM, Zhan SS, Potter H, Nilsson LN. (1999). Translation of the Alzheimer amyloid precursor protein mRNA is up-regulated by interleukin-1 through 5'-untranslated region sequences. *J Biol Chem* 274:6421-6431.

Roof RL, Schielke GP, Ren X, Hall ED. (2001). A comparison of long-term functional outcome after 2 middle cerebral artery occlusion models in rats. *Stroke* 32:2648-2657.

Rosenblum WI, Murata S, Nelson GH, Werner PK, Ranken R, Harmon RC. (1994). Anti-CD31 delays platelet adhesion/aggregation at sites of endothelial injury in mouse cerebral arterioles. *Am J Pathol* 145:33-36.

Rossner S, Lange-Dohna C, Zeitschel U, Perez-Polo JR. (2005). Alzheimer's disease beta-secretase BACE1 is not a neuron-specific enzyme. *J Neurochem* 92:226-234.

Sadowski M, Wisniewski HM, Jakubowska-Sadowska K, Tarnawski M, Lazarewicz JW, Mossakowski MJ. (1999). Pattern of neuronal loss in the rat hippocampus following experimental cardiac arrest-induced ischemia. *J Neurol Sci* 168:13–20.

Samuelsson M, Soderfeldt B, Olsson GB. (1996). Functional outcome in patients with lacunar infarction. *Stroke* 27:842-846.

Sastre M, Steiner H, Fuchs K, Capell A, Multhaup G, Condron MM, Teplow DB, Haass C. (2001). Presenilin-dependent gamma-secretase processing of beta-amyloid precursor protein at a site corresponding to the S3 cleavage of Notch. *EMBO Rep* 2:835-841.

Sastre M, Dewachter I, Landreth GE, Willson TM, Klockgether T, Van Leuven F, Heneka MT. (2003). Nonsteroidal anti-inflammatory drugs and peroxisome proliferator-activated receptor-gamma agonists modulate immunostimulated processing of amyloid precursor protein through regulation of beta-secretase. *J Neurosci* 23:9796-9804.

Sastre M, Dewachter I, Rossner S, Bogdanovic N, Rosen E, Borghgraef P, Evert BO, Dumitrescu-Ozimek L, Thal DR, Landreth G, Walter J, Klockgether T Van Leuven F, Heneka MT. (2006). Nonsteroidal anti-inflammatory drugs repress β-secretase gene promotor activity by the activation of PPARγ. *Proc Natl Acad Sci USA* 103:443-448.

Schenk D, Barbour R, Dunn W, Gordon G, Grajeda H, Guido T, Hu K, Huang J, Johnson-Wood K, Khan K, Kholodenko D, Lee M, Liao Z, Lieberburg I, Motter R, Mutter L, Soriano F, Shopp G, Vasquez N, Vandevert C, Walker S, Wogulis M, Yednock T, Games D, Seubert P. (1999). Immunization with amyloid-beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature* 400:173-177.

Schmidt-Kastner R, Szymaś J, Hossmann KA. (1990). Immunohistochemical study of glial reaction and serum-protein extravasation in relation to neuronal damage in rat hippocampus after ischemia. *Neuroscience* 38:527-540.

Schroeter M, Kury P, Jander S. (2003). Inflammatory gene expression in focal cortical brain ischemia: differences between rats and mice. *Brain Res Mol Brain Res* 117:1-7.

Selkoe DJ. (2001). Clearing the brain's amyloid cobwebs. *Neuron* 32:177-180.

Shackelford DA, Yeh RY. (1998). Dephosphorylation of tau during transient forebrain ischemia in the rat. *Mol Chem Neuropathol* 34:103-120.

Shi J, Panickar KS, Yang S-H, Rabbani O, Day AL, Simpkins JW. (1998). Estrogen attenuates over-expression of β-amyloid precursor protein messenger RNA in an animal model of focal ischemia. *Brain Res* 810:87-92.

Shi J, Yang SH, Stubley L, Day AL, Simpkins JW. (2000). Hypoperfusion induces overexpression of β-amyloid precursor protein mRNA in a focal ischemic rodent model. *Brain Res* 853:1-4.

Shinnou M, Ueno M, Sakamoto H, Ide M. (1998). Blood-brain barrier damage in reperfusion following ischemia in the hippocampus of the Mongolian gerbil brain. *Acta Neurol Scand* 98:406–411.

Simpkins JW, Rajakumar G, Zhang YQ, Simpkins CE, Greenwald D, Yu CJ, Bodor N, Day AL. (1997). Estrogens may reduce mortality and ischemic damage caused by middle cerebral artery occlusion in the female rat. *J Neurosurg* 87:724–730.

Sinigaglia-Coimbra R, Cavalheiro EA, Coimbra CG. (2002). Postischemic hypertermia induces Alzheimer-like pathology in the rat brain. *Acta Neuropathol* 103:444–452.

Smith ML, Auer RN, Siesjö BK. (1984). The density and distribution of ischemic brain injury in the rat following 2-10 min of forebrain ischemia. *Acta Neuropathol* 64:319-332.

Smith GM, Hale JH. (1997). Macrophage/Microglia regulation of astrocytic tenascin: synergistic action of transforming growth factor-beta and basic fibroblast growth factor. *J Neurosci* 17:9624-9633.

Sohrabji F. (2007). Guarding the blood-brain barrier: A role for estrogen in the etiology of neurodegenerative disease. *Gene Expression* 13:311-319.

Soto C. (2001). Protein misfolding and disease; protein refolding and therapy. *FEBS Lett* 498:204-207.

Stamer K, Vogel R, Thies E, Mandelkow E, Mandelkow EM. (2002). Tau blocks traffic of organelles, neurofilaments, and APP vesicles in neurons and enhances oxidative stress. *J Cell Biol* 156:1051-1063.

Stoll G, Jander S, Schroeter M. (1998). Inflammation and glial responses in ischemic brain lesions. *Prog Neurobiol* 56:149-171.

Stoltzner SE, Grenfell TJ, Mori C, Wisniewska KE, Wisniewski TH, Selkoe DJ, Lemere CA. (2000). Temporal accrual of complement proteins in amyloid plaques in Down's syndrome with Alzheimers disease. *Am J Pathol* 156:489–499.

Sun X, He G, Qing H, Zhou W, Dobie F, Cai F, Staufenbiel M, Huang LE, Song W. (2006). Hypoxia facilitates Alzheimer's disease pathogenesis by up-regulating BACE1 gene expression. *Proc Natl Acad Sci USA*, 103:18727–18732.

Szekely CA, Thome JE, Zandi PP, Messias E, Breitner JC, Goodman SN. (2004). Nonsteroidal anti-inflammatory drugs for the prevention of Alzheimer's disease: a systematic review. *Neuroepidemiology* 23:159-169.

Takasawa K, Kitagawa K, Yagita Y, Sasaki T, Tanaka S, Matsushita K, Ohstuki T, Miyata T, Okano H, Hori M, Matsumoto M. (2002). Increased proliferation of neural progenitor cells but reduced survival of newborn cells in the contralateral hippocampus after focal cerebral ischemia in rats. *J Cereb Blood Flow Metab* 22:299-307.

Takuma K, Baba A, Matsuda T. (2004). Astrocyte apoptosis: implications for neuroprotection. *Prog Neurobiol* 72:111–127.

Tanimukai H, Imaizumi K, Kudo T, Katayama T, Tsuda M, Takagi T, Tohyama M, Takeda M. (1998). Alzheimer-associated presenilin-1 gene is induced in gerbil hippocampus after transient ischemia. *Mol Brain Res* 54:212-218.

Tanzi RE, Moir RD, Wagner SL. (2004). Clearance of Alzheimer's Aβ peptide: the many roads to perdition. *Neuron* 43:605-608.

Tarkowski E, Issa R, Sjogren M, Wallin B, Blennow K, Tarkowski A, Kumar P. (2002). Increased intrathecal levels of the angiogenic factors VEGF and TGF-beta in Alzheimer's disease and vascular dementia. *Neurobiol Aging* 23:237-243.

Tatemichi TK, Foulkes MA, Mohr JP, Hewitt JR, Hier DB, Price TR, Wolf PA. (1990). Dementia in stroke survivors in the Stroke Data Bank cohort: prevalence, incidence, risk factors and computed tomographic findings. *Stroke* 21:858-866.

Tatemichi TK, Desmond DW, Mayeux R, Paik M, Stern Y, Sano M, Remien RH, Williams JB, Mohr JP, Hauser WA, Figueroa M. (1992). Dementia after stroke: baseline frequency, risks, and clinical features in a hospitalized cohort. *Neurology* 42:1185-1193.

Thomas T, Thomas G, McLendon C, Sutton T, Mullan M. (1996). β-amyloid-mediated vasoactivity and vascular endothelial damage. *Nature* 380:168–171.

Tomimoto H, Akiguchi I, Wakita H, Nakamura S, Kimura J. (1995). Ultrastructural localization of amyloid protein precursor in the normal and postischemic gerbil brain. *Brain Res* 672:187–195.

Touzani O, Boutin H, LeFeuvre R, Parker L, Miller A, Luheshi G, Rothwell N. (2002). Interleukin-1 influences ischemic brain damage in the mouse independently of the interleukin-1 type I receptor. *J Neurosci* 22:38-43.

Uchihara T, Nakamura A, Arai T, Ikeda K, Tsuchiya K. (2004). Microglial tau undergoes phosphorylation-independent modification after ischemia. *Glia* 45:180-187.

Ueno M, Tomimoto H, Akiguchi I, Wakita H, Sakamoto H. (2002). Blood-brain barrier disruption in white matter lesions in a rat model of chronic cerebral hypoperfusion. *J Cereb Blood Flow Metab* 22:97–104.

Van Beek J, Chan P, Bernaudin M, Petit E, MacKenzie ET, Fontaine M. (2000). Glial responses, clusterin, and complement in permanent focal cerebral ischemia in the mouse. *Glia* 31:39-50.

Walton M, Young D, Sirimanne E, Dodd J, Christie D, Williams C, Gluckman P, Dragunow M. (1996). Induction of clusterin in the immature brain following a hypoxic-ischemic injury. *Mol Brain Res* 39:137-152.

Wen Y, Onyewuchi O, Yang S, Liu R, Simpkins JW. (2004a). Increased beta-secretase activity and expression in rats following transient cerebral ischemia. *Brain Res* 1009:1-8.

Wen Y, Yang S, Liu R, Simpkins JW. (2004b). Transient cerebral ischemia induces site-specific hyperphosphorylation of tau protein. *Brain Res* 1022:30-38.

Wen Y, Yang S, Liu R, Brun-Zinkernagel AM, Koulen P, Simpkins JW. (2004c). Transient cerebral ischemia induces aberrant neuronal cell cycle re-entry and Alzheimer's disease-like tauopathy in female rats. *J Biol Chem* 279:22684-22692.

Wen Y, Yang SH, Liu R, Perez EJ, Brun-Ziukemagel AM, Koulen P, Simpkins JW. (2007). Cdk5 is involved in NFT-like tauopathy induced by transient cerebral ischemia in female rats. *Biochim Biophys Acta* 1772:473-483.

Wenk GL, Barnes CA. (2000). Regional changes in the hippocampal density of AMPA and NMDA receptors across the lifespan of the rat. *Brain Res* 885:1-5.

Willing AE, Cuevas J, Pennypacker KR. (2007). Treatment of Alzheimer's disease: New insights from treatment of stroke at delayed time points. *In Ischemia-reperfusion pathways in Alzheimer's disease.* Ed. R. Pluta. Nova Science Publishers, Inc. New York. pp. 185-203.

Wisniewski HM, Pluta R, Lossinsky AS, Mossakowski MJ. (1995). Ultrastructural studies of cerebral vascular spasm after cardiac arrest-related global cerebral ischemia in rats. *Acta Neuropathol* 90:432–440.

Wisniewski HM, Maslinska D. (1996). Beta-protein immunoreactivity in the human brain after cardiac arrest. *Folia Neuropathol* 34:65-71.

Wolfe MS, Xia W, Ostaszewski BL, Diehl TS, Kimberly WT, Selkoe DJ. (1999). Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and gamma-secretase activity. *Nature* 398:513–517.

Wyss-Coray T, Loike JD, Brionne TC, Lu E, Anankov R, Yan F, Silverstein SC, Husemann J. (2003). Adult mouse astrocytes degrade amyloid-beta in vitro and in situ. *Nat Med* 9:453–457.

Yam PS, Takasago T, Dewar D, Graham DI, McCulloch J. (1997). Amyloid precursor protein accumulates in white matter at the margin of a focal ischaemic lesion. *Brain Res* 760:150–157.

Yamasaki Y, Matsuura N, Shozuhara H, Onodera H, Itoyama Y, Kogure K. (1995). Interleukin-1 as a pathogenetic mediator of ischemic brain damage in rats. *Stroke* 26:676-680.

Yan FL, Zhang J, Guan XN, Hong Z. (2007). mRNA expression and activity of ADAM17 in hippocampus after chronic cerebral hypoperfusion: experiment with aged rats. *Zhonghua Yi Xue Za Zhi* 87:2515-2517.

Yang Y, Kinney GA, Spain WJ, Breitner JCS, Cook DG. (2004). Presenilin-1 and intracellular calcium stores regulate neuronal glutamate uptake. *J Neurochem* 88:1361-1372.

Yang F, Lim GP, Begum AN, Ubeda OJ, Simmons MR, Ambegaokar SS, Chen PP, Kayed R, Glabe CG, Frautschy SA, Cole GM. (2005). Curcumin inhibits formation of amyloid beta oligomers and fibrils, binds plaques, and reduces amyloid *in vivo. J Biol Chem* 280:5892-5901.

Yang SH, Liu R, Perez EJ, Wang X, Simpkins JW. (2005). Estrogens as protectants of the neurovascular unit against ischemic stroke. *Curr Drug Targets CNS Neurol Disord* 4:169–177.

Yang SH, Simpkins JW. (2007). Ischemia-reperfusion promotes tau and beta-amyloid pathology and a progressive cognictive impairment. In Pluta R, editor. *Ischemia-reperfusion pathways in Alzheimer's disease.* New York: Nova Science Publishers, Inc. p 113-138.

Yokota M, Saido TC, Tani E, Yamaura I, Minami N. (1996). Cytotoxic fragment of amyloid precursor protein accumulates in hippocampus after global forebrain ischemia. *J Cereb Blood Flow Metab* 16:1219–1223.

Yu C, Kim SH, Ikeuchi T, Xu H, Gasparini L, Wang R, Sisodia SS. (2001). Characterization of a presenilin-mediated amyloid precursor protein carboxyl-terminal fragment γ. Evidence for distinct mechanisms involved in γ-secretase processing of the APP and Notch1 transmembrane domains. *J Biol Chem* 276:43756-43760.

Zhang RL, Chopp M, Liu Y, Zaloga C, Jiang N, Jones ML, Miyasaka M, Ward PA. (1994). Anti-ICAM-1 antibody reduces ischemic cell damage after transient middle cerebral artery occlusion in the rat. *Neurology* 44:1747-1751.

Zhang X, Zhou K, Wang R, Cui J, Lipton SA, Liao FF, Hu H, Zhang YW. (2007). Hypoxia-inducible factor 1α (HIF-1α)-mediated hypoxia increases BACE1 expression and β-amyloid generation. *J Biol Chem* 282:10873–10880.

Zipser BD, Johanson CE, Gonzalez L, Berzin TM, Tavares R, Hultte CM, Vitek MP, Hovanesian V, Stopa EG. Microvascular injury and blood-brain barrier leakage in Alzheimer's disease. Neurobiol Aging 2007;27:977-986.

Zlokovic BV, Martel CL, Matsubara E, McComb JG, Zhang G, McCluskey RT, Frangione B, Ghiso J. (1996). Glycoprotein 330/megalin: probable role in receptor-mediated transport of apolipoprotein J alone and in a complex with Alzheimer disease amyloid beta at the blood-brain and blood-cerebrospinal fluid barriers. *Proc Natl Acad Sci USA* 93:4229-4234.

Zlokovic BV. (2005). Neurovascular mechanisms of Alzheimer's neurodegeneration. *Trends Neurosci* 28:202-208.

7

The Leukocyte Count, Immature Granulocyte Count and Immediate Outcome in Head Injury Patients

Arulselvi Subramanian, Deepak Agrawal, Ravindra Mohan Pandey, Mohita Nimiya and Venencia Albert
Jai Prakash Narayan Apex Trauma Centre, AIIMS
India

1. Introduction

Proliferation and differentiation of hematopoietic stem cells into mature white blood cells (WBC) in the bone marrow, followed by release into the circulation of mature WBC, is an extremely regulated process (Metcalf, 2008). Differentiation and maturation of the hematopoietic cells into granulocytes, monocytes, lymphocytes, megakaryocytes and erythroid cells is influenced by soluble factors including growth factors and cytokines with the bone marrow stroma, and are mediated to a certain extent through an interaction of adhesion molecules. The synchronized production of leukocytes in bone marrow is crucial for innate and adaptive immunity**.** Leukocytes encompass several subtypes including neutrophils, lymphocytes, monocytes, eosinophils, and basophils, and play a vital role in innate and adaptive immunity against invading microorganisms. They are also involved in the pathogenesis of various acute and chronic diseases. The circulating numbers of leukocytes can be influenced by stress, infection, or inflammation.

Mature neutrophils are ephemeral and localize rapidly to inflammatory sites where they deliver microbicidal activity. It takes about 14 days until it reaches the blood, of which the last 6-7 days are spent in maturation and storage pool. In less than a day after it arrives in the blood vessel, the neutrophil emigrates from the circulation in a random manner and enters the tissue. If not utilized in an inflammatory response, the neutrophils leave the body within a few days via secretions in bronchi, saliva, gastrointestinal tract and urine, or are destroyed by the reticuloendothelial system. The kinetics of eosinophils are similar to the neutrophils, they are stored in the bone marrow for several days after going through the different maturational stages. The half-life in the blood is approximately 18 hours before entering the tissues. Basophils have a life span similar to eosinophil; the maturation time in the marrow is about 7 days. Basophils circulate in the blood and are not normally found in the tissue. Monocytes share the same committed progenitor cell as neutrophils; they undergo maturation for a period of about 50-60 hours before being released in to the blood. Once monocytes enter the blood they leave randomly with a half-time of 8.4 hours. After monocytes leave the blood, they spend several months, or longer as tissue macrophages. (McPherson & Pincus, 2006).

2. Leukocyte count and immature granulocyte count as prognostic marker for traumatic brain injury

Traumatic Brain Injury persists to be a major health problem, and a recurrent cause of death and severe disability among a primarily young population. Worldwide, traumatic brain injury (TBI) is the single largest cause of death and disability following injury. Most TBI's are due to roadside accidents. According to WHO, by the year 2020, head trauma will be third largest killer in the developing world. The statistics from India are even more alarming. Studies show, on an average one person dies every six minutes, 70% of these being directly attributable to head and spinal trauma. The annual social costs of road accidents are estimated 3% of India's Gross Domestic Product (GDP). The accident rate of 35 per 1000 vehicles in India is also amongst the highest in the world (Ahmed et al., 2009). Traumatic brain injury (TBI) is also a major cause of disability, with survivors acquiring long-term cognitive, motor, behavioral or speech-language disabilities (Rutland-Brown, 2003, as cited in Namas et al., 2009). The various forms of traumatic injury therefore represent a pandemic disease that affects every nation in the world without regard for economic development, racial or religious predominance, or political ideology; this disease is acute in onset and often results in chronic, debilitating health problems affecting far beyond the individual victims (Kauvar & Wade, 2005, as cited in Namas et al., 2009).

Trauma acts as a trigger of a complex cascade of posttraumatic events that can be divided into a hemodynamic, metabolic, neuro-endocrine and immune responses leading to a multifocal pathophysiologic process (DeLong & Born, 2004, as cited in Namas et al., 2009). Inflammation is a a well-coordinated communication network operating at an intermediate time scale between neural and longer term endocrine processes which is necessary for the removal or reduction of challenges to the organism and subsequent restoration of homeostasis. (Vodovotz et al., 2008, as cited in Namas et al., 2009). Inflammation is necessary for the removal or reduction of challenges to the organism and subsequent restoration of homeostasis. (Nathan, 2002, as cited in Namas et al., 2009).

Although the inflammatory response is crucial in clearing invading organisms and offending agents and promoting tissue repair, these same responses carried out under a set of extreme conditions can also compromise healthy tissue and further exacerbate inflammation (Nathan, 2002, Jarrar, 1999, as cited in Namas et al., 2009). Thus, early identification of reliable prognostic factors for severely head-injured patients is of significance to both the practicing neurosurgeon and the clinical investigator. The ability to predict likely outcome in acutely admitted hospital patients can be beneficial in several ways. Risk assessment on the basis of laboratory investigations is also commonly used, but is usually applied in specific disease situations, and generally gives subjective assessments of risk. Several prognostic factors, such as age group, gender, pupillary reactivity, Glasgow Coma Scale (GCS) on admission, serum glucose level, total white blood cell counts, platelet counts, coagulation profile, computerised tomography (CT) scan, have been authenticated in various studies to predict outcome in adult traumatic brain injury patients.

A number of other variables have also been suggested to be important for determining the prognosis of patients with severe head injury. These include multimodality evoked potentials, electroencephalography, cerebral perfusion pressure, blood flow velocity on transcranial doppler, jugular venous oxygen saturation, brain tissue oxygenation, and specific serum biochemical markers such as creatine-kinase isoenzyme BB, neuronspecific enolase, and S-100B protein (Dings et al., 1996; Moulton et al., 1994; Nordby and Urdal, 1985;

Raabe et al., 1999; Raabe and Seifert, 2000; Sheinberg et al., 1992 as cited in Rovlias & Kotsou, 2004). Also several studies have shown that hyperglycemia and leukocytosis are associated with a worse outcome, particularly during focal ischemia or hypoxia, which are frequently found in patients with severe head injury (De Salles et al., 1987; Graham et al.,1989; Zhuang et al., 1993 as cited in Rovlias & Kotsou, 2004). Although nonreactive pupils, Sub Arachnoid Hemorrhage, acute subdural and intracerebral haematoma hold clinical significance there are routine laboratory investigations which emerge to be associated with mortality risk in hospital patients.

The complete blood count and leukocyte differential count are among the most frequently requested clinical laboratory tests. Leukocytosis, an increase in the number of circulating white blood cells, was first described by Virchow and Andral in the mid 19th century (Lawerence et al., 2007) is a common phenomenon in head injuries. High leukocyte count though nonspecific has been used more specifically as a prognostic indicator in myocardial infarction and as a predictor of plasma urinary oestrogen levels in women undergoing gonadotrophic treatment for infertility (Hughes, 1963, Cruichshank, 1970, 1972). Early trends in WBC alert the physician about the possibility of sepsis and allow prompt therapeutic response. Leukocytosis above a certain level could serve as a marker for bacterial infection despite the known physiologic leukocytosis following splenectomy (Toutouzas et al., 2002). Leukocytosis is also associated with a worse outcome, particularly during focal ischemia or hypoxia, which are frequently found in patients with severe head injury (Rovlias & Kotsou, 2004).

Leukocyte (WBC) count is considered a biomarker of inflammatory processes that actively contribute to vascular injury and atherosclerosis (Mehta et al., 1998, Alexander, 1994, as cited in Ruggiero et al., 2007). Whether elevated WBC count directly contributes to cardiovascular disease and mortality (Coller, 2005, as cited in Ruggiero et al., 2007) or is merely a marker of negative cardiovascular risk profile remains controversial (Loimaala et al., 2006, Smith et al., 2003 as cited in Ruggiero et al., 2007).

Injury educe a response from all cells of the immune system in which cytokines and other metabolic products of activated leukocytes can act either beneficially to provide for enhanced host resistance or deleteriously to depress the function of remote organs and causes systemic inflammation.

A nonspecific systemic inflammatory response occurs after both ischemic and hemorrhagic stroke, either as part of the process of brain damage or in response to complications such as deep venous thrombosis.

Inflammation alters normal leukocyte production by promoting granulopoiesis over lymphopoiesis, a response that supports the reactive neutrophilia following infection. Leukocytosis in trauma is due to neutrophilia, caused by neutrophil margination, and not due to increased marrow production or release of immature cells or bands. The phenomenon is short-lived, lasting only minutes to hours (Abramson & Beckz, 2000 as cited in Santucci et al., 2008). It is hypothesized that, patients with significant injury should have a higher degree of leukocytosis compared to patients with minor injuries (Santucci et al., 2008).

Traumatic brain injury is associated with elevated serum levels of catecholamines (Clifton et al., 1981, Hortangl et al., 1980, Rosner et al., 1984 as cited in Gürkanlar et al., 2009). Catecholamines are responsible for the release of neutrophil stores while corticosteroids cause a decrease in the egress of neutrophils from the circulation. Catecholamines increase the leukocyte count by release of the marginated cells into the circulating pool. Corticosteroids increase the neutrophil count by releasing the cells from the storage pool in the bone marrow into the blood and by preventing egress from the circulation into these

tissues (Boggs, 1967 as cited in Gürkanlar et al., 2009). Brain swelling occurring after head trauma is probably an inflammatory response due to tracerebral cytokine production and increased leukocyte adhesion as a result of a direct effect on vascular permeability and leucocyte activation (Dietrich et al., 2004, Fee et al., 2003, Gourin & Shackford, 1997, Juurlink, 2000, Lenzlinger, 2001 as cited in Gurkanlar et al., 2009). Another theory of leukocytosis after trauma can be explained as follows: In post traumatic injury, the cell body of the microglia becomes hypertrophic with long, branched and crennellated processes during the first 60 minutes post injury, as the blood brain barrier (BBB) opens at the time of the trauma and approaches closure at about 60 minutes post injury (Bednar et al., 1997 as cited in Gürkanlar et al., 2009). Microglia cells express class I and class II MHC antigens and these antigens could be presented to lymphocytes in the regional lymph nodes and trigger the activation of circulating lymphocytes in the central nervous system (Capps, 1896, Kakarieka, 1997, Neil-Dwyer & Cruickshank 1974, Rovlias & Kotsou, 2001 as cited in Gürkanlar et al., 2009). Microglia cells play a predominant role in the induction and maintenance of the immune response following head trauma (Czigner et al., 2007 as cited in Gürkanlar et al., 2009). An alternative mechanism by which leucocytes can be associated with cerebral damage is the traumatic rupture of microvessels followed by physical occlusion. The leucocytes are less deformable than the erythrocytes, and a greater pressure gradient is therefore required to force them through the capillaries with small diameter. Under conditions of reduced perfusion pressure, the capillaries may behave like a sieve and trap the leucocytes to increase the WBC count. After the entrapment, the leucocytes form a common area of contact with the endothelium and may not be dislodged even after the perfusion pressure returns to normal (Hallenbeck, 1986, Janoff, 1965, Suval, 1987, Yamakawa, 1987 as cited in Gürkanlar et al., 2009). The mechanical occlusion of the capillaries may become more evident as a result of the release of a number of cytotoxic chemicals that leads to increased leucocyte endothelial interactions (Harlan & Winn, 2007 as cited in Gürkanlar et al., 2009).

The presence of immature granulocytes (IG) in the peripheral blood may indicate bacterial sepsis, inflammation, trauma, cancer, steroid therapy or myeloproliferative diseases (Ansari-Lari et al., 2003, Briggs, 2003, 2009, Iddles, 2007.) They are also present in the later stages of pregnancy. In these cases, there is often an increased neutrophil count. Nevertheless neutrophils morphological abnormalities and automated left shift flags are notoriously unreliable as specific diagnostic features.

The measurement of the immature cells of the myeloid series, specifically "band" cells, is considered clinically useful for the diagnosis of infections, especially neonatal sepsis. Rodwell et al., 1988, Seebach et al., 1997 as cited in Buttarello & Plebani, 2008) Even though a morphologic definition of these cells exists, it is not universally accepted. (Cornbleet & Novak, 1995, as cited in Buttarello & Plebani, 2008). Immature granulocytes, normally absent from peripheral blood, are increased also in other conditions such as tissue necrosis, acute transplant rejection, surgical and orthopedic trauma. In these cases, the increase in immature granulocytes is accompanied by an increase in neutrophils, which are freed from the marginal pool and bone marrow. In some subjects, especially elderly people, neonates, and myelosuppressed patients, the increase in neutrophils may be absent, and in other conditions, such as sepsis, there can even be neutropenia. In these situations, the increase in IGs (>2%), even if isolated, can be useful for identifying an acute infection, even when not suspected (Briggs et al., 2003 as cited in Buttarello & Plebani, 2008).

Microscopic immature granulocytes counts has limits of imprecision and lack clinical sensitivity because these components are usually found in low concentrations (<10%).

Published studies agree that IG counts have a high specificity for infectious conditions (from 83% to 97%) but are accompanied by low sensitivity (between 35% and 40%) (Briggs et al., 2003, Ansari-Lari et al., 2003, as cited in Buttarello & Plebani, 2008). This low sensitivity means that this count is not indicated as a screening test for infection, even though a significant association exists between elevated IG counts and positive blood cultures (Buttarello & Plebani, 2008). The presence of low numbers of immature granulocytes is more reliably detected on automated hematology analyzers than using manual microscopy. Automated blood cell counters have undergone a formidable technological evolution owing to the introduction of new physical principles for cellular analysis and the progressive evolution of software, resulting in high number of cells being counted (Briggs et al., 2003, as cited in Briggs, 2009). This is because of the high number of cells counted and an increase of IG (>2%) can be useful in identifying infection even when not suspected (Briggs et al., 2003, as cited in Briggs, 2009).

2.1 Aim

Our aim for this study was to correlate on admission leukocyte and immature granulocyte count (IG) with the severity of head injury (according to the Glasgow coma score), computed tomography findings and pupillary reaction in trauma patients with isolated head injuries. We also intended to determine the factors influencing the immediate clinical outcome (dead or alive) in isolated head injury patients. The acute-phase response due to trauma is characterized by a leukocytosis upon admission. Therefore, an increase in the white blood cell (WBC) count might serve as an additional diagnostic and prognostic indicator in head injury. Our goal was to demonstrate that a reliable prediction of outcome based on the admission day leukocyte and immature granulocyte count is of great clinical relevance. We aimed to develop a prognostic model with readily available traditional laboratory parameters for the selection of those trauma patients who are likely to progress towards an adverse outcome, in turn ensuring their optimum management.

2.2 Materials and method

For the purpose of this study retrospective analysis of case files of patients admitted with, non penetrating head injury (mild, moderate and severe) at a level I trauma centre for duration of two months (June - July 2008) was performed. Patients with brain death, penetrating injury, infection and possible diseases that may alter the white blood cell count (myocardial infarction, cerebral vascular accident, surgical procedures etc) were excluded from the study.

Two ml of venous blood was collected in a disposable EDTA tubes, on the same day as clinical assessment or, for patients admitted to the hospital, as soon after assessment as possible, for the estimation of basic hemogram parameters and immature granulocyte count. The WBC and IG count was measured using a fully automated hematology analyzer, Sysmex XE 2100 (Sysmex, Kobe, Japan).

The results of all the routinely done, laboratory investigations were documented in the institution computerised patient record system (CPRS). The patients clinical and laboratory details were extracted from the CPRS and patient files, for the purpose of this study.

The XE-2100TM is a haematology analyser that, utilises the technology of fluorescence flow cytometry to quantitate the standard five part , immature granulocytes (metamyelocytes, myelocytes and promyelocytes), nucleated red blood cells (NRBC), reticulocyte count, immature reticulocyte fraction and "optical" fluorescent platelet

count. A hemolytic reagent causes disruption of mature WBC membranes, leaving bare nuclei, while immature myeloid cells with low cell membrane lipid content remain intact. A surfactant increases membrane permeability allowing a poly-methylene dye with high affinity for nucleic acid to enter the cells. When excited by a 633-nm laser beam, the stained cells emit fluorescence proportional to their content of nucleic acid. The combination of side scatter (inner complexity of the cell), forward scatter (volume) and fluorescence intensity of nucleated cells gives a concise but precise image of each cell detected in the peripheral blood. A well-defined physical description of the different leucocyte populations (clusters) is obtained. Immature granulocytes are recognized by their increased fluorescence emission compared with segmented neutrophils because they contain more RNA and DNA. The immature information (IMI) channel of the XE-2100 counts human progenitor cells (HPC). The reagents specifically affect the lipid components of the cell membranes; the membranes of mature cells, with a higher content of lipid are lysed while immature cells retain their membranes. In normal samples no intact cells are seen in the IMI area. The HPC has been shown to be an important parameter in the prediction of the apheresis yields of CD34+ cells in peripheral blood in patients undergoing progenitor cell mobilisation. It has been demonstrated that the use of peripheral blood HPC counts gives a more precise measurement of early cells than visual blast cell counts and allows a more quantitative assessment of the release of progenitor cells into the blood (Briggs et al., 1999).

The Sysmex XE 2100 automated analyzer can count immature granulocyte while performing the differential leukocyte (WBC) count, with notably lower imprecision [Coefficient Variance (CV) near 7%]. On comparison with microscopic examination or flow cytometry using Monoclonal antibody (MoAb) methods high accuracy was observed for the Sysmex XE 2100 automated analyzer (r between 0.78 and 0.96). (Briggs et al., 2003, Field et al., 2006 as cited in Buttarello & Plebani, 2008).

The corresponding computed tomography (CT) scan findings and pupillary reaction were extracted from the case files and analyzed. For purposes of analysis, pupillary reaction was noted as unilaterally present, bilaterally present or bilaterally absent reaction. The CT scan findings were recorded as subdural hemorrhage (SDH), extradural hemorrhage (EDH), intracerebral hemorrhage (ICH) and contusion and subarachnoid hemorrhage (SAH). The severity of head injury was graded according to the GCS as mild (GCS 14-15), moderate (GCS 8-13) and severe (GCS 3-7).

The WBC and IG counts were correlated with severity of head injury, pupillary reaction and CT scan findings (SDH, EDH, ICH and SAH). Death during the hospital stay was considered as the study immediate outcome and WBC count, IG count, pupillary reaction and severity of head injury were considered as its potential determinants.

Data was recorded on a predesigned proforma and managed on an excel spread sheet. Categorical variables such as pupillary reaction, severity of head injury (mild, moderate, severe), CT findings (SDH, SAH, EDH, ICH and contusion) and immediate outcome (dead/ alive) were summarized as frequency (%). Quantitative variables (WBC and IG counts) were summarized as mean±S.D (standard deviation) for normally distributed and median (Inter quartile range) for non-normally distributed variables Students's t-Test was used to compare mean values between two groups, while Wilcoxon rank sum test was used to compare median values between two groups. For the overall comparison of mean values between more than two groups, One Way Analysis Of Variance (ANOVA) followed by Bonferroni's correction in post-hoc analysis was applied.

To find out the statistical correlation of various clinical factors with the immediate outcome (dead/ alive), firstly, chi square test was used to measure the statistical association of these factors in the binary form with the outcome, followed by a bivariate logistic regression to compute unadjusted odds ratio (95% confidence interval) of each of the separate factors with the outcome. Lastly all the factors were considered simultaneously in the stepwise multivariate logistic regression analysis with probability to enter as 0.05 and the probability to remove as 0.1. STATA 10.0 statistical software (STATA corporation, Texas, US) was used for data analysis. In this study p value < 0.05 is considered as statistically significant.

2.3 Results

A total of eighty patients were included in the study. The mean age was 33.5±13.9 years; there were 70 (87.5%) males. The head injury was mild (GCS 14-15) in 17(21.3%) patients, moderate (GCS 8-13) in 21(26.2%) patients and severe (GCS 3-7) in 42 patients (52.5%). The overall admission day mean± S.D. leukocyte count and median (IQR) immature granulocyte counts were 14,062 ± 5383 cells/cumm and 0.07 (0-1.54) cells/cumm respectively. Mortality rate of 28.8% (23) was observed in the study group during the course of their hospital stay. The mean WBC count was associated with the severity of head injury according to the GCS scores, pupillary reaction and CT scan findings and the results were tabulated (table 1). The head injury patients with low GCS scores (3-7) had higher mean WBC counts compared to moderate and mild head injury groups ($p<0.001$). Head injured patients with bilaterally absent pupillary reaction had higher mean WBC counts compared to unilaterally present and bilaterally present pupillary reaction groups ($p<0.001$). However, the mean WBC count between the unilaterally present and bilaterally present pupillary reaction groups was not significant ($p<1.00$). Statistically significant association was not observed between the mean WBC count and CT scan findings. The results of the comparison between the median IG count and the other variables are shown in table 2. The median IG count in the, CT scan findings, SAH group was significantly lower than groups with other CT scan findings the non SAH group ($p=0.04$).

To dichotomize the variables, cut-offs were derived for the WBC and the IG counts. The mean WBC count among the survivors and non survivors was 12,096 ± 3842 cells/cumm and 18,934±1172 cells/cumm respectively. Similarly, the median range IG count among the survivors and non survivors was 0.07 (0-0.26) cells/cumm and 0.13 (0-1.54) cells/cumm respectively. The mean value of WBC and IG count among survivors was taken as the cut off and variables were entered in to bivariate analysis to derive the significant factors.The mean value of WBC and IG count among survivors was taken as the cut off and variables were entered in to bivariate analysis to determine the significant independent role of these investigations in head injury patients. The results of bivariate and multivariate logistic regression are shown in table 3. The non survivors had high WBC counts ($\geq$12,096 cells/cumm, $p<0.0001$), severe head injury (GCS 3-7, $p<0.001$) and higher abnormal pupillary reaction (unilaterally present and bilaterally absent pupillary reaction, $p<0.01$) compared to the survivors. Multivariate logistic regression analysis to determine the correlation of each individual factor with mortality revealed only high WBC count [OR (95% CI): 4.9 (0.8-29.5)] and severe head injury (GCS 3-7) [OR (95% CI): 4.4 (0.9-21.2)] to be independent significant predictors of mortality. Bilaterally absent pupillary responses was not found to be statistically significant in predicting mortality, thus it should not always be associated with a hopeless outcome.

Variables	Frequency (n)	WBC (Mean ± S.D.)	Statistical Significance	Post Hoc Analysis p value
Head injury (HI)				
Severe HI	42	17495.2 ± 4687.4	F = 34.1; p = 0.0001	Severe vs. Moderate : 0.001
Moderate HI	21	11114.2 ± 3557.7		Severe vs. Mild : 0.001
Mild HI	17	9223.5 ± 1933.7		
Pupillary reaction				
Bilaterally (B/L) absent	12	19533.3 ± 5884.8	F = 8.72; p = 0.0004	B/L absent vs. U/L absent : 0.009
Unilaterally (U/L) absent	11	13227.2 ± 6103.9		U/L absent vs. B/L present : 1.000
Bilaterally (B/L) present	57	13071.9 ± 4458.2		B/L absent vs. B/L present : 0.000
CT scan finding				
Extra Dural Hemorrhage (EDH)	14	11985.7 ± 4250.2	F = 1.46; p = 0.23	___
Sub Dural Hemorrhage (SDH)	19	15878.9 ± 6878.1		
Sub Arachnoid Hemorrhage (SAH)	11	14727.2 ± 5828.0		
Intra Cerebral Hemorrhage (ICH) and contusion	33	14063.6 ± 4582.7		

Table 1. Comparison of mean WBC counts in various Clinical parameters in Head Injury patients: Results of one way analysis of variance (ANOVA) and Bonferroni correction

Variables	Frequency (n)	IG median (range)	Statistical Significance	Post Hoc Analysis p value
Head injury(HI)				
Severe HI	42	0.85 (0-1.5)	χ^2 = 3.48; p = 0.17	-
Moderate HI	21	0.05 (0-0.5)		
Mild HI	17	0.07 (0-0.3)		
Pupillary reaction				
Bilaterally (B/L) absent	12	0.05 (0-1.3)	χ^2 = 0.4; p = 0.78	-
Unilaterally (U/L) absent	11	0.09 (0-0.3)		
Bilaterally (B/L) present	57	0.07 (0-1.5)		
CT scan finding				
Extra Dural Hemorrhage (EDH)	14	0.08 (0-0.1)	χ^2 = 9.68; p = 0.04	SAH vs. EDH- p<0.05
Sub Dural Hemorrhage (SDH)	19	0.08 (0-1.5)		SAH vs. SDH - p=0.05
Sub Arachnoid Hemorrhage (SAH)	11	0.04 (0-0.2)		SAH vs. ICH - p<0.05
Intra Cerebral Hemorrhage (ICH) and contusion	33	0.08 (0-1.3)		

Table 2. Median (Inter quartile range) Immature Granulocytes (IG) counts in head injury patients: Results of Kruskal-Wallis Test and overall comparison using one way ANOVA & Bonferroni correction

2.4 Discussion

Few authors have studied the association between WBC count, outcome and head injury. Keskil et al illustrated in a study of 153 head trauma patients that WBC count exceeding 20 x 10^6/l was associated with poor clinical grade on admission and high mortality compared to those patients with normal or slightly above normal WBC counts (Keskil et al.,1994). Rovlias & Kotsou did a prospective analysis of 125 patients of severe head injury to study the prognostic significance of WBC counts in these patients. Patients with severe head injury had significantly higher white blood cell counts than did those with moderate or minor injury ($p < 0.001$). Among the patients with severe head injury, a significant relationship was found between WBC counts and Glasgow Coma Scale score, pupillary reaction, and presence of subarachnoid haemorrhage ($p < 0.001$). WBC counts were also found to be an independent significant predictor of outcome on multivariate analysis (Rovlias & Kotsou, 2001). Our results were concordant to that demonstrated by Rovlias & Kotsou. (Rovlias & Kotsou, 2001). Our results were concordant to that demonstrated by Rovlias & Kotsou.

Akin results were also seen in the study by Kan et al, wherein 146 children with Severe TBI were evaluated in attempt to establish the prognostic factors of severe TBI. They observed that a low coma score upon admission was independently associated with poor outcome, also the presence of diabetes insipidus within 3 days post-TBI (OR: 1.9), hyperglycemia (OR: 1.2), prolonged PT ratio (OR: 2.3) and leukocytosis (OR: 1.1) were associated with poorer outcome (Khan et al., 2009). In a study by Gurkanlar et al on 59 patients of head trauma, it was shown that WBC count exceeding 17.5x10^6/l had a predictive value for poor GCS score and long hospital stay. Similarly, CT progression was significantly seen in patients with moderate and severe head injury. Other studies in the literature were done on either trauma patients overall or on specific type of trauma patients such as blunt trauma victims.

Variables	Alive (n=57)	Dead (n=23)	χ^2 Value	p value	Unadjusted odds ratio (95% CI)	Adjusted odds ratio (95% CI)
WBC count ≥12,096 cells/cumm	26 (45.6)	21(91.3)	0.001	0.0001	12.5(2.6 - 58.4)	**4.9 (0.8-29.5)**
IG count > 0.07 cells/cumm	25(43.8)	14(60.8)	1.89	0.13	2.0 (0.7 -5.3)	—
Head Injury Severe	22(38.5)	20(86.9)	15.42	0.001	10.6 (2.8-39.9)	**4.4 (0.9-21.2)**
Pupillary reaction Bilaterally and Unilaterally absent(Abnormal)	12(21.0)	11(47.8)	5.73	0.01	3.43 (1.2- 9.6)	—

WBC count Cut off = 12,096 cells/cumm; IG count Cut off = 0.07 cells/cumm

Table 3. Association of various factors with the death as outcome in head injured patients: Results of bivariate and multivariate logistic regression analysis

Chang et al did a prospective analysis of 786 trauma victims and found ISS >15, GCS≤8 and white race to be associated with increase in white cell count. Their study included all trauma patients irrespective of the site of injury (Chang et al., 2003). WBC count as a laboratory marker has also been studied in blunt trauma patients to predict the severity of injury (Santucci et al., 2008).

Schnüriger et al conducted a study to ascertain the significance of serial white blood cell (WBC) counts in trauma patients with a suspected hollow viscus injury (HVI), on an overall study population of 5,950. A significant relationship between increasing Injury Severity Score and increasing WBC count on admission was found by linear regression and they concluded that WBC count elevation on admission is nonspecific and does not predict the presence of Hollow Viscus Injury (HVI) (Schnüriger et al., 2010).

Similarly, in the study done on 805 trauma patient, to test the diagnostic use of white blood cell (WBC) count in differentiating major from minor injuries. Paladino et al. concluded that WBC count was not a useful addition as a diagnostic indicator of major trauma in their study population (Paladino et al., 2010).

While the WBC had moderate discriminatory capability for serious injury, was not considered to be a reliable independent marker to rule in or out serious injury. Nevertheless, the use of WBC on presentation to the emergency department as an adjunct for making disposition decisions is recommended.

Rovlias & Kotsou observed that, WBC counts were significantly higher in those with an unfavorable outcome ($p < 0.001$) i.e. a high mean± S.D.WBC count of 18144.93±467/cumm was seen in patients with unfavourable outcome (severe disability, persistent vegetative state, dead) in contrast to a mean± S.D. WBC count of 13711.85±415.61/cumm in patients with a favourable outcome, i.e. good recovery, moderate disability (Rovlias & Kotsou, 2001). In another study, the mean WBC count was higher i.e. $21.1x10^6/l$ in patients with Glasgow outcome score (GOS) of one (death) and comparatively low i.e. $12.3x10^6/l$ when the GOS was five (good recovery) (Gürkanlar et al., 2009). Keskil et al showed in a prospective analysis, a low mortality rate (23%) in patients with WBC counts less than 20x106/l Compared to those with counts more than $20x10^6/l$ (mortality rate 96%) (Keskil et al., 1994). The present study showed that a high WBC count (>12,096 cells/cumm) [OR (95% CI): 4.9 (0.8-29.5)] and severe head injury (GCS 3-7) [OR (95% CI): 4.4 (0.9-21.2)] to be independent significant predictors of mortality.

Rangarajan et al. in their study designed to find out the factors influencing mortality in acutely injured trauma patients receiving massive blood transfusion (MBT). They observed a total leukocyte count (TLC) ≥ 10,000 cells/cubic mm, GCS ≤ 8, the presence of coagulopathy and major vascular surgery as four independent determinants of mortality in multivariate logistic regression analysis (Rangarajan et al., 2011).

Immature granulocyte count is a recently introduced parameter in the automated hematology analyzers that provides the number of promyelocytes, myelocytes and metamyelocytes in the peripheral blood. The presence of low numbers of immature granulocytes is more reliably detected on automated hematology analyzers than using manual microscopy (Ali Ansari-Lari et al., 2003; Briggs, 2003, 2009; Iddles et al., 2007). The performance analysis of the automated hematology analyzer for this parameter has been performed earlier (Walters & Garrity, 2000). The immature granulocyte parameter measured using Sysmex-2100 is presently used only for research purposes and in the context of clinical decision making requires more prospective

trials. There are no accredited external quality assessment schemes (EQAS) available for this parameter. Nevertheless, this instrument has internal quality control material available for this parameter and has been proven to be accurate, precise and highly suitable as a screening analyzer reducing the need for manual differentials.

Bruegel et al. analyzed immature granulocytes in 156 healthy donors by using IG count and IMI channel. Men and women showed comparable values for IGs with the highest value of 0.03 $x10^9$/l for men and 0.06 $x10^9$/l for women. No age dependency for the IG counts was reported (Bruegel et al., 2004).

In the study to determine the usefulness of immature granulocyte measurement as a predictor of infection or positive blood culture. Blood samples from 102 infected and 69 non infected patients were analyzed using the Sysmex XE-2100 automated blood cell counter (Sysmex, Kobe, Japan). The percentage of immature granulocytes was found to be significantly higher ($P < .001$) in infected than in non infected patients and in patients with positive than patients with negative blood cultures ($P = .005$). Also, a percentage of immature granulocytes of > 3 was observed to be a very specific predictor of sepsis. On comparing the results of immature granulocyte measurement with total WBC count and absolute neutrophil count (ANC), receiver operating characteristic curves (ROC) showed that the percentage of immature granulocytes was a better predictor of infection than the WBC count and comparable to the ANC. They concluded that immature granulocyte measurements reflect a biologically and clinically relevant phenomenon but are not sensitive enough to be used as screening assays for prediction of infection or bacteremia. However, although infrequently encountered, a percentage of immature granulocytes of more than 3 might help expedite microbiologic laboratory evaluation of a subset of patients. (Ali Ansari-Lari et al., 2003)

In the present study, the role of admission WBC and IG count as prognostic determinants of mortality in isolated head injury patients was investigated. The study revealed that the mean WBC count was high in patients with severe (GCS 3-7) head injury and bilaterally absent pupillary reaction groups. The independent significant determinants of mortality due to head injury were high WBC count ($\geq$12,096 cells/cumm) and severe head injury (GCS 3-7). IG count was not found to be a potential determinant of mortality in this study.

We observed high WBC counts in the non survivors compared to the survivors and the difference was statistically significant ($p<0.001$). Similar results were observed by Rovlias et al.

We found that the IG count in patients with subdural haemorrhage, extradural hemorrhage, intracerebral hemorrhage and contusion was significantly higher than those patients who had subarachnoid haemorrhage.

3. Conclusion

Leukocytosis at initial examination is associated with adverse prognosis in trauma patients. High admission WBC count (>12,096 cells/cumm) and low GCS scores (3-7) portends a worse prognosis in isolated head trauma patients. Percentage of immature granulocytes correlates with CT findings ($p=0.04$) of Head injury patients, but its association with severity of injury and mortality is clinically insignificant. More prospective studies would be

required to evaluate the role of IG count as a marker of head injury in a larger study population, also to assess whether immature granulocyte measurements could be combined with other markers to create an algorithm with better diagnostic sensitivity or specificity.

4. Acknowledgment

We would like to thank Dr Kanchana Rangarajan, Senior Resident at the department of laboratory medicine, Trauma Center, AIIMS, for dedicated work and support. Mr Bhupender for helping in the statistical analysis of the study.
Prof. Dr. Mahesh Chandra Misra , Chief of Trauma Center for providing the ammenities and facilities, that made this study possible.

5. References

Ali Ansari-Lari M, Kickler TS, Borowitz MJ. (2003). Immature granulocyte measurement using the sysmex XE-2100. Relationship to infection and sepsis. *Am J Clin Pathol*, Vol. 120, pp (795-99)

Ahmed S, Khan S, Agrawal D, Sharma B S. (2009). Out come in Head Injured patients :Experience at a level 1 Trauma Centre. *Indian Journal of Neurotrauma, Vol. 6, No. 2, n.d., pp. (119-122)*

Briggs C, Harrison P, Grant D, Staves J, Chavada N, Machin S J. (1999). Performance Evaluation of the Sysmex XE-2100™ Automated Haematology Analyser. *Sysmex Journal International,* Vol.9, No.2, n.d., pp.(113 – 119)

Briggs C, Kunka S, Fuijimoto H, Hamaguchi Y, Davies B, Machin SJ.(2003).Evaluation of the immature granulocyte counts by the XE-IG Master: upgraded software for the XE-2100 automated hematology analyser. *Laboratory Hematology*, Vol. 9, No. , n.d., pp: 117-24.

Bruegel M, Fiedler GM, Matthes G, Thierry J. (2004). Reference values for immature granulocytes in healthy blood donors generated on the Sysmex XE2100 automated hematology analyzer. *Sysmex Journal International,* Vol 14, pp (5-7)

Buttarello M, Plebani M.(2008). Automated blood cell counts: state of the art. *Am J Clin Pathol*, Vol. 130, No. 1, n.d, pp (104-16)

Briggs C.(2009). Quality counts: new parameters in blood cell counting. *Int J Lab Hematol*. Vol. 31. , n.d., pp.(277-97)

Cruichshank JM, Morris R, Butt WR, Crooke AC.(1970). The relationship of total and differential leukocyte counts with urinary oestrogen and plasma cortisol levels. *J Obstet Gynaec Br Commonw*, Vol 77, No. ,n.d, pp.(634-9)

Cruichshank JM, Morris R, Butt WR, Corker CS. Interrelationships between levels of plasma oestradiol, urinary total oestrogens and blood haemoglobin and neutrophil counts.(1972). *J Obstet Gynaec Br, Vol.* 79, No. , n.d, pp.(450-4)

Chang DC, Cornwell EE 3rd, Phillips J, Paradise J, Campbell K.(2003).Early leukocytosis in trauma patients: what difference does it make?. *Curr Surg*, Vol. 60, No. , n.d., pp.(632-5)

Gürkanlar D, Lakadamyali H, Ergun T, Yilmaz C, Yücel E, Altinörs N.(2009). Predictive value of leucocytosis in head trauma. *Turkish Neurosurgery, Vol: 19, No: 3, pp. (211-215)*

Hughes WL, Kalbfleisch JM, Brandt EN, Costiloe JP.(1963).Myocardial infarction prognoses by discriminant analysis. *Arch Intern Med*, Vol. 111, No. , n.d, pp. (338-45)

Iddles C, Taylor J, Cole R, Hill FGH. (2007).Evaluation of the immature granulocyte count in the diagnosis of sepsis using the sysmex XE-2100 analyser. *Sysmex J Int* , Vol. 17, No. , n.d., pp. (20-29)

Keskil S, Baykaner MK, Ceviker N, Aykol Ş.(1994).Head Trauma and Leucocytosis. *Acta Neurochir (Wien)*, Vol. 131, No. , n.d., pp.(211-14)

Kan C H, Saffari1 M, Khoo T H.(2009).Prognostic Factors of Severe Traumatic Brain Injury Outcome in Children Aged 2-16 Years at A Major Neurosurgical Referral Centre. *Malaysian Journal of Medical Sciences*, Vol. 16,No. 4, pp (25-33)

Lawrence Y R, Raveh D, Rudensky B, Munter G. (2007). Extreme leukocytosis in the emergency department. *QJM.* Vol. 100, No. 4, n.d., pp. (217-223)

McPherson, R A. Pincus, M R.(August 18, 2006). Henry's Clinical Diagnosis and Management by Laboratory Method (21st Edittion), W. B. Saunders Company, ISBN-13: 978-1-4160-0287-1, ISBN-13: 978-1416002871, USA.

Metcalf D (2008) Hematopoietic cytokines. *Blood, Vol.*111. pp (485–491)

Namas R, Ghuma A, Hermus L, Zamora R, Okonkwo DO, Billiar TR, Vodovotz Y. (2009). The Acute Inflammatory Response in Trauma / Hemorrhage and Traumatic Brain Injury: Current State and Emerging Prospects. *Libyan J Med.* Vol. 4, No. 3,.n.d., pp. (97-103)

Paladino L, Subramanian A R, Bonilla E, Sinert R H.(2010). Leukocytosis as Prognostic Indicator of Major Injury. *Western Journal of Emergency Medicine*. Vol. 11, No. 5. Pp. (450-455)

Rangarajan K, Subramanian A, Pandey R M. (2011). Determinants of mortalityin trauma patients following massive blood transfusion. *Journal of emergency trauma and shock*, Vol. 4, No. 1, n.d., pp.(58-63)

Rovlias A, Kotsou S.(2001) The Blood Leucocyte count and its prognostic significance in severe head injury. *Surg Neurol* , Vol. 55, No. , n.d., pp.(190-96)

Rovlias A, Kotsou S. (2004). Classification and Regression Tree for Prediction of Outcome after Severe Head Injury Using Simple Clinical and Laboratory Variables. *Journal of Neurotrauma.* Vol. 21, No. 7, n.d., Pp. (886–893), ISSN 0897-7151

Ruggiero C, Metter EJ, Cherubini A, Maggio M, Sen R, Najjar SS, Windham GB, Ble A, Senin U, Ferrucci L.(2008). White Blood Cell Count and Mortality in the Baltimore Longitudinal Study of Aging. *J Am Coll Cardiol*. Vol. 49, No. 18,n.d., pp. (1841-50)

Santucci CA, Purcell TB, Mejia C.(2008). Leukocytosis as a predictor of severe injury in blunt trauma *Western Journal of Emergency Medicine*. Vol. 9, No.2, pp. (81-85)

Schnüriger B, Inaba K, Barmparas G, Barbara M. Eberle B. M., Lustenberger T., Lam L., Talving P.,Demetriades D.(2010). Serial White Blood Cell Counts in Trauma: Do They Predict a Hollow Viscus Injury?.*J Truma of Injury, infection and critical care*, Vol. 69, No. 2, n.d., pp. (302-307)

Toutouzas K G, Velmahos G C, Kaminski A, Chan L, Demetriades D. (2002). Leukocytosis After Posttraumatic Splenectomy A Physiologic Event or Sign of Sepsis?. *Arch Surg*.Vol. 137, No. 8, n.d.,pp. (924-928)

Walters J, Garrity P.(2000). Performance evaluation of the Sysmex XE-2100 hematology analyzer. *Lab Hematol* , Vol. 6, No. , n.d., pp.(83-92)

8

Animal Models of Retinal Ischemia

Gillipsie Minhas and Akshay Anand*
Neuroscience Research Lab, Department of Neurology
Postgraduate Institute of Medical Education and Research, Chandigarh
India

1. Introduction

Retinal ischemia is a frequent source of irreparable visual impairment and even loss of sight, affecting over a hundred million individuals in the world. It is associated with a wide range of clinical retinal disorders, like ischemic optic neuropathies, obstructive retinopathies, carotid occlusive disorders, diabetic retinopathy and glaucoma. Retinal ischemia occurs when the blood supply to retina is inadequate to meet the metabolic requirements of the retina. If treatment is not given to fix this imbalance, the outcome is irreversible, ischemic and apoptotic cascades resulting in cell death. Appropriate study models, particularly animal models, are necessary for further understanding the etiology, pathology, and evolution of retinal ischemia and also in order to help in the evaluation, development, and improvement of therapeutic strategies. Accordingly, quite a few *in-vivo* and *ex-vivo* mammalian models have been developed to study this syndrome. The rat models of retinal ischemia are frequently used, because the distribution of retinal and choroidal blood supply is quite similar to that in humans.

The retina has been extensively used for the study of pathophysiology of ischemia and mechanism of damage triggered by ischemia and excitotoxicity. Compared to all the other tissues, retina has a higher metabolic rate; any disturbance in blood supply can have an effect on the supply of oxygen and the substrates leading to retinal ischemia. The retina has a dual blood supply. The photoreceptors and most of the outer plexiform layer (OPL) are nourished by choriocapillaries, while the inner retinal layers are nourished by the central retinal artery. The actual effects of retinal ischemia vary, depending on the position of the occlusion. It is clear that occlusion of the retinal artery leads to inner retinal ischemia only, but occlusion of ophthalmic artery leads to global retinal ischemia, as it supplies blood to the central retinal artery as well as choriocapillaries.

2. Retinal architecture

The retina of mammals is a functionally specialised tissue. It is capable of light detection and perception as well as processing and transmission of the information received to the central nervous system. It has two major elements – the neurosensory retina and the pigment epithelium (RPE). During the embryonic development, the RPE and neural development are

* Corresponding Author

derived from the same layer, i.e. the neuroectoderm, although they are morphologically not similar. Hence, they are considered collectively as "retina".

The retina is made up of three principal layers of nuclei, which are, from internal to external, the thin ganglion cell layer, the inner nuclear layer and the outer nuclear layer (Figure 1). The ganglion cell layer consists of cell bodies of various classes of ganglion cells and amacrine cells. The inner nuclear layer is the layer with nuclei of bipolar cells, amacrine cells, horizontal cells and Muller cells. And the outer layer is the one which contains the nuclei of rod and cone photoreceptors. There are also two plexiform or synaptic layers that are not filled with any cell nuclei. The inner plexiform layer (IPL) lies between the ganglion cells and inner nuclear layer and the outer plexiform layer (OPL) is sandwiched between the outer and inner nuclear layer. These synaptic layers contain axons and dendrites, which aid in early visual processing and also help in adjusting to different light intensities.

The retina contains a rich assortment of cell types including light-sensing photoreceptors. The outermost layer of the cells in the retina is the RPE. It is a simple cuboidal epithelium, containing melanosomes that help in quenching photons that are not absorbed and therefore, minimise light scattering. RPE also has other biological functions like maintenance of choroidal vasculature and blood-retinal barrier. The retinal photoreceptor cells are specialised neurons found in the outer retina. The diversity of inner retinal neurons is really complex. Bipolar cells span from the OPL to the IPL, in which they form synapses with photoreceptor cells and ganglion cells respectively. The nuclei of bipolar cells are found in the inner nuclear layer. Amacrine cells in themselves as a group are considerable in number as well as diversity. These are present in both inner nuclear layer plus the ganglion cell layer where they are involved in relaying impulses (Masland, 1988). Another type of cells that also occur in the retina are the ganglion cells. Ganglion cells have long axons that are pass through the optic nerve (Berson, 2007). Glial cells are also found in the retina. These cells support the retinal microenvironment. These include Muller cells, astrocytes and microglia. Another type of cell found in the ganglion cell layer and optic nerve head are the astrocytes. These cells contribute to the blood retinal barrier (Kaur *et al*., 2008).

3. Retinal blood supply

As mentioned earlier, the cause of retinal ischemia is insufficient supply of blood, which is unable to meet the metabolic demands of the retina. When occlusion occurs in any tissue, anatomy of blood supply plays a significant role. The retina has a higher metabolic rate, even than that of the brain. The retina is a specialised extension of central nervous system and has a complex and dual blood supply, i.e the choroidal and the retinal. The choroid gets the maximum blood supply (around 65-85% of total supply to the eye), whereas the retina gets just 20-30%blood (Henkind, *et al*., 1979). The photoreceptors in the outer nuclear layer and the outer plexiform layer are nourished indirectly from the choriocapillaries; whereas, the inner retinal layers are nourished by branches of central retinal artery, which arises from the ophthalmic artery as the central retinal artery enters the retina, it divides into four main branches. The retinal blood vessels also help in maintaining the blood-retinal barrier. Outer retinal layer ischemia is caused by occlusion in choriocapillaries. On the other hand, complete retinal ischemia and infarction require ophthalmic artery occlusion (Saint-Geniez and D'Amore, 2004).

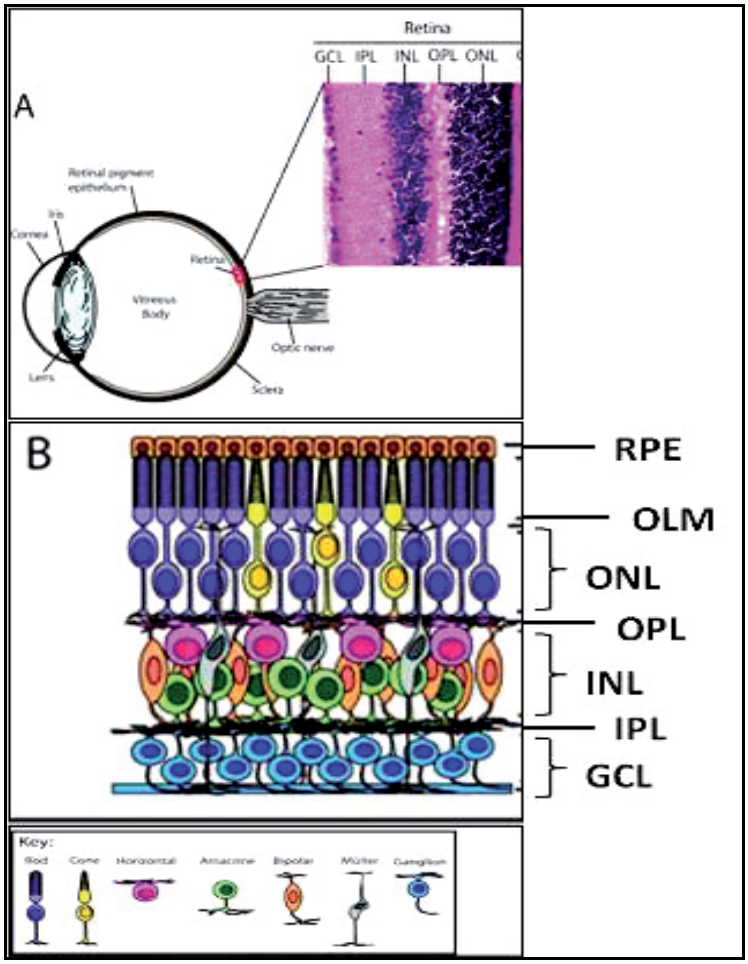


Fig. 1. Different cell layers in retina (where RPE – Retina Pigmented Epithelium, OLM – Outer Limiting Membrane, ONL – Outer Nuclear Layer, OPL – Outer Plexiform Layer, INL – Inner Nuclear Layer, IPL – Inner Plexiform Layer, GCL – Ganglion Cell Layer) *(reproduced and readapted with permission – Poche & Reese, 2009)*

4. Correlation between the brain and the retina

The retina shares many functional, embryological and anatomic characteristics with the brain. In humans, the eye starts developing at about 3 weeks of pregnancy. The eye is mainly derived from three types of embryonic tissue – neuroectoderm forms retina, pigment cell layers and optic nerves, mesoderm leads to cornea, sclera and blood vessels and the ectoderm forms the lens. At 22 days of embryonic stage, a pair of optic vesicles is formed on each side of the forebrain. These vesicles form connections with the developing central nervous system through stalk-like structures. As the development progresses, these stalks become thinner and form optic nerves. Thus, it is a representative of the CNS. The mesoderm in the embryo forms the blood vessels – the hyaloid artery and vein, that nourishes the developing lens, which later on in development transform to the central artery

and the vein. The retina can be visualised directly, thus, it can be used to study stroke. Many retinal conditions are associated with stroke, such as occlusion of middle cerebral artery also leads to retinal ischemia, as can be seen in figure 2, where it shows that the ophthalmic artery originates nearby to the MCA. Thus, the mechanisms that affect the eye and the brain are linked to some extent. But, the examination of retina to predict future stroke incidence is still doubtful.

Retinal neurons and glia show same response to ischemia as the neurons in the other part of the CNS. Retina also has a blood-retinal barrier similar to the blood-brain barrier (Tso & Jampol, 1982). These two also differ in the resistance to the ischemic injury. The retina can survive much longer than the brain. Also, the retina shows geographical difference in sensitivity to ischemia, the outer retinal layers being more prone to the injury than the inner ones.

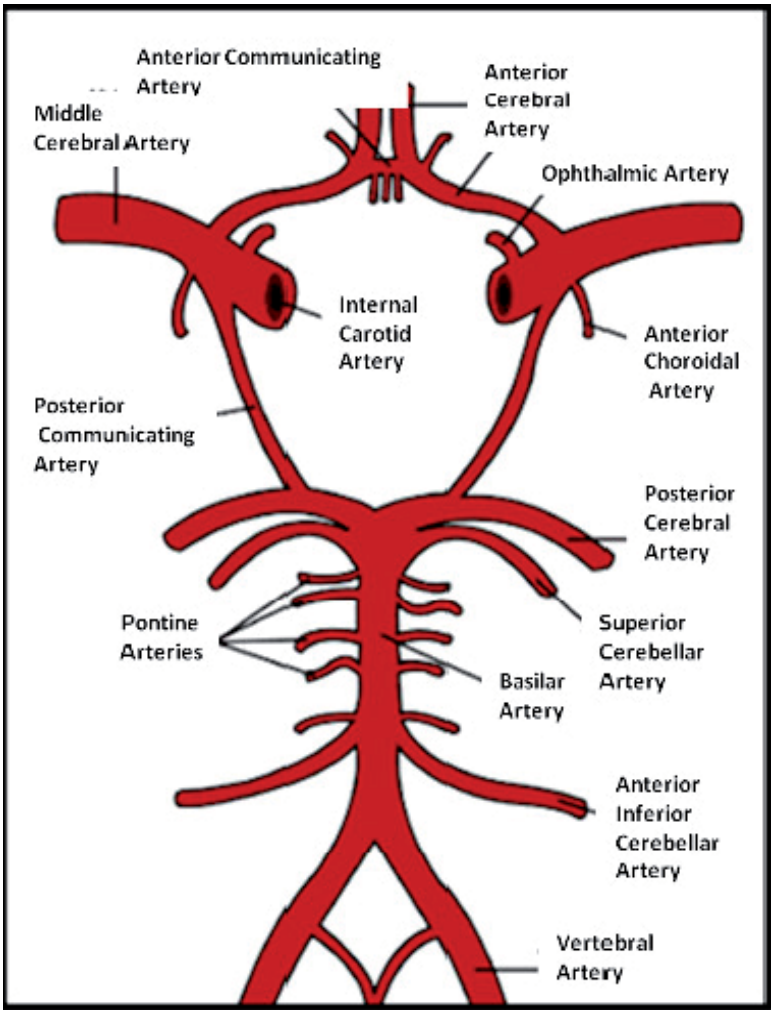


Fig. 2. The figure depicting Circle of Willis showing blood supply to brain and retina (*Reproduced with permission from M. F. Block, 1997)*

5. Susceptibility to retinal ischemia

Various changeable and non-changeable factors are involved in ischemia, such as age, family history, ethnic group, and previous medical history. Kawai *et al* studied the different risk factors related to neuronal injury using an intraocular pressure rat model. The number of retinal ganglion cells (RGCs) keeps on decreasing with age and the residual RGCs become more susceptible to damage. Calorie intake has also been proven to be a factor involved in ischemia. The diet restriction has a neuroprotective effect and thus, leads to lesser damage to RGCs. As seen in the case of glaucoma, pre-existing diabetes is a reason for greater harm to RGCs (Kawai *et al*, 2001).

Genetic background is one of the principal determinants of susceptibility to retinal neovascularisation and breakdown of blood retinal barrier. Vulnerability to ischemia-induced retinal neovascularisation depends on the strain of animal model used. Strain difference in rat model leads to variation in expression of VEGF and thus, causes an increase in permeability and leakage of fluid and plasma proteins, resulting in edema. It has been demonstrated that Brown-Norway rats are more susceptible to Sprague-Dawley rats (Gao *et al*., 2002).

It is seen that a certain percentage of cell death that occurs in transient cerebral and retinal ischemia occurs by means of apoptosis. An apoptosis cascade involves both pro-apoptotic and anti-apoptotic genes. One of these is p53, which is a DNA-binding transcription factor involved in DNA damage and repair. Ischemia leads to increase in expression of p53. The p53 causes selective vulnerability of inner retina to transient ischemia. Also, it is observed that transgenic mice lacking in p53 are resistant to excitotoxicity. Mice heterozygous for null mutation in p53 gene are resistant to retinal ischemia. From the above discussion, it is safe to say that p53 can be one more target for therapeutic strategy for retinal ischemia (Zhang *et al*., 2005).

6. Pathophysiology of retinal ischemia

Retina has a really high metabolic rate. Glucose and oxygen deprivation can harm the whole retina, but all cells are not equally vulnerable. The loss of cells due to ischemia is irregular. The retinal cells that lie near the blood vessels are exposed to an environment rich in oxygen and thus, are more prone to the ischemic damage. But, another thing to consider is that these cells are the first one to be cured on reperfusion. Temporary interruption of blood circulation prevents the exchange of metabolic substrates and products, affecting cells in retina. However, in addition, there are many indirect effects which are sustained even after restoration of blood supply. These effects may be systemic, such as respiratory or vasomotor centre failure or the outcome can also be localised, i.e. impaired reperfusion, edema or breakdown of blood-brain barrier. Thus, evidently, these effects are so complicated that it is hard to identify the order of events leading to damage.

Retinal ischemia causes a number of morphological and functional changes. These changes are a product of combined and inter-related pathophysiological pathways – leading to imbalance in ion transport, changes in neurotransmitter levels, neuronal depolarisation, oxidative stress, and energy failure. The occurrence of ischemia leads to a complex cascade of response to energy failure and ATP depletion which eventually causes cell death. Studies have shown that substrate deprivation is the less damaging as compared to oxygen deprivation, which reduces protein synthesis. The irreversible damage increases with an increase in the duration of oxygen deprivation. The cells which are the most affected are the

photoreceptor cells as these have the maximum oxidative metabolic rates. But when both substrate and oxygen deprivation are combined, a reduction in the ATP synthesis is observed. This leads to energy metabolism failure. The process can be explained as follows - decrease in ATP levels disrupt the Na^+/K^+ ATPase transporter, leading to disruption of membrane potential and ion gradients, preventing the repolarisation of axons and synaptic membranes. There are many studies which testify to the discharge of various neurotransmitters, e.g. GABA, glycine, dopamine, acetylcholine, after the occurrence of ischemia. During the ischemia the receptors for different neurotransmitters, present on the retina, are opened in response to the elevated levels of their ligands, e.g. GABA, glycine, extracellularly. Under normal circumstances, neurotransmitter levels are low extracellularly. Glutamate is recognized as the major excitatory retinal neurotransmitter. It is released by photoreceptor bipolar cells and the ganglion cells. During retinal ischemia glutamate gets accumulated in the extracellular space (Louzada-Junior *et al*., 1992). Lucas and Newhouse showed the occurrence of glutamate excitotoxicity in ischemia. Neurons in inner retina and ganglion cells are more susceptible to ischemia due to the incidence of high levels of glutamate receptors. Glutamate causes neurotoxicity by several different mechanisms, i.e., increase in Ca^{2+} ion levels, Na^+ influx, which depolarises the plasma membranes. There are a number of receptors which can activate glutamate neurotoxicity. These can be NMDA as well as non-NMDA based receptors (Lucas & Newhouse, 1957). In 1992, Osborne stated the role of NMDA – based excitotoxicity in retinal ischemia. NMDA receptors are Ca^{2+} permeable, and thus, increase in glutamate levels raises the Ca^{2+} ion levels intracellularly. Excess glutamate causes Na^+ influx, which is followed by Cl^- influx, which cannot be countered by outward efflux as the membranes are impermeable to most intracellular anions. Therefore, transport of cations and Cl^- ions increases the intracellular osmolarity, causing osmotic shock, edema and cell lysis and death (Osborne *et al*., 1992). The voltage gated Ca^{2+} channels are also opened, leading to rise in intracellular levels of Ca^{2+} ions, which inhibit the mitochondrial metabolism. Another group also validated the involvement of NMDA as well as non-NMDA based receptors in retinal degeneration. Romano *et al.,* in an *in-vitro* model of ischemia in the retina from a chick embryo reported by using the blockers of both NMDA and non-NMDA receptors that the damage to the retina is due to overexcitation of the receptors (Romano *et al*., 1998). Ueda *et al* also showed that the NMDA caused damage to blood vessels in the retina. They created a rat model by injecting NMDA into the eye, which led to loss of retinal ganglion cells and thinning of the inner plexiform layer, thus damaging the inner retinal layers and also led to loss of endothelial cells in the blood vessels in the retina (Ueda *et al*., 2010).

Reperfusion, i.e. restoration of blood supply after the ischemic injury, can also lead to cell damage. Oxygen restoration to the deprived tissue can add up to the injury caused by ischemia (Jennings *et al*., 1960). Also, it has been shown that LDH, marker of cell death increases after oxygen restoration (Sims *et al*., 1992).

7. *In-vitro* models of retinal ischemia

Most of the cell culture models for ischemia utilise primary nerve cell cultures, that are exposed to insults associated with ischemia *in-vivo*, e.g., glutamate neurotoxicity, glucose and oxygen deprivation. One of the approach involved chemical ischemia in immortalised rat retinal ganglion cell line (RGC-5). They used iodoacetic acid (IAA), a known inhibitor of enzyme glyceraldehyde 3-phosphate dehydrogenase. This IAA treatment induces changes

seen in retinal ischemia, such as disturbance in membrane potential, ATP loss and reactive oxygen species generation (Malur *et al*., 2008). Another way of inducing ischemic-like changes *in-vitro* is to incubate retinal pigmented epithelium (RPE) cells with oligomycin (an ATP synthase inhibitor) and sodium cyanide (inhibits cytochrome-c oxidase), along with IAA (Palmero *et al*., 2000). In-vitro model can also be induced by glucose/oxygen deprivation.

These in-vitro models can be used for testing and identifying novel neuroprotective compounds. Many herbal products are nowadays being used, the Chinese herbal medicines, in a large number of disorders, e.g. coronary heart disease, cardiovascular disease and traumatic wounds. Romano *et al* examined the neuroprotective activity of an extract from Chinese safflower (*Cartnamus tinchoris*, Honghua). An *in-vitro* model of retinal ischemia was made from chick embryo retina. The ischemia was generated by removing glucose from the media and growing the culture in nitrogen atmosphere. It was seen that Honghua protected the retina from the effects of toxins like NMDA and also from the ischemic conditions (Romano, *et al*., 1993).

Another herbal extract that has been used in Korea and China are derived from the shrub, *Thuja orientalis*. It has been shown to be effective in disorders such as, gout, diarrhoea and rheumatism. It was demonstrated by Jung *et al* in the transformed retinal ganglion cell line (RGC-5) *in vitro* that the extract of *Thuja orientalis* has anti-oxidant properties. In this study, RGC-5 cells were exposed to H_2O_2 to create oxidative stress. The major component found in this extract with anti-oxidant properties is the isoquercitrin, which can be in future used for treating glaucoma, but requires further investigation (Jung, *et al*., 2010). The roots of another plant, *Scutellaria baicalensis*, are also used in China. It contains three flavnoids – wogonin, baicalin, baicalein. These flavenoids are natural free-radical scavengers. Out of the three flavenoids found, baicalin has been shown to have neuroprotective action, but the mode of its action is unknown. The role of baicalin was observed in *in vitro* model in RGC-5 cell line, where it reduces the damage caused by reactive species and apoptosis (Jung *et al*., 2008). Matteucci *et al* have also tested the curcumin, the phenolic extract obtained from *Curcuma longa* in primary retinal cell cultures. It showed protective effect for both the retinal as well as hippocampal neurons from NMDA excitotoxicity. The mode of action of curcumin may be through the increase in production of NMDA receptor subunits (Matteucci, *et al*., 2011). But these *in-vitro* models have some limitations of their own, such as getting sufficient quantities of cells and obtaining reproducible and comparable data. Also, the *in vitro* models do not correlate much to the *in vivo* conditions as these do not provide apt physiological environment and are based on chemical interactions.

8. Need for animal models

Animal models have been a mainstay of basic and applied research. Animal testing has been used since second century where early writings by Greeks discuss its use. Dissection and experimentation has been used to get knowledge about anatomy and physiology in humans. The use of animal models has allowed the fast progression of scientific discovery. Animal models have had a central place in medical research, in developing new therapeutic strategies for treating human diseases as well as in preclinical trials. The aim of using animal models is to achieve better understanding of pathways involved in the disease without causing any harm to the human being. A number of animal models are available for studying the mechanisms of retinal ischemia. The vascular supply and pathways involved

in retinal ischemia in animal models must be better understood for developing new therapies for human disorders.
In-vitro models have always been used to get insight of biochemical and molecular events caused by ischemia; but animal models are essential in understanding pathophysiology of retinal ischemia. In all animal models of retinal ischemia, the retinal circulation is obstructed to study the balance between energy supply and demands, vascular and neuronal changes. Different species such as monkeys, rabbits, rodents, cats, dogs have been used as animal models. In choosing an animal model, several factors are considered like anatomy of vascular circulation and retina, relevance to humans and also the availability of the animals.

9. Desirable characteristics in animal models

Animal models are used to understand the mechanism behind a particular disease and also to discover new as well as validate the already therapeutics for it. Animal models can either be spontaneous or induced and can be developed in any species which fits best for the disease to be studied and the purpose of our study.
The model should show up same characteristics and symptoms as seen in human disorders. The model should have common features with the humans, such as anatomy, vascular system and retina in case of animal models for retinal ischemia. But at the same time these animals should be easy to manipulate, i.e. they should have small size, high reproducibility and easy for genetic manipulation. For testing the therapeutics, the animal models being used should mimic the humans and give same response as desired in the humans, so that the humans will show similar response. Other desirable characteristics include low cost, easy availability, easy to handle and breed and less prone to infections or diseases other than the desired one.

10. Animal models of retina ischemia

10.1 Raising the intraocular pressure

It is the most widely used method to study the mechanisms involved in retinal ischemia. Peachey *et al*. demonstrated that the high intraocular pressure (IOP) induced retinal ischemia model has been established as an important model system. IOP model creates ischemia by elevating and maintaining the intraocular pressure above the systemic arterial pressure (Peachey *et al*., 1993). Flower *et al* described the retinal ischemia model in cats. The IOP was raised to 110mm Hg by cannulation of anterior chamber with a 26-gauge needle, which is connected through nylon tubing to an elevated container with normal saline. This increase in intraocular pressure blocks the retinal blood circulation and thus, leads to ischemia (Flower *et al*., 1971). Following a similar procedure, a model of pressure-induced retinal ischemia/reperfusion injury was established in rats (Buchi *et al*., 1991).
It has been used to study changes in protein expression, excitotoxicity and alteration in membrane properties in various different models. IOP animal models have also been used to study changes in serum antibody reactivities after ischemia. Joachim *et al*. created IOP model by raising the pressure to 130mm Hg for an hour to check the antibody response to ischemia/reperfusion injury (Joachim, *et al*., 2011). On the other hand, Hirelinger *et al*. investigated the involvement of ion imbalance and role of Muller cells in degeneration of retina in a mouse IOP model (Hirelinger, *et al*., 2010).

Baicalin, a flavenoid found in roots of *Scutellaria biacalensis*, when was administered in rats intraperitoneally showed protection from the retinal ischemia'reperfusion injury, as was seen *in vitro.* The baicalin was given before subjecting the animals to raised intraocular pressure of 120mm Hg for 50 minutes (Jung, *et al*., 2008).
The principle limitation of this model include that the raised intraocular pressure itself can contribute to the resulting retinal damage. Thus, this model is complicated by a combination of ischemic as well as pressure-induced injury.

10.2 Cerebral artery occlusion

Acute thrombotic/embolic stroke in humans are often associated with temporary diminishment (amaurois fugax) or even permanent loss of vision. Middle cerebral artery occlusion (MCAO) is a purely vascular model of retinal ischemia that reproduces transient human vision loss. MCAO in rodents is one of the most widely used experimental paradigms to induce focal cerebral ischemia. This model occludes arterial blood flow intraluminally and allows reperfusion by removing the inserted filament. The ophthalmic artery that mainly supplies the inner retina originates from the internal carotid artery proximal to the origin of the middle cerebral artery. Therefore, it is expected that MCAO simultaneously obstructs blood flow in the ipsilateral retina. Block *et al* demonstrated the first evidence of retinal ischemia by MCAO in rats (Block *et al*., 1992). Steele *et al* have shown for the first time that MCAO simultaneously obstructs blood flow in ipsilateral retina in mice (Steele *et al*., 2008).
Kaja *et al* indicates that the MCAO/ reperfusion model is more appropriate than other retinal ischemia models such as high intraocular pressure or optic nerve ligation models to study the cellular and molecular changes in retina after stroke (Kaja *et al*., 2003). This model is non-invasive with respect to the eye and does not induce blood-retina barrier disruption or mechanical damage to retina. The model is reproducible and easily reversible and involves vascular structure of the entire eye. Therefore, MCAO is a more relevant model for studying changes and testing the efficacy of therapeutic strategies for retinal ischemia.
This model has been used to validate the effect of a various herbs of Chinese origin. The extracts from wolfberries *(Lycium barbarum,* Gougizi*)* mostly consist of polysaccharides and are believed to be good for the eye. It has also been shown previously in many studies that these extracts have protective effect against liver damage, ageing and oxidation (Ha, *et al*., 2005, Li, *et al*., 2007, Yu, *et al*., 2007).The group created a model by occluding the internal carotid artery in rats. The extracts were given orally for 1 week before the occlusion. This study showed that this pre-treatment with the extracts protected the retina from various conditions linked with retinal ischemia, such as, neuronal death, apoptosis, glial cell activation and blood-retinal barrier disruption (Li, *et al*., 2011).

10.3 Chronic carotid ligation

In two-vessel occlusion model, the occlusion is permanent and long-lasting and reperfusion does not occur. This model reduces the cortical/hippocampal blood flow to 25-50% of the normal levels in 2.5 hours post occlusion (Yamamoto *et al*., 2006). The blood flow in the retina may be more severely reduced than in the brain. Davidson *et al* have also shown that the two-vessel occlusion, i.e. the bilateral carotid artery occlusion causes an early loss of the pupillary reflex in 50% of the animals (Davidson *et al*., 2000). The 2-VO model mimics the ocular pathology of human carotid artery disease. The internal carotid artery (ICA) which

begins at the bifurcation of the common carotid artery (CCA) provides the major blood supply to brain. It also provides the blood supply to the eye through the ophthalmic artery.
Many studies have shown that bilateral common carotid artery occlusion in rat causes functional impairment of retina (Block *et al*., 1992). The electroretinogram have also demonstrated that b-wave amplitude representing bipolar and Muller cell activity in response to light exposure is decreased 7 days after the onset of 2VO (Barnett and Osborne, 1995). These functional changes are accompanied by structural damage. In animals that lost their pupillary reflex, the total retinal thickness decreased from approximately 120 µm to around 87 µm. The most affected layers being the synaptic zones in inner plexiform as well as the outer plexiform layer (Lavinsky *et al*., 2006).
In this model, occlusion of both CCAs cuts off blood supply to the retina, however, some retinal perfusion is maintained by retrograde blood flow to ophthalmic artery through the Circle of Willis. The degree of retinal damage also varies greatly within the same experiment, due to heterogeneity in tolerance towards ischemia in individual animal.

10.4 Photocoagulation of retinal vessels

There are only a few studies at present that report the ischemia of less than 5 minutes, which is often observed during the ocular surgeries. The faults that are present in other methods, such as invasiveness and inflammation in raising the intraocular pressure or ligation of optic vessels that causes changes in retina unrelated to ischemia, can be reduced by direct laser exposure of the main retinal vessels.
Kalamkarov *et al.* induced the characteristics of retinal ischemia in rats by direct laser coagulation of blood vessels using argon laser (Kalamkarov *et al*., 2000). Selective occlusion of vessels using laser permits creation of local and extensive retinal ischemia by choosing various retinal vessels and by modifying the exposure dose. In this technique, Rose Bengal, an iodinated photosensitive dye is injected intravenously through tail vein. The eyes are then exposed to 7 minutes of intense light (550nm, which is the absorption peak for this dye). Retinal vein occlusion was simulated in non-human primate model, i.e. cynomolgus monkey (*Maccaca fascicularis*). Dye yellow (577nm) laser light was used to occlude all branch retinal veins in the eye (Miller *et al*., 1994). Photodynamic thrombosis with green argon laser light and Rose Bengal dye was also used to create retinal ischemia model in pigs by occluding the retinal veins.
Laser coagulated Sprague Dawley rats were used to validate the effect of Hongua *in vivo*. In this model, Honghua extract was injected intravitreally, before they were subjected to Rose Bengal dye and laser (550nm). It is hypothesised that as the major component in the Honghua extract is glucose, the neuroprotective effect of the same could be due to availability of energy source after ischemia (Romano, *et al*., 1993). Another Chinese herbal medicine – Fufang Xuehuantong capsule was also studied by Yuan *et al*. They created a rat model for retinal vein occlusion by using laser photocoagulation and then validated the therapeutic benefits Chinese herbal medicine by quantitating the expression of various growth factors in the animal model (Yuan *et al*., 2011).

10.5 Central retinal artery occlusion

In humans, CRAO results in severe retinal ischemia, resulting in irreversible damage within hours. A minimal invasive model of transient retinal ischemia was introduced by Dangeliene *et al*., which involves photothrombotic central retinal artery occlusion (CRAO)

using intravenous injection of Rose Bengal and green laser irradiation of CRA in rats (Dangeliene *et al*., 2000). Rose Bengal is a photosensitive dye that releases oxygen free radicals when irradiated by the laser. This active oxygen results in intraluminal thrombus formation and thus, occlusion of CRA. Another retinal ischemia-reperfusion model through the occlusion of central retinal artery involves placing a suture behind the eye globe, including the CRA and ciliary artery. Both ends of the suture were then passed through a small plastic tube and ischemia is caused by pressing the tube against the artery. Prasad *et al* used the central retinal artery ligation model in rats to compare between different occlusion times as well as different time-period of reperfusion. They studied gene expression of various transcription-related genes after 30 and 90 minutes of occlusion and at 3 hours and 12 hours of reperfusion (Prasad *et al*., 2010).

10.6 Endothelin administration

Transient obstruction of central retinal artery can also be obtained by injecting vasoconstrictive drug. This method is less invasive, simple and does not require any special equipment. Endothelin-1 (ET-1) is a potent vasoconstrictive peptide produced by the vascular endothelial cells. Endothelin is found naturally in various tissues and is involved in a variety of biological activities. It has been linked with pathophysiology of various human disorders, e.g. cardiovascular, renal and ocular. Endothelin-1 can cause apoptosis of neurons in the CNS (Syed, *et al*., 2006). Endothelin-1 causes cell death through mechanism involving free-radicals (Oku, *et al*., 2008). Sugiyama *et al* have shown the association of endothelin with glaucoma. The authors investigated the effect of ET-1 on rabbit eye and observed that the intravenous as well as intravitreal administration of ET-1 reduces both the intraocular pressure and the blood flow in the optic nerve (Sugiyama, *et al*., 1995). Granstam *et al* demonstrated similar effects of endothelin in a cat model (Granstam, *et al*., 1992). Masuzawa *et al* have shown that a high dose of ET-1 when injected under the conjunctiva obstructs the central retinal artery without any damage to other tissues. Endothelin-1 causes retinal ganglion cell loss and activates glial cells (Masuzawa *et al*., 2006). It has also been seen that the intravitreal administration of ET-1 affects the retinal arteries directly. A dose of 10^{-7} M ET-1 led to decrease in the diameter by 17% (Bursell, *et al*., 1995).

Endothelin-1 also causes constriction of arteries *in-vitro.* Yu *et al* showed that ET-1 dose causes constriction even in cryopreserved human retinal arterioles. But the relation between the dose and the related activity is still not known (Yu, *et al*., 1998).

Thus, in this method there is no problem regarding inflammation or infection. But, like any other method being used, this method too is not completely free from drawbacks. The dose of endothelin-1 used is quite high, which may pass into the systemic circulation and exert some undesired effects in other tissues.

11. Current and potential therapeutic strategies for retinal ischemia

Many strategies have been used but have not been successful or have shown various limitations and are at experimental stage. Current treatments available for retinal ischemia include intravitreal or retinal vein administration of tissue-plasminogen activator (t-PA), hemodilution, pan-retinal laser photocoagulation or anti-VEGF antibodies or medication (Lucentis or Avastin). Occlusion of retinal vessels or retinal ischemia leads to retinal neovascularisation. Laser photocoagulation is used to decrease the neovascularisation in retina and thus, the oxygen demand. The decrease in overall oxygen requirement will stop

ischemia and hence, further damage to the eye. Ischemic conditions also cause up-regulation of expression of angiogenic factors, such as vascular endothelial growth factor or VEGF. VEGF is involved in angiogenesis and causes abnormal vessels growth or neovascularisation. To reduce the damage of ischemia, oxygen supply to retina has to be improved. Therefore, anti-VEGF drugs can be directly injected in the eye, e.g. the drugs that have been most tested in animal models include, bevacizumab, ranibizumab, pegaptanib sodium. Corticosteroids, such as, dexamethasone, are also under experimentation, as these inhibit VEGF and inflammatory factors (Lattanzio, *et al*., 2011). Another technique that holds promise is the use of anti-VEGF antibodies. Neutralising anti-VEGF monoclonal antibodies have been demonstrated to block neovascularisation when administered in a primate model of laser-induced retinal ischemia. Aiello *et al* showed the in-vivo inhibition of VEGF with the help of VEGF- neutralising proteins-containing extracellular domain of human (Flt) or mouse (Flk) VEGF receptors attached to IgG. These chimeric proteins showed 100% reduction in neovascularisation with human Flt and 95% with murine Flk domains. The suppression of VEGF was dose-dependent (Aeillo *et al*., 1995).

There are many other drugs and chemical compounds which have been tested in animal models of retinal ischemia with positive results and hope for future therapeutics. Cao *et al* in 1994 provided the evidence for the neuroprotective effect of NMDA antagonists in retinal ischemia. They showed that the NMDA receptor antagonist, dextromethorphan has a protective effect after retinal ischemia. However, it is not still clear whether dextromethorphan works via NMDA receptors. Other NMDA antagonists, such as MK-801 or memantine also protect from retinal ischemia (Lam *et al*., 1997 and Osborne, 1999). Blockers of voltage-gated Ca^{2+} channels, e.g. nifediprine and betaxolol also decrease neurotoxicity by reducing Ca^{2+} ions influx (Melena *et al*., 1999).

Another approach can be the use of the free- radical scavengers. Free-radicals play an important role in the damage caused by retina ischemia. Free-radicals are formed when reduced compounds, which accumulate during ischemia, are reoxidised during reperfusion. This free radical burst causes oxidative stress (Gilgun Sherki, 2002). Intravenous injection of SOD reduces the development of edema in rat model. SOD or superoxide dismutase is a well-known scavenger of superoxide radicals. Another compound that can be used is dimethylthiourea or DMTU, which is a synthetic compound that traps $OH^{\cdot}$, H_2O_2 and other free radicals. Intravitreal injections of both SOD and DMTU have been shown to lead to recovery in IOP-induced ischemia rat model. DMTU (75µg/eye) resulted in 40% functional recovery when assessed through electroretinogram. SOD, on the other hand, leads to 99% functional recovery on post-treatment and 81% on pre-treatment. Thus, all these chemical compounds and drugs that decrease or reverse the cause of ischemia can be helpful in reducing the damage to some extent.

RNA interference (RNAi) is a natural phenomenon in mammals, which is involved in silencing of gene expression. It involves a double-stranded RNA which cleaves any RNA complimentary to it. RNAi has been proposed to be used in therapeutics by downregulating the expression of specific genes. Reich *et al* used the technique of RNA interference in retinal cells *in vitro*, as well as *in vivo* in the mouse retina. They used this technique to downregulate the VEGF expression, which is known to be upregulated in retinal ischemia (Reich *et al*., 2003). RNAi can be further investigated and tested for other genes, e.g. cytokines that are upregulated in the pathophysiology of retinal ischemia.

Stem cell therapy is a promising technique for tissue repair and regeneration. Advances in the field of stem cells have lead to their use in treatment of various disorders (Lenka &

Anand, 2010, Rajarathna, 2009). Stem cells basically are unspecialised cells which are capable of self – renewal and under specific defined environment these cells can form functionally specialised cells. The stem cells can either be obtained from early embryos or certain tissues in adults, such as umbilical cord and peripheral blood, bone marrow. They work through either replacing damaged cells or through the factors released by them. Stem cells have been used in various vascular neurodegenerative diseases and most of the ocular disorders involve problem in either of the two. Eye is an accessible organ and with large number of animal models available, the use of stem cells poses a promise for preserving functionality (Cogliati & Swaroop, 2009).

Adult bone marrow contains hematopoietic stem cells (HSCs) and hematopoietic progenitor cells (HPCs), which can differentiate into various cell types of myeloid and endothelial lineages. Bone-marrow contains stem cells which can either differentiate into Lin+ (hematopoietic lineage) or Lin- (non-hematopoietic lineage). Lin- population contains progenitor cells that differentiate into vascular endothelial cells, i.e. the endothelial progenitor cells (EPCs). Many preclinical and clinical studies have shown bone-marrow derived cells contribute to neoangiogenesis during wound healing, retinal ischemia, myocardial infarction, neonatal growth and tumor growth. Lin- population have been shown to express neuronal markers after transplantation in brain or retina in various mouse models. Bone-marrow cells differentiate into neuronal cells, astrocytes *in-vitro* and also *in-vivo* when injected intravenously into brain of mouse model (Mezey, *et al*., 2000). Also, it has been shown that BMCs can differentiate into retinal neural cells *in-vivo* (Woodbury, *et al*., 2000). Lin- bone-marrow stem cells when injected intravitreally into photocoagulated retina of a mouse model, migrated to astrocytes and formed retinal vessels. Ischemic conditions release cytokines that recruit EPCs to the site. Ischemia results in up-regulation of angiogenic factor, vascular endothelial growth factor or VEGF-A, which has its receptors Flk-1 and Flt-1 on EPCs, HSCs and HPCs, thus leading to their migration to the site (Kalka, *et al*., 2000). But their importance in clinics is still unknown as the success depends on their functional incorporation.

Mesenchymal stem cells (MSCs) also found in bone marrow and other tissues such as cord blood, peripheral blood, fallopian tube, and fetal liver and lung have been used in over a range of different clinical trials (US NIH clinical trial database – www.clinicaltrials.gov), including those in fractures, diabetes, heart and liver disease and neurological disorders. MSCs have the potential to differentiate into neurons, especially retinal neurons. These cells also secrete molecules that modify the environment for the surrounding cells. MSCs for instance, express a number of neuroprotective factors, such as BDNF, CNTF, IGF, bFGF and NGF, which protect the injured retina. MSCs have another remarkable property, i.e. the homing potential; they can migrate to pathological areas (Prabhakar, *et al*., 2010). They can migrate from blood circulation to brain, spinal cord, and eye (Kan, *et al*., 2005). Thus, MSCs have shown neuroprotection in various neurodegenerative models, but clinical translation is still questionable.

Another source of stem cell therapy is the embryonic stem cells (ESCs), obtained from the inner cell mass of blastocyst. ESCs can differentiate into various cell types, such as hematopoietic cells, astrocytes, hepatocytes, glial cells, neurons. Wei *et al*., 2005, showed that the transplantation of human embryonic cells in MCAO stroke model led to structural as well as functional recovery. Transplanted ESCs differentiated into neurons, astrocytes, oligodendrocytes and endothelial cells. These ESC – derived endothelial cells can form vascular-like structures *in-vivo* as well as *in-vitro*, thus induce angiogenesis (Levenberg, *et*

al., 2002). Embryonic stem cells have issue of ethical restrictions as well as immune rejection. Thus, alternate source of stem cells was identified from non-pluripotent cells. Every nucleated cell in an individual has identical genome, except the gametes. Different cell types are identified on the basis of the genes that are expressed. In 2006, four transcription factors – *Oct 3/4, Sox-2, Klf-4* and *c-myc*, that are capable of reprogramming DNA were identified (Takahashi & Yamanaka, 2006). Forced expression of these specific genes in a non-pluripotent cell lead to a pluripotent stem cell, known as induced pluripotent stem cell (iPC). Human iPCs have been derived successfully from patients with neurological disorders – Parkinson's disease, muscular dystrophy, Huntington's (Park *et al*., 2008). iPC from the skin cells of an amylotrophic lateral sclerosis (ALS) patient have been differentiated into motor neurons (Dimos *et al*., 2008). Takahashi group for the first time generated photoreceptor cells from the embryonic stem cells (Takahashi & Yamanaka, 2006). The same method has been used to create human photoreceptor and retinal pigmented epithelium phenotype (Hirami *et al*., 2009). Human neuronal cells can also be generated from iPC (Karumbayaram *et al*., 2009). Human umbilical cord blood is a well known source of hematopoietic stem cells and has been used in various disorders. Umbilical cord blood contains higher percentage of hematopoietic stem cells than the bone marrow and also poses a lesser risk of immune rejection. The cells from cord blood have the potential to form retinal neuronal cells.

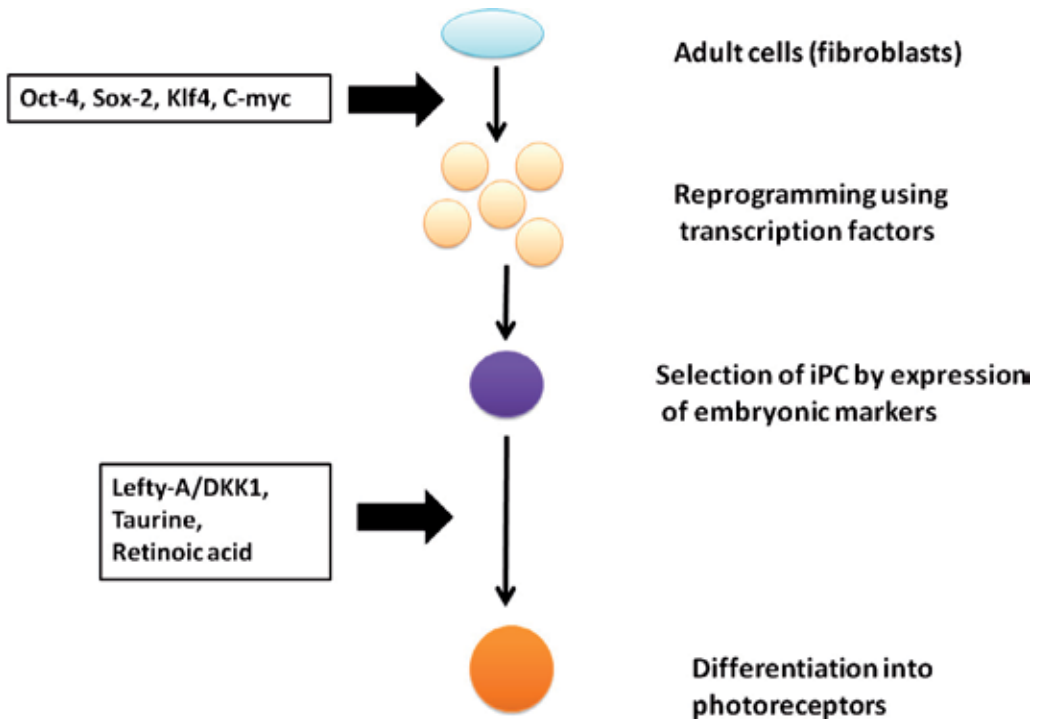


Fig. 3. Formation of iPC from adult somatic cell

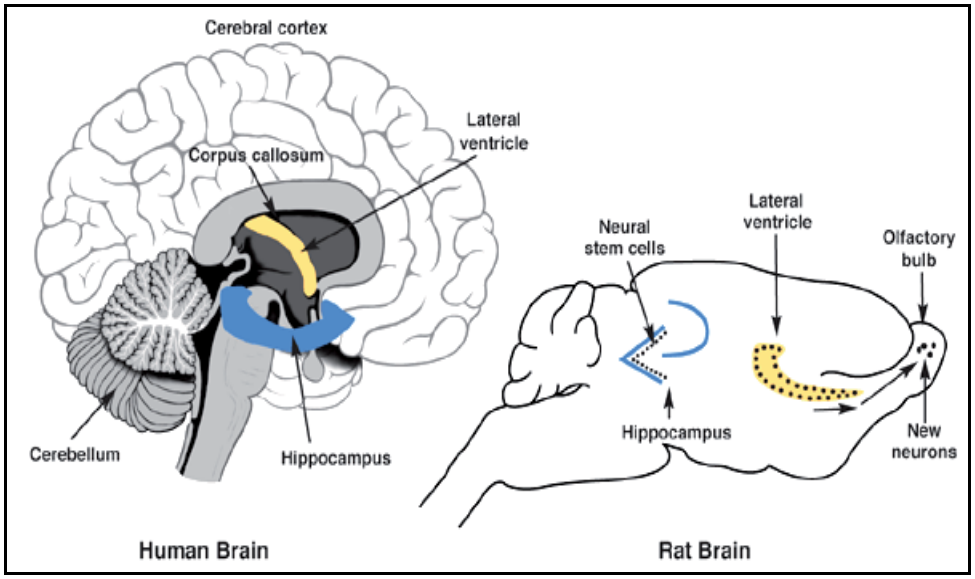


Fig. 4. Different sources of neural stem cells (NSCs) in human and rat brain *(Reproduced with permission http://pubs.niaaa.nih.gov/publications/arh27-2/197-204.htm)*

The mammalian central nervous system was considered is a non-renewable tissue. But studies have demonstrated that neural stem cells (NSCs) do exist not only in developing CNS, but also in adult nervous system of all mammals and are capable of differentiating into neurons, astrocytes and oligodendrocytes. Neural stem or progenitor cells can be isolated from various parts of CNS, such as hippocampus, subventricular zone, spinal cord and ependyma. Palmer *et al* for the first time isolated NSCs from the hippocampus of an adult rat. These hippocampus-derived NSCs have an ability to migrate and differentiate into neuronal lineage in injured retina, but are unable to form retina-specific cells (Nishida *et al*., 2000). Embryonic retina derived neural progenitors can differentiate into photoreceptors *in-vitro* (Ahmad *et al*., 2004). NSCs have an advantage over the embryonic stem cells with respect to their clinical translation, i.e. NSC can be expanded through numerous passages in-vitro and can be easily manipulated (English & Anand, 2010) . But, further studies are required to derive retinal neurons from the NSCs.

But there are some limitations and barriers for stem cell transplantation in retina, as retina shows poor cell integration. Like any other part in the central nervous system, the retina too is rigid to cell migration. Thus, most of the cells that are transplanted do not reach the retina. It has been shown that only 1% of intraocularly transplanted cells reach the retina (Johnson, *et al*., 2010).The stem cell therapy holds a promising future in retinal disorders, but the problems need to be dealt with before clinical translation.

Another therapeutic approach that shows a promising future is the concept of personalised therapy, where the candidate genes linked with various eye disorders can be identified and the genetic make-up of an individual can be used for disease prediction and treatment.

12. Conclusion

Retinal ischemia is a common cause for visual impairment and vision loss. It is a condition related with many different human disorders, such as diabetic retinopathy, glaucoma, and it

occurs when the blood supply is not sufficient to meet the demand. The retina and brain share common development pathway. Retina, like CNS, originates from ectoderm, however, it can be non-invasively studied. Also, as the retinal blood vessels share many features with the cerebral blood vessels, the investigations can be extrapolated to brain pathology. Thus, studying the retina, through the retinal ischemia models can help in understanding the mechanism and pathophysiology of stroke as well as in validating potential therapeutics. But all the methods discussed here have their own strengths and limitations. Out of all the above mentioned animal models, pressure elevation model is commonly used as it is easily reproducible and it mimics many human disorders – central retinal artery occlusion (CRAO), glaucoma, occlusion of ophthalmic artery. For example, some methods require penetration of a needle through the cornea, and must be fixed in the anterior chamber for one hour. The invasiveness can lead to inflammation and other damages. Similarly, unilateral and bilateral occlusion of carotid artery requires specialised skills in vascular surgery. It may cause incomplete ischemia and all of these procedures alter blood flow to the brain. Likewise, photocoagulation is simple but has many disadvantages including variable degrees of exposure and hence, variable damage. Besides, the ischemic damage caused is permanent because of which reperfusion cannot be studied. The choice of animal models for pre-clinical testing, therefore, depends on the research questions which have been raised.

13. References

Ahmad, I., Das, A. V., James, J., Bhattacharya S. & Zhao, X. (2004). Neural stem cells in the mammalian eye: types and regulation. *Seminar in Cell and Developmental Biology*, Vol. 15, No. 1, pp. 53-62

Barnett, N. L. & Osborne, N. N. (1995). Suppression of retinal neovascularization in vivo by inhibition of vascular endothelial growth factor (VEGF) using soluble VEGF-receptor chimeric proteins. *Experimental Eye Research*, Vol. 61, pp. 83–90

Berson, D. M. (2007). Phototransduction in ganglion - cell photoreceptors. *Pflugers Archives*, Vol. 454, pp. 849-855

Block, F., Grommes, C. & Kosinski, C. (1997). Retinal ischemia induced by the intraluminal suture methods in rats. *Neuroscience Letters*, Vol. 232, pp. 45-48

Block, F. & Schwarz, M. (1997). Effects of antioxidants on ischemic retinal dysfunction. *Experimental Eye Research*, Vol. 64, pp. 559–564

Block, F., Schwarz, M. & Sontag, K.H. (1992). Retinal ischemia induced by occlusion of both common carotid arteries in rats as demonstrated by electroretinography. *Neuroscience Letters*, Vol. 144, pp. 124–126

Buchi, E. R., Suivaizdis, I. & Fu, J. (1991). Pressure-induced retinal ischemia in rats: an experimental model for quantitative study. *Ophthalmologica*, Vol. 203, pp. 138–147

Bursell, S. E., Clermont, A. C., Oren, B. & King, G. L. (1995). The *in vivo* effect of endothelins on retinal circulation in nondiabetic and diabetic rats. *Investigative Ophthalmology and Visual Science*, Vol. 36, No. 3, pp. 596–607

Cao, W., Zaharia, M., Drumheller, A., Casanova, C., Lafond, G., Brunette, J. R. & Jolicoeur, F. B. (1994). Effects of dextromethorphan on ischemia induced ERG changes in rabbit. *Current Eye Research*, Vol. 13, No. 2, pp. 97-102

Cogliati, T. & Swaroop, A. (2009). Stems cells and neuronal repair. *Annals of Neurosciences*, Vol. 16, No. 4, pp. 143-145

Daugeliene, L. Niwa, M. & Hara, A. (2000). Transient ischemic injury in the rat retina caused by thrombotic occlusion-thrombolytic reperfusion. *Investigative Ophthalmology and Visual Science*, Vol. 41, pp. 2743–2747

Davidson, C. M., Pappas, B. A., Stevens, W. D., Fortin, T. & Bennett, S. A. L. (2000). Chronic cerebral hypoperfusion: loss of papillary reflex, visual impairment and retinal neurodegeneration. *Brain Research*, Vol. 859, No. 1, pp. 96-103

Dimos, J. T., Rodolfa, K. T. & Niakan, K. K. (2008). Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. *Science,* Vol. 321, pp. 1218–1221

English, D. & Anand, A. (2010). Neural stem cell therapy – how the hype began. *Annals of Neurosciences*. Vol. 17, No. 1.

Flower, R .W. & Patz, A. (1971). The effect of hyperbaric oxygenation on retinal ischemia. *Ophthalmology and Visual Science*. Vol. 10, No. 8, pp. 605-616.

Gao, G., Li, Y. , Fant, J., Craig, E., Crosson, S., Becerra, P. & Ma, J. (2002). Difference in ischemic regulation of vascular endothelial growth factor and pigment epithelium-derived factor in Brown Norway and Sprague Dawley rats contributing to different susceptibilities to retinal neovascularization. *Diabetes*. Vol. 51, No. 4, pp. 1218-1225.

Gilgun-Sherki, Y., Rosenbaum, Z., Melamed, E. & Offen, D. (2002). Antioxidant therapy in acute central nervous system injury: current state. *Pharmacological Reviews*. Vol. 54, No. 2, pp. 271-284.

Granstam, E., Wang, L. & Bill, A. (1992). Ocular effects of endothelin-1 in the cat. *Current Eye Research*. Vol. 11, No. 4, pp. 325-332.

Ha, K. T., Yoon, S. J., Choi, D. Y., Kim, D. W. & Kim, J. K. (2005). Protective effect of *Lycium chinense* fruit on carbon tetrachloride-induced hepatotoxicity. *Journal of Ethnopharmacology*. Vol. 96, pp. 529–535.

Hein, T. W. , Rosa, R. H., Jr., Yuan, Z. , Roberts, E. & Kuo, L. (2010). Divergent roles of nitric oxide and rho kinase in vasomotor regulation of human retinal arterioles. *Investigative Ophthalmology and Visual Sciences.* Vol. 51, No. 3, pp. 1583-1590.

Henkind , P., Hansen, R. I. & Szalay, J. (1979). Ocular circulation. In "Physiology of the human eye and visual system" (Ed. Records, R.E.), pp. 98-155. Harper & Row, New York.

Hirami, Y., Osakada F, Takahashi K, *et al*. (2009). Generation of retinal cells from mouse and human induced pluripotent stem cells. *Neuroscience Letters.* Vol. 458, pp. 126–131.

Hirrlinger, P. G., Elke, U., Ianors, I., Andreas, R. & Thomas, P. (2010). Alterations in protein expression and membrane properties during Müller cell gliosis in a murine model of transient retinal ischemia. *Neuroscience Letters*. Vol. 472, pp. 73–78.

Jennings, R. B., Sommers, H. M., Smyth, G. A., Flack, H. A. & Linn, H. (1960). Myocardial necrosis induced by temporary occlusion of a coronary artery in the dog. *Archives of Pathology*. Vol. 70, pp. 68–78.

Joachim, S. C., Wax, M. B., Boehm, N., Dirk, D., Pfeiffer N & Grus FH. (2011). Up-regulation of antibody response to heat shock proteins and tissue antigens in an ocular ischemia model. *Investigative Ophthalmology and Visual Sciences*

Jung, S. H., Kang, K. D., Ji, D., Fawcett, R. J., Safa, R., Kamalden, T. A. & Osborne, N. N.(2008). The flavonoid baicalin counteracts ischemic and oxidative insults to retinal cells and lipid peroxidation to brain membranes. *Neurochemistry International*. Vol. 53, No. 6-8, pp. 325-337.

Jung, S. H., Kim, B. J., Lee, E. H. & Osborne, N. N. (2010). Isoquercitrin is the most effective antioxidant in the plant *Thuja orientalis* and able to counteract oxidative-induced damage to a transformed cell line (RGC-5 cells). *Neurochemistry International*. Vol. 57, No. 7, pp. 713-721.

Kaja, S., Yang, S. H. & J. Wei *et al*. (2003). Estrogen protects the inner retina from apoptosis and ischemia-induced loss of Vesl-1L/Homer-1c immunoreactive synaptic connections. *Investigative Ophthalmology and Visual Science*, Vol. 44, pp. 3155-3162, .

Kalamkarov, G.R., Tsapenko, I. V., Zueva, M.V., Ivanov, A. N., Konstantinova, T. S., Burgova, A. E., Rezvykh S. V., Fedorov, A. A. & Shevchenko, T. F. (2008). Experimental model of acute ischemia of the retina in rats. *Bulletin of Experimental Biology and Medicine*. Vol. 145, No. 6, pp. 688-691.

Kalka, C., Masuda, H. & Takahashi, T. (2000). Vascular endothelial growth factor (165) gene transfer augments circulating endothelial progenitor cells in human subjects. *Circulation Research*. Vol. 86, No. 1198-1202.

Kan, I., Melamed, E. & Offen, D. (2005). Integral therapeutic potential of bone marrow mesenchymal stem cells. *Current Drug Targets*. Vol. 6, No. 1, pp. 31-41.

Karumbayaram, S., Novitch, B. G., Patterson, M. (2009). Directed differentiation of human-induced pluripotent stem cells generates active motor neurons. *Stem Cells,* Vol. 27, pp. 806–811.

Kaur, C., Foulds, W. S. & Ling, E. A. (2008). Blood-retinal barrier in hypoxic ischemic condition: basic concepts, clinical features and management. *Progress in Retina and Eye Research*, Vol. 27, pp. 622-647

Kawai, S., Vora, S., Das, S., Gachie, E., Becker, B. & Neufeld, A. H. (2001). Modeling of risk factors for the degeneration of retinal ganglion cells after ischemia/reperfusion in rats: effects of age, caloric restriction, diabetes, pigmentation, and glaucoma. *The FASEB Journal,* Vol. 15, pp. 1285-1287

Lam, T. T., Siew, E., Chu, R. & Tso, M. O. (1997). Ameliorative effect of MK-801 on retinal ischemia. *Journal of Ocular Pharmacology Therapeutics*, Vol. 13, pp. 129–137

Lavinsky, D., Arterni, N. S., Achaval, M. & Netto, C. A. (2006). Chronic bilateral common carotid artery occlusion: a model for ocular ischemic syndrome in the rat. *Graefe's Archive for Clinical and Exaperimental Opthalmology*, Vol. 244, No. 2, pp. 199-204

Lenka, N. & Anand, A. (2010). Advancements in stem cell research: an Indian perspective – I. *Annals of Neurosciences*, Vol. 17, No. 3

Levenberg, S., Golub, J. S., Amit, M., Itskovitz-Eldor, J. & Langer, R. (2002). Endothelial cells derived from human embryonic stem cells. *Proceedings of National Academy of Sciences, USA*, Vol. 99, No. 7, pp. 4391-4396

Li, S-Y., Yang, D., Yeung, C-M., Yu, W-Y., Chang, R. C-C., So, K-F., Wong, D. & Lo, A. C. Y. (2011). *Lycium barbarum* polysaccharides reduce neuronal damage, blood-retinal barrier disruption and oxidative stress in retinal ischemia/reperfusion injury. *PLoS ONE*, Vol. 6, No. 1, pp. e16380

Li, X. M., Ma, Y. L. & Liu, X. J. (2007). Effect of the *Lycium barbarum* polysaccharides on age-related oxidative stress in aged mice. *Journal of Ethnopharmacology*, Vol. 111, pp. 504–511.

Louzada-Junior, P., Dias, J. J., Santos, W. F., Lachat, J. J., Bradford, H. F. & Coutinho-Netto, J. (1992). Glutamate release in experimental ischaemia of the retina: an approach using microdialysis. *Journal of Neurochemistry*, Vol. 59, pp. 358–363

Lucas, D. R., Newhouse, J. P. (1957). The toxic effect of sodium L-glutamate on the inner layers of the retina. *Archives of Ophthalmology*, Vol. 58, pp. 193–201

Masland, R. H. (1998). Amacrine cells. *Trends in Neuroscience*, Vol. 11, pp. 405-410

Masuzawa, K., Jesmin, S., Maeda, S., Kaji, Y., Oshika, T., Zaedi, S. & Shimojo, N. (2006). A model of retinal ischemia-reperfusion injury in rats by subconjuctival injection of Endothelin-1. *Experimental Biology and Medicine*, Vol. 231, pp. 1085–1089

Matteucci, A., Cammarota, R. , Paradisi, S. , Varano, M. , Balduzzi, M. , Leo, L., Bellenchi, G.C. , de Nuccio, C., Carnovale-Scalzo, G. , Scorcia, G., Frank, C., Mallozzi, C. , di Stasi, A. M. , Visentin, S. & Malchiodi-Albedi, F. (2011) . Curcumin Protects against NMDA-Induced Toxicity: A Possible Role for NR2A Subunit. *Investigative Ophthalmology and Visual Sciences,* Vol. 52, No. 2, pp. 1070-1077

Melena, J., Wood, J. P. M. & Osborne, N. N. (1999). Betaxolol, a B1- adrenoceptor antagonist, has an affinity for L-type Ca^{2+} channels. *European Journal of Pharmacology*, Vol. 378, pp. 317–322

Mezey,E., Chandross, K. J., Harta, G., Maki, R. A. & McKercher, S. R. (2000). Turning blood into brain: cells bearing neuronal antigens generated *in vivo* from bone marrow, *Science*, Vol. 290, pp. 1779-1782

Miller, J. W., Adamis, A. P., Shima, D. T., D'Amore P. A., Moulton, R. S., O'Reilly, M. S., Folkman, J., Dvorak, H. F., Brown, L. F., Berse, B., Yeo, T-K., & Yeot, K-T. (1994) . Vascular endothelial growth factor/vascular permeability factor is temporally and spatially correlated with ocular angiogenesis in a primate model. *American Journal of Pathology*, Vol. 145, No. 3

Mishida, A., Zakahashi, M., Tauihara, H., Nakamo, I., Tahahashi, J. B., Mizoguchi, A., Ide, C. & Honda, Y. (2000). Incorporation and differentiation of hippocampus-derived neural stem cells transplanted in injured adult rat retina. *Investigative Opthalmology and Visual Sciences*, Vol. 41, No. 13, pp. 4268-4269

Osborne, N. N., DeSantis, L., Bae, J. H., Ugarte, M., Wood, J. P. M., Nash, M. S. & Chidlow, G. (1999a). Topically applied betaxolol attenuates NMDA-induced toxicity to ganglion cells and the effects of ischaemia to the retina. *Experimental Eye Research*, Vol. 69, pp. 331–342

Osborne, N. N., Safa, R., Nash & M. S. (1999b). Photoreceptors are preferentially affected in the rat retina following permanent occlusion of the carotid arteries. *Vision Research*, Vol. 39, pp. 3995–4002

Palmer, T. D., Takahashi, J. & Gage, F. H. (1997). The adult rat hippocampus contains primordial neural stem cells. *Molecular and Cellular Neuroscience*, Vol. 8, No. 6, pp. 389-404

Palmero, M., Bellot , J. L., Castillo, M., Garcia-Cabanes, C., Miquel, J. & Orts, A. (2000). An *in-vitro* model of ischemic-like stress in retinal pigmented epithelium cells:

protective effects of antioxidants. *Mechanisms of Ageing and Development*, Vol. 114, No. 3, pp. 185-190

Park, I. H., Arora, N., Huo, H., Maherali, N., Ahfeldt, T., Shimamura, A., Lensch, M. W., Chad, C., Hochedlinger, K. & Daley, G. Q. (2008). Disease-specific induced pluripotent stem cells. *Cell,* Vol. 134, pp. 877–86

Paul, A. L., Piercet, E. A., Foleyt, E. D., Takagittil, H., Chen, H., Riddle, L., Ferrara, N., Kingttii, G. L. & Smitht, L. E. H.. (1995). Suppression of retinal neovascularization in vivo by inhibition of vascular endothelial growth factor (VEGF) using soluble VEGF-receptor chimeric proteins. *Proceedings of National Academy of Science, USA,* Vol. 92, pp. 10457-10461

Peachey, N. S., Green, D. J. & Ripps, H. (1993). Ocular ischemia and the effects of allopurinol on functional recovery in the retina of the arterially perfused cat eye. *Investigative Ophthalmology and Visual Science*, Vol. 34, pp. 58–65

Plate, K. H., Breier, G., Weich, H. A., Risau, W. (1992). Vascular endothelial growth factor is a potential tumour angiogenesis factor in human gliomas *in vivo*. *Nature*, Vol. 359, pp. 845-848

Poché, R. A. & Reese, B. E. (2009).Retinal horizontal cells: challenging paradigms of neural development and cancer biology. *Development*, Vol. 136, pp. 2141-2151

Prabhakar, S., Muthaian, R., Chhabra, R. & Anand, A. (2010). Analysis of homing potential of marrow-derived mononuclear cells in an experimentally-induced brain stroke mouse model. *Brain Injury,* Vol. 24, No. 12, pp. 1485-1490

Prasad, S. S., Kojic, L., Wen, Y. H., Chen, Z., Xiong, W., Jia, W. & Cyander, M. S. (2010). Retinal gene expression after central retinal artery ligation: Effects of ischemia and reperfusion. *Investigative Ophthalmology and Visual Science*, Vol. 51, No. 12, pp. 6207-6219

Rajarathna,T. (2009). Advancements in stem cell research: an Indian perspective – II. *Annals of Neurosciences*, Vol. 16, No. 3, pp. 97

Reich, S. J. , Fosnot, J. , Kuroki, A. , Tang, W. , Yang , X. , Maguire, A. M. , Bennett, J. & Tolentino, M. J. (2003) . Small interfering RNA (siRNA) targeting *VEGF* effectively inhibits ocular neovascularization in a mouse model. *Molecular Vision*, Vol. 9, pp. 210-216

Rios, L., Jacques, C., Vennat, J-C., Menerath, J-M., & Doly, M. (1999). Comparison of intraocular treatment of DMTU and SOD following retinal ischemia in rats. *Journal of Ocular Pharmacology and Therapeutics,* Vol. 15, No. 6, pp. 547-556

Romano, C., Price, M. T., Almli, T. & Olney, J. W. (1998). Excitotoxic neurodegeneration induced by deprivation of oxygen and glucose in isolated retina. *Investigative Ophthalmology and Visual Science,* Vol. 39, pp. 416-423

Romano, C., Price, M., Bai, H. Y. & Olney, J. W. (1993). Neuroprotectants in Honghua: glucose attenuates retinal ischemic damage. *Investigative Ophthalmology and Visual Science*, Vol. 34, No. 1, pp. 72-80

Saint-Geniez, M. & D'Amore, P.A. (2004). Development and pathology of the hyaloid, choroidal and retinal vasculature. *International Journal of Developmental Biology*, Vol. 48, pp. 1045-1058

Shima, D. T., Adamis, A. P., Ferrara, N., Allende, R., Yeo, K., & D'Amore, P. A. (1995). Hypoxic induction of endothelial cell growth factors in retinal cells: identification

and characterization of vascular endothelial growth factor as the mitogen. *Molecular Medicine,* Vol. 2, pp. 64-75

Shweiki, D., Itin, A., Softer, D. & Keshet, E. (1992). Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. *Nature*, Vol. 359, pp. 843-845

Sims, S. R. (1992). Energy metabolism and selective neuronal vulnerability following global cerebral ischemia. *Neurochemical Research*, Vol. 17, pp. 923–931

Steele, E. C., Guo, Q. & Namura, S. (2008). Filamentous middle cerebral artery occlusion causes ischemic damage to retina in mice. *Stroke*, Vol. 39, No. 7, pp. 2099-2104

Sugiyama, T., Moriya, S., Oku, H. & Azuma, I. (1995). Association of endothelin-1 with normal tension Glaucoma – clinical and fundamental studies. *Survey of Opthalmology*, Vol. 39, No. 1, pp. S49-S56

Syed, H., Safa, R., Chidlow, G. & N. N. Osborne. (2006). Sulfisoxazole, an endothelin receptor antagonist, protects retinal neurones from insults of ischemia/reperfusion or lipopolysaccharide. *Neurochemistry International*, Vol. 48, No. 8, pp. 708–717

Szabo, M. E., Droy-Lefaix, M. T., Doly, M. & Braquet, P. (1991). Free radical-mediated effects in reperfusion injury: a histologic study with SOD and EGb 761 in rat retina. *Ophthalmic Research*, Vol. 23, pp. 225-234

Szabo, M. E., Droy-Lefoix, M. T., Doly, M., Carre, C. & Braquer, P. (1991). Ischemia and reperfusion-induced histologic changes in the rat retina: demonstration of a free radical-mediated mechanism. *Investigative Ophthalmology & Visual Science*, Vol. 32, No. 5, pp. 1471-1478

Takahashi, K. & Yamanaka, S. (2006).Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell,* Vol. 126, pp. 663–676

Tso, M. O. & Jampol, L. M. (1982). Pathophysiology of hypertensive retinopathy. *Ophthalmology*, Vol. 89, pp. 1132-1145

Ueda, K., Makahara, T., Hoshino, M., Mori, A. & Sakamoto, K. (2010). Retinal blood vessels are damaged in rat model of NMDA-induced retinal degeneration. *Neuroscience Letters*, Vol. 485, No. 1, pp. 55-59

Wei, L., Cui, L., Snider, B. J., Rivkin, M., Yu, S. S., Lee, C. S., Adams, L. D. Johnson, E. M., Yu, S. P. & Choi, D. W. (2005). Transplantation of embryonic stem cells overexpressing Bcl-2 promotes functional recovery after transient cerebral ischemia. *Neurobiology of Disease,* Vol. 19, No. 1-2, pp. 183-193

Woodbury, D., Schwarz, E. J., Prockop, D. J. & Black I. B. (2000). Adult rat and human bone marrow stromal cells differentiate into neurons. *Journal of Neuroscience Research*, Vol. 61, pp. 364-370

Yamamoto, H., Schimdt-Kasmer, R., Hamasaki, D. I., Yamamoto, H. & Parel, J. M. (2006). Complex neurodegeneration in retina following moderate ischemia induced by bilateral common carotid artery occlusion in Wistar rats. *Experimental Eye Research*, Vol. 82, No. 5, pp. 767-779

Yu, D. Y., Su, E. N., Cringle, S. J., Alder, V. A., Yu, P. K. & Desantis, L. (1998). Effect of betaxolol, timolol and nimodipine on human and pig retinal arterioles. *Experimental Eye Research*, Vol. 67, pp. 73–81

Yu, M. S., Lai, C. S., Ho, Y. S., Zee, S. Y. & So, K. F.(2007). Characterization of the effects of anti-aging medicine *Fructus lycii* on beta-amyloid peptide neurotoxicity. *International Journal of Molecular Medicine*, Vol. 20, pp. 261–268

Yuan, Y-Z., Yuan, F., Xu, Q-Y., Yu, J., Li, L. & Zhang, J-L. (2011). Effect of Fufang Xuehuantong capsule on a rat model of retinal vein occlusion. *Chinese Journal of Integrative Medicine*. Vol. 17, No. 4, pp. 296-301

Zhang, S. X., Ma, J., Sima, J., Chen, Y., Hu, M. S., Ottlecz, A., & Lambrou, G. N. (2005). Genetic difference in susceptibility to the blood-retina barrier breakdown in diabetes and oxygen-induced retinopathy. *American Journal of Pathology*, Vol. 166, No. 1, pp. 313–321

Part 2

Cerebral Blood Flow and Metabolism

9

Cerebral Blood Flow in Experimental and Clinical Neurotrauma: Quantitative Assessment

Hovhannes M. Manvelyan
Yerevan State Medical University
Armenia

1. Introduction

In a modern world the traumatic brain injury (TBI) becomes one of major health problems, with annually 500.000 cases in the US alone (Narayan et al., 2002), it poses the largest threat and considerably the most common cause of both the morbidity and mortality of the human young and pediatric population, and it is one of the major cases of disability and long capability in all ages (Langfitt et al., 1977; Levin et al., 1982; Fife & Jagger, 1984; Luerssen et al., 1988; Kraus et al., 1990). Moreover, TBI to children younger than 4 years of age has been shown to cause significantly more severe neurological, cognitive and motor deficits than in older children (Khoshyomn & Tranmer 2004). Thus TBI is a serious social, medical and scientific problem as well. New data, available to improve both efficacy of treatment and life quality after TBI would benefit each patient with TBI.
Despite many successful clinical and laboratory investigations on TBI, many questions of pathophysiology of development and an effective treatment remain open and demand further investigation and understanding (Potts et al., 2006). There is experimental evidence that the inflammatory response may differ in the developing brain as compared to the adult (Fan et al., 2003; Claus et al., 2010; Yoneyama-Sarnecky et al., 2010), another possible factor could be age-dependent differences in posttraumatic cerebral blood circulation (Bayir et al., 2003).

2. Cerebral blood flow in TBI

The comprehensive understanding of pathophysiological processes developing in an injured brain always demands proper understanding of the cerebral blood flow (CBF) alterations as one of the most important factors, which plays an influental role both in disease development and prognosis prediction. In many cases of clinical and animal research, it has proved the importance of monitoring blood flow violations and interesting data were found involving CBF disturbances following the injury. Vascular events after TBI involve hemorrhage, breakdown or increased permeability of the blood brain barrier and edema, and certain changes in cerebral blood flow and local perfusion to the injured area. The exact role of each of these events after trauma is complex and has yet to be fully elucidated.
Post mortem studies of patients who died of severe TBI (90% of these patients had histologic evidence of ischemia) a few decades years ago (Graham & Adams, 1971; Graham et al., 1978; Graham et al., 1989), partly stimulated the interest of CBF; whether

the ischemia was a factor of mortality or a reflection of the severity of injuries is still unknown. Ever since that time, despite many investigations, there is no universally accepted model of CBF changes in TBI. Therefore, the role of cerebral perfusion, both local and general, as well vasoreactivity in development of a cascade of biochemical reactions after the injury, could be paramount.

Significant changes, perturbations and impairment in CBF and brain metabolism are very common following severe TBI, and the severity of impaired CBF correlate with the severity of TBI and poor outcome (Adelson et al., 1997; Muizelaar et al., 1989). The main peculiarities of cerebral circulation are the ability to autoregulation and high vulnerability to ischemia. Due to unique biological organization, as an extremely high metabolic activity and absence of metabolic stores, the brain requires a constant its supply of blood. Ischemia is poorly tolerated by a non injured brain, and TBI can lead to devastating impairment of autoregulation and profound ischemia (Bayir et al, 2003).

Historically, investigations of CBF were done mostly in adults and mainly without including of the important factor of the timeline. Clinical studies have reported inconsistent and sometimes confusing, findings as decreased blood flow-hypoperfusion, increased flow–hyperemia, and even cerebral vasospasm (Meixensberger, 1993). It was considered that in children with TBI hyperemia is the cause of increased intracranial pressure and cerebral swelling (Bruce et al., 1981), those studies were completed using the intravenous Xenon 133 technique and external probes, in only six people. Animal studies in immature rats as the model of child TBI supported that point of view (Biagas et al., 1996). The authors demonstrated prominent increase of pericontusional CBF 24 hrs after injury, however, the increase was not observed immediately after.

Muizelaar et al. performed 72 measurements of CBF in 32 children with TBI (Muizelaar et al., 1989). CBF is lowest after injury and correlates with the Glasgow Coma score, but founded increase of CBF at some point in their course leads authors to statement the prevalence of hyperemia. Obrist et al. performed serial CBF studies of 75 head-injured adults using Xe133 technique (Obrist et al., 1984). They found that 55% of patients had hyperemia at some point after their injury, and 45% had consistently low CBF, accordingly, hyperemia was associated with elevated intracranial pressure. Kelly et al (Kelly et al., 1996; Kelly et al., 1997), evaluated the role of hyperemia and CBF in outcome and intracranial hypertension, and observed the highest CBF on post injury days 1 to 5, when the lowest blood flows were documented on the day of injury. Significant decrease of posttraumatic CBF has been demonstrated both in human and laboratory investigations (Adelson et al., 1997; McQuire et al., 1998; Chan et al., 1992; Marion et al., 1991; Yamakami & McIntosh, 1991; Bryan et al., 1995; Cherian et al., 1999; Armstead, 1996). In the G. Bouma study of 186 adults after severe TBI, using Xe133 radioactive labeling was shown relatively low CBF in the first 6 hours after injury (Bouma et al., 1991). The average CBF in this study at 4 to 6 hours after injury was 22 mL/100 g/min, and about one third of patients had CBF low enough to be considered ischemic (<18 mL/100 g/min) (Diringer et al., 2002; Astrup et al., 1981), when normal CBF in adults ranges from 33 to 55 mL/100 g/min (Obrist et al., 1984; Chiron et al., 1992). There was a strong correlation between low CBF and poor outcome, with a higher mortality rate for those with ischemia than those without. Thus, ischemia after TBI seems to occur early after injury. These findings were confirmed in a subsequent study using the inhaled stable Xe133 enhanced CT scan CBF technique (Bouma et al., 1995). Another study of 32 adults shows the significant changes of CBF from 27 cc/100 g/min at admission to 44 cc/100 g/min by 24 hrs (Marion et al., 1991).

Infants during the first 6 months of life also have a CBF of about 40 mL/100 g/min, which steadily increases during childhood to reach a peak of about 108 mL/100 g/min between the ages of 3 and 4 years, then goes down to 71 mL/100 g/min after the age of 9 years (Suzuki, 1990), and decreases to adult levels in the late teen age (Chiron et al., 1992). Therefore, it seems that CBF rises through early childhood, then decreases to reach adult levels by late adolescence (Chiron et al., 1992; Mansfield, 1997). Considering that large range of age depending variations, comparisons of CBF data in children could be valid only in small, well-defined age selected ranges, and some investigators of CBF revisited their opinion on prevalence of hyperemia in pediatric TBI (Zwienenberg & Muizelaar, 1999). In the largest report of CBF in 30 children with TBI (Adelson et al., 1997) it isobserved, that hypoperfusion was common in admission (about 25 mL/100 g/min) and increased by 24 hrs up to 55 mL/100 g/min. Young age (<24 months) and low CBF in the early period after TBI were associated with poor outcomes. In the most recent publication of the same group of authors it was proven that a younger age, early or later low CBF are correlated with poorer outcomes in children, a relationship of low CBF and an unfavorable outcome (Adelson et al., 2011).

3. CBF Doppler studies in humans

The application of investigative methods based on Doppler Effect allows the performance of continuous monitoring of cerebral blood flow. Due to non-invasiveness and simplicity of application they become one of the first choice investigative methods in clinical and animal research, as Transcranial Doppler, Laser Doppler, etc.

Investigations of CBF with Transcranial Doppler (TCD) techniques allowed the performance of noninvasive and more frequent studies, and as it was shown in study of 57 patients with severe TBI (GCS =<8), that decreased flow was most pronounced during first eight hrs after injury, and significantly increased after that time period (van Santbrink et al., 2002). Correlation between impaired flow and GCS and outcome on admission, was founded in another study in 121 patients with TBI (Chan et al., 1992). Experimental TBI investigations show reduced response of CBF to endogenous vasodilators as nitric oxide (NO) (Cherian et al., 2004; Hlatky et al., 2003).

There are several investigations on Cerebral Blood Flow (CBF) in TBI. It is well established that CBF decreases significantly following TBI in clinical patients (Golding et al., 1999; Robertson et al., 1992) and in experimental animal models (Cherian et al., 1999). Moderate to severe TBI leads to severe reduction of CBF, causing secondary ischemia to already injured brain tissue with increased metabolic demands (Bayir et al., 2003; Potts et al, 2006; Manvelyan, 2006). Clinical research proves the dependence of TBI mortality to primary or secondary ischemia during autopsy (Adams et al., 1983).

It is suggested that trauma roughly disturbs the brain autoregulation, the fine relations between vasoconstrictor and vasodilator production, and accordingly with increased cerebral metabolic demands, makes the brain more vulnerable to ischemia. But, it is very speculative to suggest that the hypoperfusion is connected with vasodilator activity, following hyperemia with vasoconstrictors. Blood flow in the brain is influenced by a wide variety of vasodilators as arachidonic acid metabolites, prostacyclin (Bayir et al., 2003), acetylcholine, calcitonin gene-related peptide, adenosine, and many others (Golding et al., 1999; Bayir et al., 2003), and vasoconstrictors as serotonin, thromboxane, endothelin, and others (Golding, 2002).

The reductions in CBF and further hyperemia after TBI may have been due to the production or reduction of one or more of these cerebral vasoconstrictors or vasodilators. TBI increases the synthesis of cerebral vasoconstrictors including thromboxane (DeWitt et al., 1988) and endothelin (Armstead, 1996) and decreases the release or impairs the vasodilator action of prostacyclin (Al-Turki & Armstead, 1998). Greater TBI-induced release of endothelin-1 in newborn pigs vs. juvenile as age dependent differences in cerebral autoregulation response was shown in Armstead study (Armstead, 1999).
So, insufficient and sometimes controversial data on CBF, demands further investigation, and proper understanding of the role of regional vascular changes beyond vasoactive mediators, as a result of local inflammatory and angiogenesis. This will be appreciated in understanding mechanisms of cerebral autoregulation impairment and CBF disturbances in TBI and then CBF effective regulation will become a therapeutic real target.
We started our own investigations of CBF changes in 69 patients with severe TBI using Ultrasound Transcranial Doppler measurements by strictly considering the timeline. The measurements were started immediately after patient admission to the hospital, in initial hours of TBI onset, and then monitored during the subsequent two week period. Thus, letting us perform the monitoring of the CBF changes in the injured brain. We recorded the mean blood flow velocity in Median Cerebral Artery (MCA), as the biggest artery of the Willis Circle, providing the main blood volume of anterior circulation. The selection of patients was done according to their severity of injury; patients with intracranial hematoms due to possible vascular external compressions were excluded from the study.
The most interesting finding was the total drop in the blood flow during initial hours after injury onset. If it is considered that the mean velocity in MCA, measured in healthy volunteers, is equal to 62±12 cm/s, in patients with acute TBI it was notably reduced down to 36±14 cm/s, which is on the border for development of cerebral primary ischemia. This condition is described as hypovolemia, which means reduced blood flow to the brain.
The following next two days (post-injury 2-3) were marked with notable increase of the CBF velocity in MCA, reaching and overcoming the mean numbers, up to 87±14 cm/s, and continuously growing during a week after injury, up to 120 cm/s, which is Doppler sign of hypervolemia. Some exceptional cases recorded blood flow velocity of 174 cm/s, which exceeds the mean flow velocity three times (!), and develops exclusively as the vasospasm, on postinjury days 6-8. Later on, by the end of two week monitoring period, the CBF velocity drops to mean levels, 66±8 cm/s.
Two patients did not survive the injury; one with the lowest flows recorded, passed on Day 1, and another with the severe cerebral vasospasm, passed on Day 8 post-injury.
Clinical data suggests that during severe neurotrauma cerebral blood flow goes through succeeding processes of hypovolemia, hypervolemia and in most uncontrolled and/or severe cases, vasospasm. Hypovolemia develops immediately after injury and lasts one-two days, and could lead to primary brain ischemia, then cerebral perfusion trends to recover and increasing intensity, reaching hypervolemia also. Development of the arterial spasm (on days 7-8) leads to secondary ischemia.

4. Laser Doppler animal experimental studies

Clinical investigations proved the key role of CBF in primary (due to hypoperfusion) and secondary (mostly due to vasospasm) ischemia in TBI. The animal studies show the correlation between trauma severity and reduction of CBF. The pathological pathways of

CBF reductions are impairments of cerebral autoregulation and cerebral perfusion pressure, and release endogenous vasoconstrictors, responsible also for inflammatory response, serotonin, endothelin, alteration in brain tissue metabolism and ions transport, etc. These experiments demonstrate that TBI leads to significant reduction in cortical CBF of mature and immature experimental animals.

Reduced CBF has been shown in many models of TBI. Moderate fluid percussion injury caused up to 50% reduction in CBF within 15-30 min after injury (Yamakami & McIntosh, 1989). Controlled cortical impact reduced cortical CBF by about 35% (Cherian et al., 1994) to 50% (Bryan et al., 1995).

The recent data of different investigators shows the one-time only continuous monitoring of CBF in animals with TBI model during a few minutes and up to hours. Due to technical difficulties there was no data on the subsequent days and weeks, whenever clinical data shows significant importance of CBF monitoring and repeated investigation during at least a two week period after TBI onset.

For our investigation of cerebral circulation in TBI a rodent model of cortical impact injury was used including adult mice and 21 day immature mice as a model of adult and baby brain injury (a total of 50 animals). For the CBF investigations we used the laser Doppler device LASERFLO BPM2 (Vasamedics, US). Laser Doppler flowmeter (LDF) measures relative perfusion continuously, using a transmitter/receiver probe that emits monochromatic laser light which is reflected by moving RBC according Doppler effect (Stern et al., 1977; Wardel et al., 1993). The power and frequency of the reflected are proportional to the blood volume and blood velocity. Perfusion is calculated as a product of blood volume and velocity in 1mm3 tissue volume under the probe (Haberl et al., 1989). The blood flow in the scanned area is presented in absolute or relative numbers. The power and CBF was monitored using a LDF with the fiber optic needle probe mounted on a stereotactic manipulator and placed approximately 0.5 mm above the dura mater or the pial surface, carefully positioned away from the visible large vessels. Once a suitable placement was obtained, the probe was left at that point for the duration of the experiment, and removed and replaced on the same position after cortical injury.

Measurements were recorded and compared between mice based on a percentage change from baseline values (intact cortex) after experimental procedures. LDF monitored the cortical blood flow in different time points: from the intact skull, intact cortex right after craniotomy, from injured cortex after TBI, and prior to scarification of the animal on 5 different groups (five animals in each) on Day 1, Day 3, Day 7, Day 14 and Day 28. Prior to euthanasia all animals under surgical dosage of anesthesia were placed on a warming pad (to keep the animal under the same condition as during initial investigation) and after a skin incision right above the craniotomy site, the Doppler probe was maintained above the cortex. The measurements show the sufficient drop of flow right after the injury in both groups of animals, CBF falls down about 31% in adults and 33% in babies (*t-test*: $P<0.05$, the difference between two groups is not significant). The next investigation was performed on the next day, and it is more likely the tendency to restoration of the CBF, when the Flow in adults were reduced about 15% of the Pre-Injury level, and increased about 15 % in babies (*t-test*: $P<0.05$, the difference is not significant). On the Day 3 blood flow continues the tendency to restoration and the differences were less prominent in adults (decreased about 5% of the Pre Injury level) and babies (increased about 8%), there was no statistically significant difference between that values again (*t-test*: $P<0.05$, the difference is not significant).

The most significant changes of CBF were recorded on Day 7, when in both groups the Flow reached the highest values during all investigation time points, 43% in adults and 56% in babies comparing the Pre-Injury level of the flow. On days 14 and 28 we saw the restoration of the Flow in both groups. The mean values of the flow presented by those numbers: the flow in the intact cortex is about 25 *mlLD/min/100gTissue* +/- 8 (in adults) and +/- 5 (in immature) (*t-test*: P<0.05, the difference is not significant). When tissue perfusion was less then 10-12 mlLD/min/100gTissue right after injury, it is an indication of severe cortical ischemia, leading to death of the animal.

Performing 35-day long monitoring of cortical local perfusion gives the following data. Cortical perfusion in an intact brain is similar in both mature and immature CNS. The mean absolute values of the perfusion in the intact cortex are 25 +/- 8 *mlLD/min/100gTissue* (in adults) and 25 +/- 5 *mlLD/min/100gTissue* (in immature) (*t-test*: P<0.05, the difference is not significant). The consequent measurements show the sufficient drop of perfusion immediately after the injury in both groups of animals, falls down about 31% in adults and 33% in immature (*t-test*: P<0.05, the difference between two groups is not significant). During the following posttraumatic days one and three local cortical perfusion in both groups had tendency toward the restoration of flow in pre-injury level, as it was measured from the intact brain, then significant change in perfusion was recorded in both groups on day seven, when perfusion reached its highest values during all investigative time points. On following days 14 and 28 the restoration of the perfusion in the both groups were recorded.

Next we examined brain slices stained for vascular proliferation with *Esculentum Lectin*. Enormously large dilated blood vessels appeared at the 7 days post injury in both groups, coinciding with the maximal perfusion, registered by Doppler monitoring. Moreover, on the same day activated microglia/macrophages were prominent within the ipsi-lateral cortex and hippocampus of both age groups (figure 1).

The fact of decreasing blood flow immediately after injury still demands further understanding. Recent papers show the connection between the CBF decreases and NO level reduction after moderate TBI, increase of the cytokines expression (Ahn et al., 2004). One of the probable ways of understanding it is cerebral circulation. Cerebral microcirculation depends on very delicate interaction of vasoconstrictors and vasodilators. TBI is roughly interacting with that system, which leads to the release of cerebral vasoconstrictors (DeWitt et al., 2000) and decreases the production or affect of the activity of vasodilators (Al-Turki & Armstead, 1998). The other findings associated with CBF reduction on early time points after TBI, is the increased cytokine expression and production. However, the role of their changes in activity is not clear yet (Ahn et al., 2004).

So, the reduction of CBF immediately after severe TBI onset could be explained by the changing histochemistry of the brain and vessels, but the symmetric changes in CBF in immature and mature brain on day 7 demands further investigation and understanding. One of the most probable reasons of it could be prevalence of vasodilatation effect due to impairment of cerebral autoregulation. Clinical studies shows the presence of vasospasm on days 7-9, and it shows increased blood flow in main arteries of the brain, Willis circle, whenever the microcirculation in cerebral cortex could be altered due to local conditions. It remains speculative since simultaneous measurement of cerebral hemodinamics and vasoactive agents *in vivo* studies is limited.

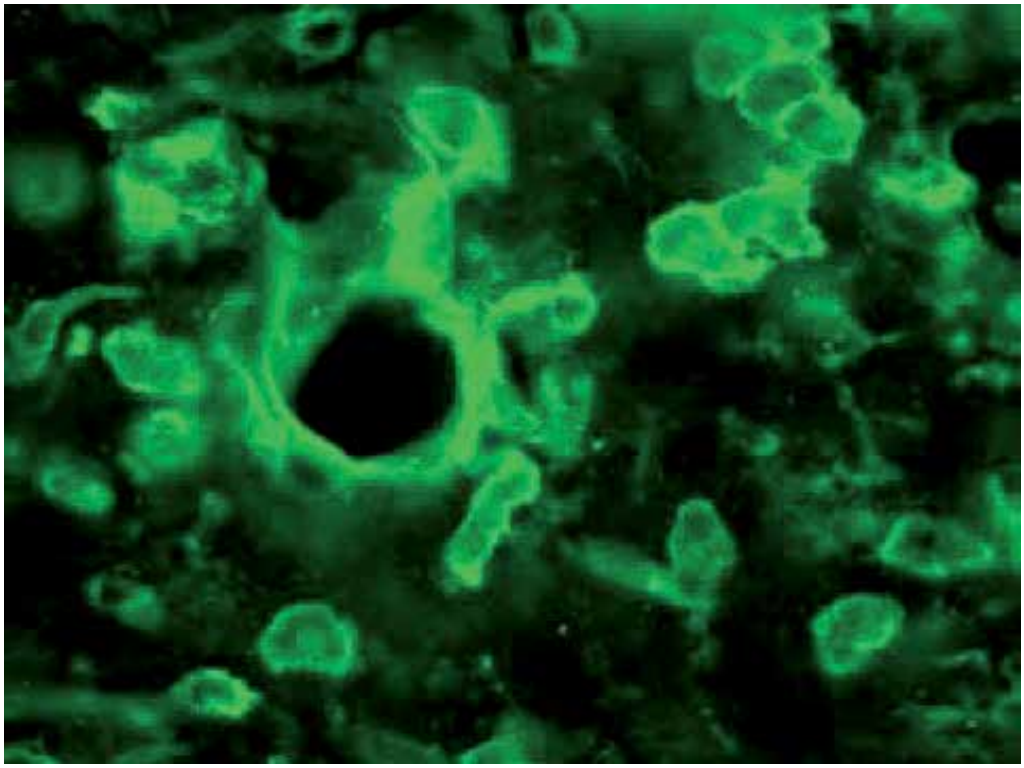


Fig. 1. Enormously enlarged vessel with activated microglial cells.

5. Conclusion

Cerebral Blood Flow is an important chain in the development of cascade of multiple biochemical and vascular events in TBI, contributing to both severity and prognosis. Despite the lack of complete data and existing controversies, and the level of brain maturity, CBF changes in brain injury in both human and experimental research are similar: immediate drop after injury onset (hypovolemia), recovery up to initial levels on following days, and further developement of hyperperfusion and vasospasm.

In normal condition the cerebral circulation has the ability to maintain a stable CBF over a wide range of cerebral perfusion pressures (CPP); this phenomenon is designated as cerebral autoregulation and mainly represents the capacity of the brain's resistance vessels to dilate in response to a decrease in CPP or to constrict in response to an increase in CPP. The caliber changes of the autoregulatory vessels are mediated by myogenic, metabolic, or neurogenic mechanisms (Brownlee & Langille, 1991; Bryan et al., 1995; Busto et al., 1997). The smaller arterioles dilate proportionately more than larger arterioles at a mean arterial blood pressure below physiological levels (Ellis et al., 1979; Engelborghs et al., 2000). However, larger arterioles tend to be more responsive than smaller arterioles at normal and increased levels of arterial pressure (Engelborghs et al., 2000). The other findings associated with CBF reduction at early time points after TBI is the increased cytokine expression and production, however the role of their activity changes is not clear yet (Ahn et al., 2004). Destruction of cortical regions could effectively produce deafferentation in subcortical and

cortical target regions resulting in reduced energy requirement and metabolic rates. The blood vessel can participate in the regulation of blood flow by changing its own structure, a process known as vascular remodeling (Langfitt et al., 1977; Langille et al., 1989; Brownlee & Langille, 1991). It is characterized by changes in vessel wall thickness and organization, which allows the vasculature to cope with physiological or pathological conditions. The processes involved in vascular remodeling include cellular hypertrophy and hyperplasia, as well as enhanced protein synthesis (Golding et al., 1999; Golding, 2002). So, the reduction of regional CBF immediately after severe TBI onset could be explained by the changing histochemistry of the brain and caliber of the vessels, but the symmetric changes in CBF in immature and mature brain on day 7 demand further investigations and understanding. The most probable reason for it could be the reverse effect of changed levels of vasoactive agents due to impairment of cerebral autoregulation, or expressed production of vasoconstrictors. Clinical studies show the presence of vasospasm on days 7-9 (Hlatky et al., Robertson, 2003; Voulgaris et al., 2005) and it shows increased blood flow in the main arteries of the brain, Willis circle, whenever the microcirculation in cerebral cortex could be different and altered due to local conditions.

Being an influent factor in outcomes of TBI, cortical perfusion is not the determinant of increased vulnerability of immature to neurotrauma.

CBF must be monitored in TBI, as an important participating factor in pathophysiology, strong predictor of prognosis and a tool to prevention of primary and secondary ischemia.

6. Acknowledgment

Author expresses his sincere gratitude to Prof. Linda Noble-Haeusslein, Professor, Department of Neurological Surgery and Department of Physical Therapy and Rehabilitation, Co-Director and Principal Investigator, Brain and Spinal Injury Center (BASIC), Co-Director, Neurobehavioral Core for Rehabilitation Research, UCSF, for her unlimited support and counseling in conducting of the experimental part of this work.

7. References

Adams, JH, Graham, DI & Gennarelli, TA (1983). Head Injury in man and experimental animals: neuropathology. *Acta Neurochir Supl*. 32, S15-S30

Adelson, PD, Srinivas, R, Chang, Y, Bell, M & Kochanek, PM (2011). Cerebrovascular response in children following severe traumatic brain injury. Childs Nerv Syst. DOI: 10.1007/s00381-011-1476-z

Adelson, PD, Clyde, B., Kochanek, PM, Wisniewski, SR, Marion, DW & Yonas, H. (1997). Cerebrovascular response in infants and young children following severe traumatic brain injury: a preliminary report. Pediatr Neurosurg 26:200-207

Ahn, MJ, Sherwood, ER, Prough, DS, Lin, CY & DeWitt, DS (2004). The effects of traumatic brain injury on cerebral blood flow and brain tissue nitric oxide levels and cytokine expression. *J Neurotrauma*, Vol 21, 10, 1431-1442

Al-Turki, A. & Armstead, WM. (1998). Altered release of prostaglandins by opioids contributes to impaired cerebral hemodynamics following brain injury. *Crit Care Med*. 26(5):917-25

Armstead, WM. (1996). Role of endothelin in pial artery vasoconstriction and altered responses to vasopressin following brain injury. *J Neurosurg*. 85:901–907

Armstead, WM. (1999). Cerebral hemodynamics after traumatic brain injury of immature brain. *Exp Toxicol Pathol*. 51(2):137-42

Astrup, J., Siesjö, BK & Symon, L. (1981). Thresholds in cerebral ischemia - the ischemic penumbra. *Stroke*. 12(6):723-5

Bayir, H., Kochanek, PM & Clark, RS. (2003). Traumatic brain injury in infants and children: mechanisms of secondary damage and treatment in the intensive care unit. *Crit Care Clin* 19:529-549

Biagas, KV, Grundl, PD, Kochanek PM, Schiding, JK & Nemoto, EM. (1996). Posttraumatic Hyperemia in Immature, Mature, and Aged Rats: Autoradiographic Determination of Cerebral Blood Flow. *Journal of Neurotrauma*. 13(4): 189-200.

Bouma, GJ & Muizelaar, JP. (1995). Cerebral blood flow in severe clinical head injury. *New Horiz*. 3(3):384-94

Bouma, GJ, Muizelaar, JP, Stringer, WA, Choi, SC, Fatouros, P. & Young, HF. (1992). Ultra-early evaluation of regional cerebral blood flow in severely head-injured patients using xenon-enhanced computerized tomography. *J Neurosurg*. 77(3):360-8.

Brownlee, RD & Langille, BL. (1991). Arterial adaptations to altered blood flow. *Can J Physiol Pharmacol*. 69(7):978-83

Bruce, DA, Alavi, A. & Bilaniuk, L. (1981). Diffuse cerebral swelling following head injuries in children: the syndrome of "malignant brain edema". *J Neurosurg* 54:170–8

Bryan, R., Cherian, L. & Robertson, C. (1995). Regional cerebral blood flow after controlled cortical impact injury in rats. *Anesth. Analg*. 80, 687-695

Busto, R., Dietrich, WD, Blobus, MY, Alonso, M. & Ginsberg, WD. (1997), Extracellular release of serotonin following fluid-percussion brain injury in rats. *J Neurotrauma*. 14:35–42

Chan, KH, Dearden, NM, Miller, JD, Midgley, S. & Piper, IR. (1992). Transcranial Doppler waveform differences in hyperemic and nonhyperemic patients after severe head injury. *Surg Neurol*. 38(6): 433-6

Cherian, L., Chacko, G., Goodman, JC & Robertson, CS. (1999). Cerebral hemodynamic effects of phenylephrine and L-arginine after cortical impact injury. *Crit Care Med.* 27:2512–2517

Cherian, L., Hlatky, R. & Robertson CS. (2004). Nitric Oxide in Traumatic Brain Injury. *Brain Pathol*, 14:195-201

Cherian, L., Robertson, CS, Contant, CF & Bryan, RM (1994). Lateral cortical impact injury in rats: cerebrovascular effects of varying depth of cortical deformation and impact velocity. *J. Neurotrauma.* 11, 573-585

Chiron, C., Raynaud, C. & Maziere, B. (1992). Changes in regional cerebral blood flow during brain maturation in children and adolescents. *J Nucl Med* 33: 696– 703

Claus, CP, Tsuru-Aoyagi, K., Adwanikar, H., Walker, B., Manvelyan, H., Whetstone, W. & Noble-Haeusslein, LJ (2010). Age is a determinant of leukocyte infiltration and loss of cortical volume after traumatic brain injury. *Dev Neurosci*; 32(5-6): 454-65

DeWitt, DS & Prough, DS (2000). Should pressors be used to augument cerebral blood flow after traumatic brain injury? *Crit Care Med.* 28(12):3933-4

DeWitt, DS, Yuan, XQ, Becker, DP & Hayes, RL (1988). Simultaneous, quantitative measurement of local blood flow and glucose utilization in tissue samples in normal and injured feline brain. *Brain Inj*. 2(4):291-303

Diringer, MN, Videen, TO, Yundt, K., Zazulia, AR, Aiyagari, V., Dacey, RG, Grubb, RL & Powers, WJ (2002). Regional cerebrovascular and metabolic effects of hyperventilation after severe traumatic brain injury. *J Neurosurg*. 96(1):103-8

Ellis, EF, Wei, EP & Kontos, HA (1979). Vasodilation of cat cerebral arterioles by prostaglandins D2, E2, G2, and I2. *Am J. Physiol*. 237 H381–385

Engelborghs, K., Haseldonckx, M., Van Reempts, J., Van Rossem, K., Wouters, L., Borgers, M. & Verlooy, J. (2000). Impaired autoregulation of cerebral blood flow in an experimental model of traumatic brain injury. *J Neurotrauma*. 17(8):667-77.

Fan, P., Yamauchi, T. & Noble-Haeusslein, LJ & Ferriero, DM (2003). Age-dependent differences in glutathione peroxidase activity after traumatic brain injury. *J Neurotrauma.* 20: 437-445

Fife, D. & Jagger J. (1987). Head injury with and without hospital admission: comparisons of incidence and short-term disability. *Am J Public Health*. 77(7): 810–812.

Golding, EM (2002). Sequelae following traumatic brain injury. The cerebrovascular perspective. *Brain Res Rev*. 38(3): 377-388

Golding, EM, Robertson, CS & Bryan, RM (1999). The consequences of traumatic brain injury on cerebral blood flow and autoregulation: a review. *Clin Exp Hypertens*. 21:299–332

Graham, DI & Adams, JM (1971). Ischemic brain damage in fatal head injuries. *The Lancet*. Vol. 297 (Issue 7693), 265-266

Graham, DI, Adams, JH & Doyle, D. (1978). Ischaemic brain damage in fatal non-missile head injuries. *J Neurol Sci*. 39(2-3):213-34

Graham, DI, Ford, I., Adams, JH, Doyle, D., Lawrence, AE, McLellan, DR & Ng, HK (1989). Fatal head injury in children. *J Clin Pathol*. 42(1):18-22.

Haberl, RL, Heizer, ML, Marmarou, A. & Ellis, EF (1989). Laser-Doppler assessment of brain microcirculation: effect of systemic alterations. *Am J Physiol*. 256(4 Pt 2):H1247-54.

Hlatky, R., Valadka, AB & Robertson, CS (2003). Intracranial hypertension and cerebral ischemia after severe traumatic brain injury. *Neurosurg Focus*. 15;14(4)

Kelly, DF, Kordestani, RK, Martin, NA, Nguyen, T., Hovda, DA, Bergsneider, M., McArthur, DL & Becker DP (1996). Hyperemia following traumatic brain injury: relationship to intracranial hypertension and outcome. *J Neurosurg*. 85(5):762-71

Kelly, DF, Martin, NA, Kordestani, R., Counelis, G., Hovda, DA, Bergsneider, M., McBride, DQ, Shalmon, E., Herman, D. & Becker, DP (1997). Cerebral blood flow as a predictor of outcome following traumatic brain injury. *J Neurosurg*. 86(4):633-41

Khoshyomn, S. & Tranmer, BI (2004). Diagnosis and management of pediatric closed head injury. *Semin Pediatr Surg*. 13:80-86

Langfitt, TW, Obrist, WD, Gennarelli, TA, O'Connor, MJ & Weeme, CA (1977). Correlation of cerebral blood flow with outcome in head injured patients. *Ann. Surg*. 186: 411–414

Levin, HS, Aldrich, EF, Saydjari, C., Eisenberg, HM, Foulkes, MA & Bellefleur, M. (1992). Severe head injury in children: experience of the Traumatic Coma Data Bank. *Neurosurgery*. 31:435-443

Luerssen, TG, Klauber, MR & Marshall, LF (1988).Outcome from head injury related to patient' age. A longitudinal prospective study of adult and pediatric head injury. *Journal of Neurosurgery*. 68(3):409-16

Mansfield, RT (1997). Head injuries in children and adults. *Crit Care Clin*. 13(3):611-28.

Manvelyan, HM (2006). Cortical perfusion is an important factor, but not a determinant of increased vulnerability of immature to traumatic brain injury. *Georgian Medical News*, No 12 (141), p. 24 – 28

Marion, DW, Darby, J. & Yonas, H. (1991) Acute regional cerebral blood flow changes caused by severe head injuries. *J Neurosurg* 74:407-414

McQuire, JC, Sutcliffe, JC & Coats, TJ (1998). Early changes in middle cerebral artery blood flow velocity after head injury. *J Neurosurg*. 89(4):526-32

Meixensberger, J., Dings, J., Kuhnigk, H. & Roosen, K. (1993). Studies of tissue PO2 in normal and pathological human brain cortex. *Acta Neurochir Suppl (Wien)*. 59:58-63

Muizelaar, JP, Marmarou, A., DeSalles, AF, Ward, JD, Zimmerman, RS, Li, Z., Choi, SC & Young, HF (1989). Cerebral blood flow and metabolism in severely head-injured children. *Journal of Neurosurgery*. Vol 71(1): 63-71

Narayan, RK, Michel, ME, Ansell, B., Baethmann, A., Biegon, A., Bracken, MB, Bullock, MR, Choi, SC, Clifton, GL, Contant, CF, Coplin, WM, Dietrich, WD, Ghajar, J., Grady, SM, Grossman, RG, Hall, ED, Heetderks, W., Hovda, DA, Jallo, J., Katz, RL, Knoller, N., Kochanek, PM, Maas, AI, Majde, J., Marion, DW, Marmarou, A., Marshall, LF, McIntosh, TK, Miller, E., Mohberg, N., Muizelaar, JP, Pitts, LH, Quinn, P., Riesenfeld, G., Robertson, CS, Strauss, KI, Teasdale, G., Temkin, N., Tuma, R., Wade, C., Walker, MD, Weinrich, M., Whyte, J., Wilberger, J., Young, AB & Yurkewicz L. (2002). Clinical trials in head injury. *J Neurotrauma*. 19(5):503-57

Obrist, WD, Langfitt, TW, Jaggi, JL, Cruz J. & Gennarelli TA (1984). Cerebral blood flow and metabolism in comatose patients with acute head injury. *Journal of Neurosurgery*. Vol. 61, No. 2, 241-253

Potts, MB, Koh, SE, Whetstone, WD, Walker, BA, Yoneyama, T, Claus, CP, Manvelyan, HM & Noble-Haeusslein LJ (2006). Traumatic injury to the immature brain: inflammation, oxidative injury, and iron-mediated damage as potential therapeutic targets. *NeuroRx*.(2):143-53

Robertson, CS, Contant, CF, Gokaslan, ZS, Narayan, RK & Grossman, RG (1992). Cerebral blood flow, arteriovenous oxygen difference, and outcome in head injured patients. *J. Neurol. Neurosurg. Psych*. 55: 594–603

Stern, MD, Lappe, DL, Bowen, PD, Chimosky, JE, Holloway, GA, Keiser, HR & Bowman, RL (1977). Continuous measurement of tissue blood flow by laser Doppler spectroscopy. *Am.J. Physiol*. 232, H441-H448

Suzuki, K. (1990). The changes of regional cerebral blood flow with advancing age in normal children. *Nagoya Med J* 34:159– 70

van Santbrink, H., Schouten, JW, Steyerberg, EW, Avezaat, CJ & Maas, AI (2002). Serial transcranial Doppler measurements in traumatic brain injury with special focus on the early posttraumatic period. *Acta Neurochir (Wien)*. Nov;144(11):1141-9

Voulgaris, SG, Partheni, M., Kaliora, H., Haftouras, N., Pessach, IS & Polyzoidis, KS (2005). Early cerebral monitoring using the transcranial Doppler pulsatility index in patients with severe brain trauma. *Med Sci Monit*. 24;11(2):CR49-52

Wardell, K., Jakobsson, A. & Nilsson, GE (1993). Laser Doppler perfusion imaging by dynamic light scatttering. *IEEE Trans Biomed Eng*. 40, 309-316

Yamakami, I. & McIntosh, TK (1989). Effects of traumatic brain injury on cerebral regional blood flow in rats as measured with radiolabeled microspheres. *J Cereb. Blood Flow Metab*. 9, 117-124

Yamakami, I. & McIntosh, TK (1991). Alterations in regional cerebral blood flow following brain injury in the rat. *J Cereb Blood Flow Metab*. 11(4):655-60

Yoneyama-Sarnecky, T, Olivas, AD, Azari, S, Ferriero, DM, Manvelyan, HM & Noble-Haeusslein, LJ (2010). Heme oxygenase-2 modulates early pathogenesis after traumatic injury to the immature brain. *Dev Neurosci*. 32(1):81-90

Zwienenberg, M. & Muizelaar, JP (1999). Severe pediatric head injury: the role of hyperemia revisited. *J Neurotrauma*. 16(10):937-43

Part 3

Investigative Approaches and Monitoring

10

MRI Characterization of Progressive Brain Alterations After Experimental Traumatic Brain Injury: Region Specific Tissue Damage, Hemodynamic Changes and Axonal Injury

Riikka Immonen and Nick Hayward
University of Eastern Finland
Finland

1. Introduction

In this chapter we describe magnetic resonance imaging (MRI) findings in a rat model of traumatic brain injury (TBI). Although studied in a rodent model, these findings correspond closely to those seen in patients. The experimental study setup allows mapping of the spatio-temporal interrelations of different pathological features in the injured brain as well as correlation of non-invasive MRI results with tissue histochemistry data. We combine the information provided by some selected MRI techniques to characterize the interplay between structural and hemodynamic changes.

1.1 Non-invasive imaging and animal models are needed for understanding the complex and progressive brain alterations that occur after traumatic brain injury (TBI)

TBI is a devastating disease with a variety of cognitive and motor function deficits that manifest from immediately after the impact and even up to several years later. The mechanisms of TBI are complex and there is a great unmet medical need to find neuroprotective treatments. Primary TBI damage is caused by the shear forces of the impact itself, which initiates ionic, molecular, and cellular alterations within seconds (McIntosh et al., 1994; Rink et al., 1995) followed by immediate cytotoxic edema and later vasogenic edema (Faden et al., 1989). Thereafter, the secondary injury begins to develop and continues to worsen for days, months, or even years. The neurodegenerative cascades of secondary injury are composed of complex combinations of cellular and metabolic alterations (Pitkänen et al., 2009). The cortical contusion site suffers most, but other cortical areas and sub-cortical gray matter regions are exposed to the destructive cascades as well. The impact forces stretch the white matter tract network and adjacent vasculature, which causes microbleeds within the white matter bundles. These microbleeds are one early indicator of diffuse axonal injury (DAI), which involves progressive demyelination and axonal damage throughout the brain. DAI is recognized as a key factor in the consequential progressive cognitive impairment. Histological studies of TBI report the co-occurrence of chronic inflammation, glial hypertrophy, and axonal injury (Lenzlinger et al., 2001; Morganti-Kossmann et al., 2002; Soares et al., 1995). We know that eventually the combination of these gray matter and white matter associated cascades leads to functional disabilities including

motor impairment, cognitive decline, emotional disturbance, or epilepsy (Kharatishvili et al., 2006; Thompson et al., 2005), but the exact mechanisms remain unrevealed.

Animal studies permit control of the inter subject variability, elimination of environmental un-known factors, long-term follow-up of the same subject, multimodal testing, and accurate correlation of radiological, behavioural, histological and molecular findings. The following chapter focuses on describing the multimodal MRI findings in a *rat model of lateral fluid percussion injury (LFPI) induced TBI*, which is the most widely used and best characterized experimental model of human closed head injury (Kharatishvili et al., 2006; Thompson et al., 2005).

Magnetic resonance imaging (MRI) methods offer a variety of approaches to study the different features of brain pathologies non-invasively. Particularly, in complex nervous system diseases that progress slowly and carry unknown mechanisms, the application of multimodal MRI techniques that target different underlying phenomena can provide crucial information about the spatio-temporal developments of the tissue damage and thereby provide added insights into the disease mechanisms.

2. Multimodal MRI of the injured brain: Access to morphology and tissue atrophy, transient edema, neurodegeneration, white matter damage, intracerebral hemorrhages and hemodynamics

2.1 Characterization of damage extent and region specific pathological features

MRI has an important role after head injury in detecting the primary lesion, tissue atrophy and lysis, microbleeds and hematomas, transient oedema coverage and other morphological features. In particular, ultra high magnetic fields (such as 7 T – 16 T) can access the fine details in cortical and sub-cortical structures and map the entire brain of a mouse in, for example, a resolution of tens of micrometers. Clinical scanners (1.5 T - 3 T) are able to pick up the same features in patients at the sub-millimeter scale. Figures 1 and 2 demonstrate the extent and sparse distribution of pathological morphological MRI findings post experimental TBI and therefore stress the importance of both the whole-brain coverage and high resolution agenda in imaging.

In the lateral fluid percussion injury rat model, the direct damage caused by the impact forces (pressure and shear stress) depends upon the impact pressure and exact direction. Figures 1 and 2 show example cases of severe injury caused by higher than 3 atm fluid mediated pressure. Figure 1 shows how the atrophy gradually worsens at 3 hours, 3 days, 9 days, 23 days, 2 months, 3 months and 6 months after injury in one representative animal. Figure 2 shows a higher resolution image set of another example animal at 2 months post injury and how the hematomas, atrophy and calcifications extend through the brain rostro-caudally. Immediately after impact the integrity of the blood brain barrier (BBB) is compromised both in gray matter at the contusion site and inside/next to major white matter tracts that are exposed to and transduce the mechanical shear forces. This creates hematomas in cortical gray matter at the contusion site and microbleeds along more distal white matter bundles. Thereafter, the contusion site and ipsilateral surrounding regions suffer from transient edema (hyperintense, swollen cortical area in Figure 1, visible already at 3 hours, maximal at 3 days and partially resolving after 9 days). The impact launches destructive cellular cascades, and the area of neurodegeneration and subsequent atrophy can be first identified at the core of the contusion site as very high intensity (i.e. high free water content) T_2 weighted signal. The tissue degradation area expands from 23 days post

injury onwards and is called here 'the focal lesion'. At later time points a glia scar is found to be outlining this cerebrospinal fluid filled cavity. Macroscopically, it is evident that the hippocampus ipsilaterally to the impact is deformed as atrophy progresses. Microscopically, very pronounced and well described loss of hilar interneurons has been reported in this model (Lowenstein et al., 1992; Hunt et al., 2011). In long follow-up studies of rats, repeated MRI scans have shown that the atrophy volume continues to increase up to 6 months post injury, which correspond to decades in humans (when comparing the relative age).

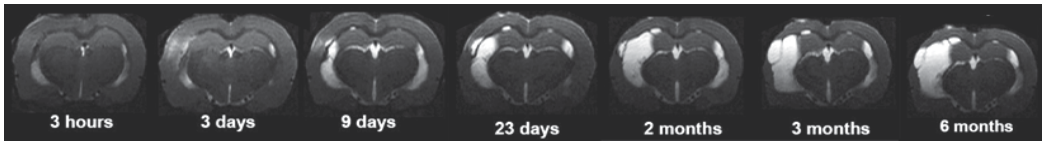


Fig. 1. Progressive atrophy mapped by non-invasive MRI. T_2 weighted MRI made at a 4.7 T scanner. Long-term follow-up studies of the LFPI rat model for TBI demonstrate how tissue atrophy continues until 6 months. This is one time series from the same animal imaged from 3 hours to 6 months post-injury. Sub-acute progression continues for over 2 months and a 'plateau' state is reached 6 months post-injury. The corresponding time scale for humans could be from years to decades. (Immonen et al., 2009b)

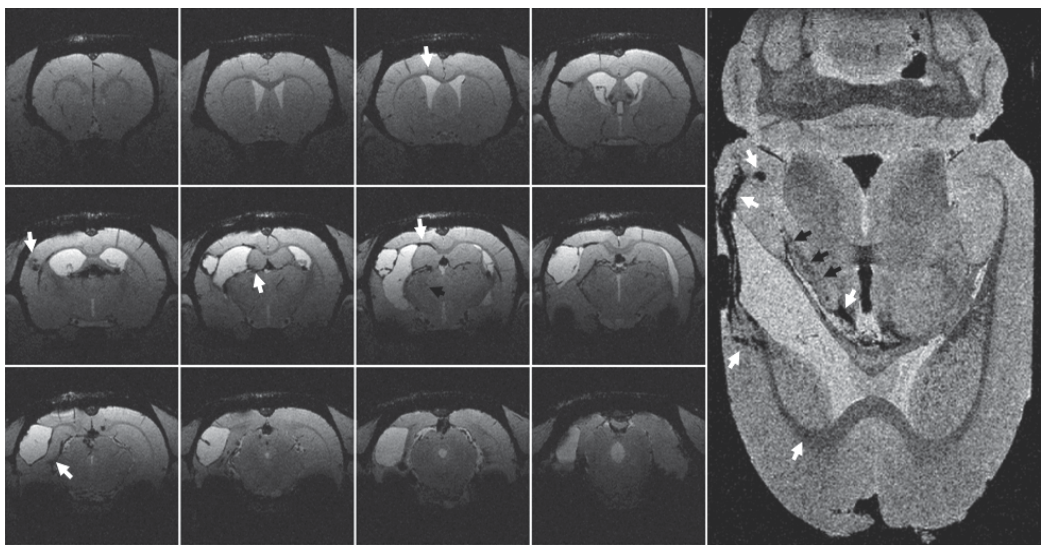


Fig. 2. T_2/T_{2*} weighted images of a rat brain after TBI. The high resolution achievable in an ultra high 9.4 T magnetic field with small animal models characterizes the morphological brain pathology in detail. Left: In vivo MRI of a living mouse at 5 weeks after fluid percussion injury: a series of transverse images showing every fifth slice of 200 μm-thick slices that cover the entire brain (in-plane resolution 70*70 μm). Right: Ex vivo MRI of an intact, fixed rat brain 12 weeks post injury acquired at 50*50*60 μm resolution, coronal view. Anatomical high resolution MRI demonstrates the extent of atrophy (tissue lysis at the primary lesion site and enlargement of ventricles, cerebrospinal fluid appears bright), hematomas and microbleeds (white arrows) and thalamic calcifications (black arrows)

The literature concerning clinical and experimental MRI findings as well as histological correlates after TBI shows good compatibility with our data. In patients, intracerebral hemorrhage, lesion and edema formation at the acute phase are common clinical MRI findings (Caroli et al., 2001; Kurth et al., 1994; Morais et al., 2008; Scheid et al., 2007). Axonal damage following TBI has been frequently detected using diffusion tensor imaging (DTI) and the changes in diffusion anisotropy have been reported in both acute and chronic TBI patients (Sidaros et al., 2008).

In experimental studies, MRI findings resemble the clinical findings with accelerated temporal progression. MRI alterations associated with edema and hemorrhage have been reported (Graham et al., 2000b; Iwamoto et al., 1997) and axonal damage has been linked to changes in diffusion MRI (Mac Donald et al., 2007). Studies in rats have described prolonged relaxation times due to increased water content and decreased cell density. For example, the hyperintensity in T_2 weighted images has been used to show edema, neuronal loss or, the consequence of tissue atrophy and partial volume effect. The hippocampal T_2 relaxation time has been reported to increase during the first 7 days after TBI induced by controlled cortical impact (Obenaus et al., 2007). A 3 month follow-up showed enlarged ventricles, cisterns, and that the necrotic tissue in the primary contusion site was absorbed and replaced by cerebrospinal fluid (Iwamoto et al., 1997). Diffusion weighted imaging has shown decreased apparent water diffusion (ADC) acutely (<24 hours) after TBI followed by increased diffusion days or weeks after TBI (Albensi et al., 2000; Obenaus et al., 2007; Onyszchuk et al., 2007; Van Putten et al., 2005; Vink et al., 2001).

Histological studies in rat models have verified these aforementioned MRI findings and the robust cellular loss and cavity formation in the primary cortical contusion site during the early weeks post-injury, the degeneration has been found to be selective for certain cell types and brain regions (Conti et al., 1998; Cortez, McIntosh, Noble 1989a; Hallam et al., 2004; Raghupathi et al., 2002; Rink et al., 1995; Sato et al., 2001) and it has been demonstrated to go on up to 1 year (Bramlett et al., 1997; Pierce et al., 1998; Smith et al., 1997).

2.2 Progressive brain alterations: Quantitative mapping of relaxation properties

Slow degradation and plastic neuronal processes after trauma may not show in anatomical images in their early phase but they cause alterations in magnetic relaxation properties of tissue locally and can thereby be detected and followed by quantitative MRI. Particularly the gray matter areas surrounding the primary contusion site appear visually completely normal in conventional anatomical images (both in T_2 or T_1 weighted MRI) but the quantitative mapping is sensitive enough to pick out the regions that are undergoing microstructural changes. Figure 3 shows how the perifocal area and ipsilateral hippocampus can appear healthy initially and then later display increased relaxation and diffusion values at 23 days post-injury. Only a 5-10% change in absolute relaxation and diffusion values in perilesional cortical regions at the sub-acute phase (secondary increase after resolution of edema) post injury reveals regions that later progress to atrophy. Maps of T_1, T_2, $T_{1\rho}$ and diffusion (ADC/D_{av}) parameters discern regions of irreversible and reversible tissue damage, and are sensitive enough to monitor the progression and potential treatment responses in different brain regions. Both the magnitude and the temporal pattern of the observed MRI changes differ between the primary lesion site (irreversible damage) and in the perilesional regions (tissue at risk but potentially salvageable), including the hippocampus. Figures 3 and 4 describe the alteration pattern of quantitative MRI data in the acute phase 3 hours post-trauma associated with the immediate mechanical consequences of

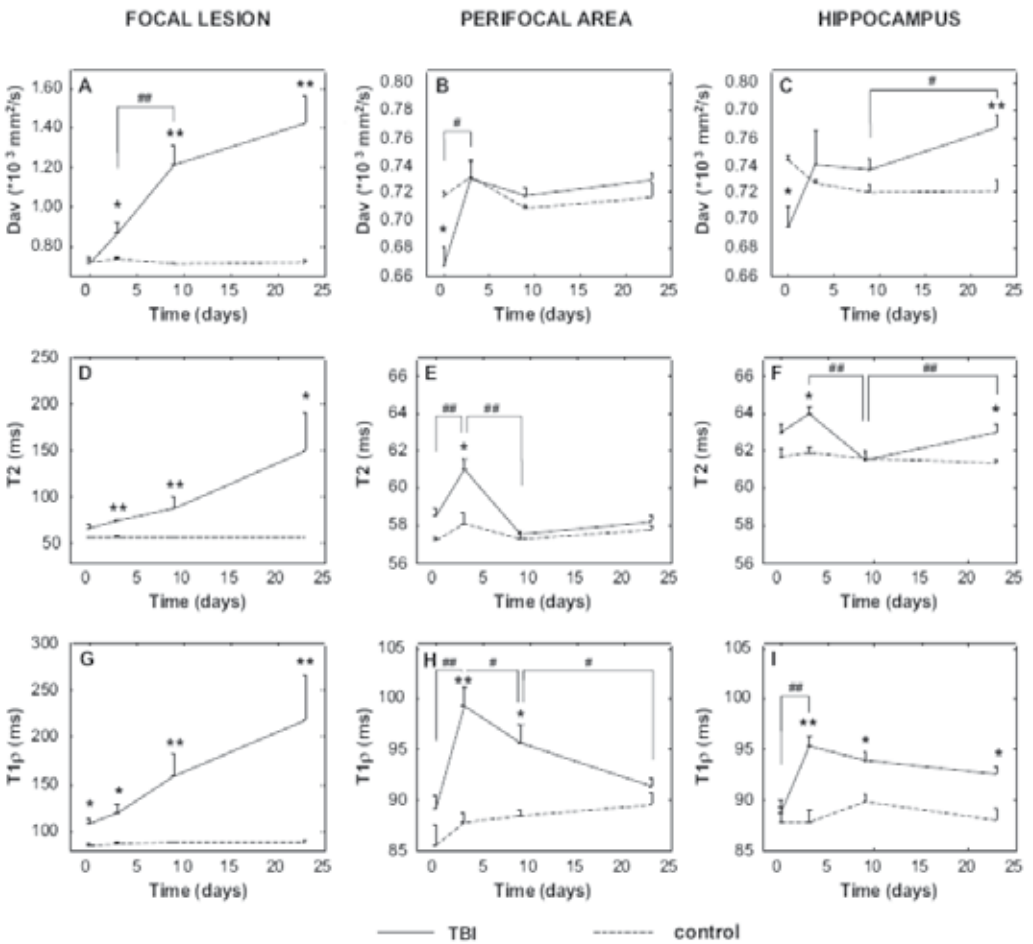


Fig. 3. Early progression of D_{av}, T_2 and $T_{1\rho}$ in the lesion (left column), in the perifocal area (middle column) and in the ipsilateral hippocampus (right column). Graphs compare the quantitative relaxation and average diffusion values from TBI and sham groups at 3 hours, 3 days, 9 days and 23 days after TBI induction. Data points are group averages from region of interest (ROI) analysis of quantitative maps. Each ROI shows a characteristic pattern. **The lesion:** D_{av} increased rapidly in the lesion area starting from day 3. Both T_2 and $T_{1\rho}$ also increased steadily, and $T_{1\rho}$ was significantly elevated already at 3 hours after TBI induction. **The perifocal area** (*i.e.* ipsilateral cortex excluding the lesion): D_{av} dropped acutely (3 hours) after TBI. T_2 and $T_{1\rho}$ show a transient peak at day 3 post-TBI, but unlike T_2, $T_{1\rho}$ remained elevated still at day 9. **The ipsilateral hippocampus**: D_{av} dropped acutely (3 hours) after TBI and increased 23 days later. Both T_2 and $T_{1\rho}$ peaked at day 3 post TBI and were elevated at day 23, but T_2 recovered at day 9 while $T_{1\rho}$ remained elevated. Differences between groups are indicated as **, $p<0.01$ and *, $p<0.05$ (Mann Whitney *post hoc* test), and the differences between time points as #, $p<0.05$ and ##, $p<0.01$ (Wilcoxon *post hoc* test). Note that the scale on lesion graphs differs from that on the perifocal and hippocampus graphs. (Immonen et al., 2009ab)

impact injury and cytotoxic edema (1), in the sub-acute phase 1-5 days after trauma associated with some recovery processes and vasogenic edema (2), during secondary injury cascades associated with neurodegeneration, gliosis and inflammation (and more) that become detectable around 2 weeks after trauma by quantitative MRI and show persistent progression up to 6 months (3), and in the chronic, apparently stable stage 7-12 months after trauma (4). Note again, that this time scale in rats can correspond to a period of tens of years in patients.

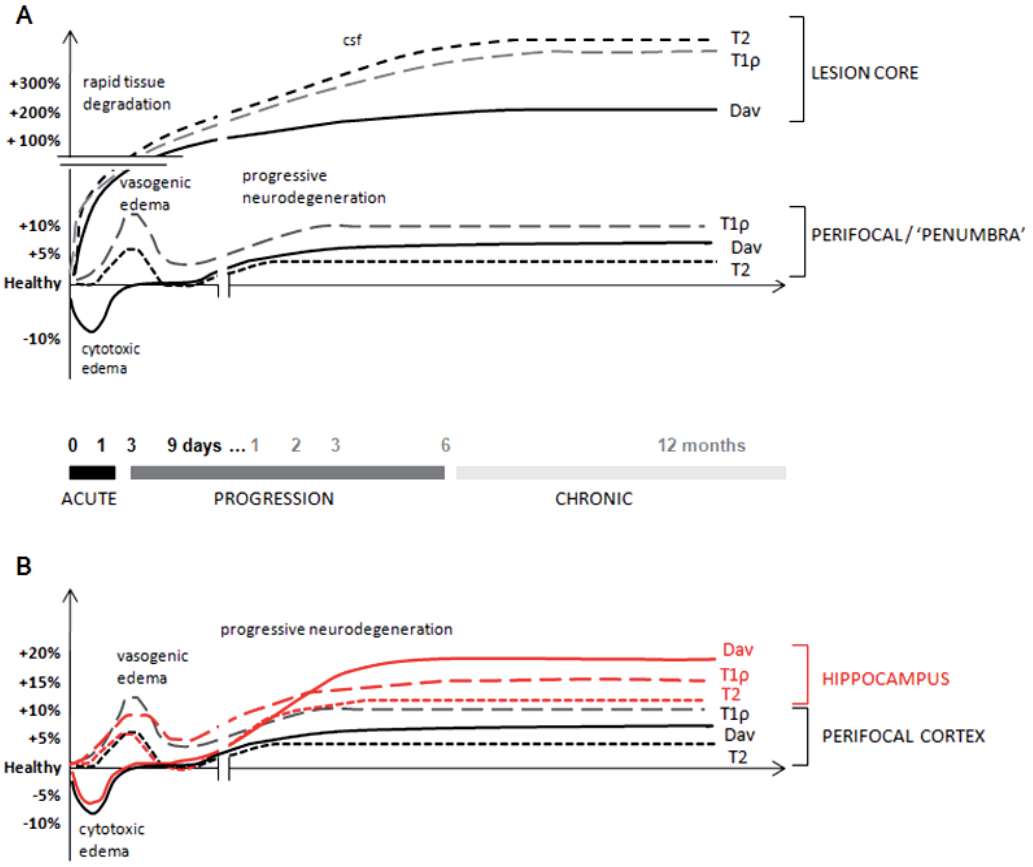


Fig. 4. Schematic graph of the temporal development of average diffusion (D_{av}), T_2 and $T_{1\rho}$ relaxation times over a 1 year period. **A)** Note the differences in both the magnitude and the temporal pattern at the lesion core (irreversible fast damage, values increase by 100-400%) compared to the perilesional region (initial fluctuating values due to transient edema, secondary increase with slow progression, potentially reversible, values increase 5-10%). **B)** When comparing the two main regions at risk: perifocal cortex and ipsilateral hippocampus, the patterns are very similar. The observation that the hippocampus displays somewhat higher secondary alterations (values increase 10-20%) than the perifocal cortical region may partly be a consequence of the perifocal ROI selection (the ROI may include some well preserved cortical tissue). In both cases, the D_{av} and $T_{1\rho}$ seem to be more sensitive than T_2, and when focusing on the time point around day 9 (edema is resolving), the only parameter that does not appear normal is $T_{1\rho}$.

Thus, quantitative MRI has the capability to probe two important characteristics of TBI. First, it is sensitive enough to detect that gray matter alterations continued for several months. While the volume of the primary focal lesion continued to expand for 3 months, the quantitative MRI observations showed progressive changes at the primary cortical lesion site for 6 months. In the perifocal region and in the hippocampus the slow secondary increase in quantitative D_{av}, T_2 and $T_{1\rho}$ continued steadily for the first 2-3 months (significant further increase in the hippocampus until 3 months and in the perifocal area until 2 months).

Secondly, the quantitative MRI is specific enough to discern the irreversible damage and tissue at risk. In the lesion site the drastic increase of all T_2, $T_{1\rho}$ and D_{av}, detectable already 3 hours or 3 days post-injury and rapidly increasing henceforth, was several fold in magnitude as compared to the more subtle alterations detected in the perilesional regions. For example, when considering the situation 9 days after injury, the observed changes at that time in the perilesional regions were maximally +8% (for $T_{1\rho}$) or even undetectable (for T_2 and D_{av}) while in the primary lesion the irreversible damage was revealed by a 70% increase in diffusion, 54% increase in T_2, and 80% increase in $T_{1\rho}$. Thereafter, the lesion values rose up to the values equal to those in CSF, that is, the tissue was absorbed and the cavity filled with CSF was generated (Fig. 4). In addition to the magnitude differences the temporal pattern of MRI changes differed between lesion and surroundings. In the primary lesion the T_2, $T_{1\rho}$ and D_{av} values simply shot up, continuing the rapid irreversible increase, while in the perilesional regions (the perifocal cortical area and the hippocampus) the values first displayed acute edema related increase, recovery, and then a delayed, slow, persistent secondary increase. The magnitude of quantitative MRI contrast parameter deviations during the delayed secondary increase remained around 10-13% for the relaxation times in the ipsilateral hippocampus (4-7% in the contralateral hippocampus and 3-9% in the perifocal area) and around 7-15% for the diffusion in the ipsilateral hippocampus (6-8% in the contralateral hippocampus and 4-6% in the perifocal area). (Immonen et al., 2009ab)

2.3 Diffuse axonal injury

After studying the visually detectable morphological and more subtle gray matter alterations post-TBI, the next major subject of interest is the white matter integrity. White matter shear injury and myelin damage can be selectively probed by MRI. One MRI technique that provides information about diffuse axonal injury (in addition to diffusion tensor imaging and magnetization transfer imaging that are not discussed here) is susceptibility weighted imaging (SWI) that has been reported to detect diffuse axonal injury associated microbleeds after TBI, detailed vascular properties, fine structure of lesions and myelin content related features (particularly in high > 3Tesla field strengths) (Haacke, 2004; Akiyama, 2009). It also distinguishes calcifications due to their diamagnetic properties. SWI utilizes the phase information of MR images in combination with magnitude information, but this phase content can be presented separately to provide phase contrast images or phase maps. This phase contrast is one hot topic in current experimental MRI method development, and even though it is easily transferable to clinical scanners, it is not yet in general use for patient care. Figure 5 shows some recent unpublished preliminary data (by Immonen) with a very robust phase mapping approach in the fluid percussion injury rat model for TBI, and Figure 6 compares the MRI magnitude and phase information to tissue myelin histochemistry of brain sections.

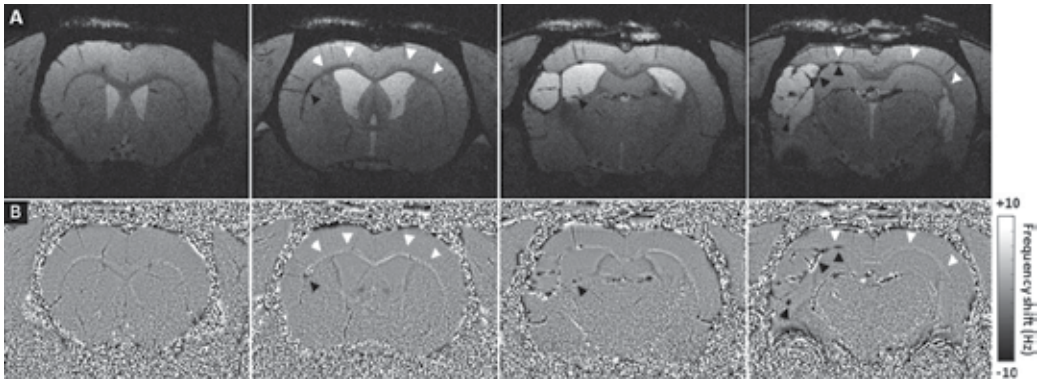


Fig. 5. A) T_2/T_{2^*} weighted magnitude images of a representative rat made one month after lateral fluid percussion injury, and B) corresponding phase maps. The contrast in the phase maps highlights the major white matter tracts (positive frequency shift, white arrowheads) and picks out dark hematomas and microbleeds (negative frequency shift, black arrowheads), while the contrast in surrounding tissue and accross ventricles appears very flat. The main magnetic field B_0 orientation is through the plane and the shown axial slices are 0.2 mm thick.

2.4 From morphology to function - Hemodynamic alterations after TBI

Autoregulation of brain perfusion is impaired after traumatic brain injury. The immediate and focal effects of the impact pressure and stretching of the vessels cause tears and BBB leakage. Blood clots can form and they may cause occlusions that seize the delivery of blood to some regions, which causes ischemia. Both the acute and sub-acute tissue edema and the later developing hydrocephalus can apply extra compression locally or through elevated intracranial pressure. The later changes in regional circulation can be beneficial if they are a controlled physiological response to the increased metabolic demands of the recovery processes. However, if the vascular system is unable to respond to these needs then the situation further deteriorates and can launch adverse cascades. The causal relations and exact mechanisms of autoregulation failure, structural tissue degradation, and functional brain injury development over the course of TBI are still largely unknown.

A valuable MRI method that probes cerebral hemodynamics non-invasively is arterial spin labeling (ASL) technique. This technique yields cerebral blood flow (CBF) maps. In ASL, no exogenous contrast agent is used because the protons in the blood-stream are labeled by an 180° inversion RF-pulse applied at a location covering the incoming vasculature upstream from the level of interest. This technique determines the perfusion rate by quantifying the labeled portion of blood that moves into/through the region of interest (Williams et al., 1992).

In severely brain injured patients three distinct cerebral hemodynamic phases have been recognized based on cerebral blood flow (CBF) changes (Martin et al., 1997): hypoperfusion (decreased CBF during the day of the injury), hyperemia (i.e. increased perfusion in the tissue capillary bed and vasculature during the next 3 days), and vasospasm that invokes a fall in the CBF that lasts for the next two weeks. Particularly, decreased CBF acutely after impact has been documented (Kelly et al., 1996; Kelly et al., 1997; Martin et al., 1997). Long term deviations from the normal CBF level have been depicted, and even patients with symptomatic mild traumatic brain injury but without any other abnormal MRI findings have shown persistent regional hypoperfusion (Bonne et al., 2003).

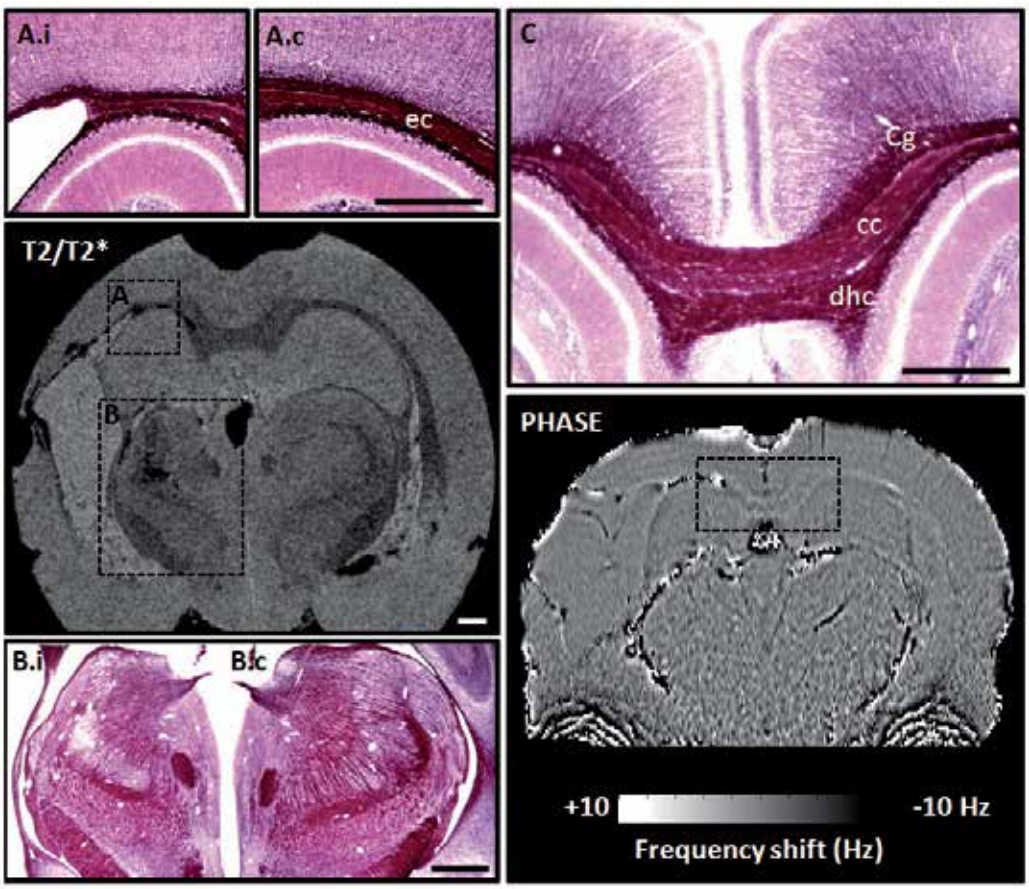


Fig. 6. Histology of the myelinated structures and how they appear in MRI: findings in a TBI rat model. T_2/T_{2^*} weighted contrast of fixed trauma brain and myelin stained brain sections of the same animal are shown on the left. The two regions selected (dashed squares on top of T_2/T_{2^*} weighted MRI) are highlighting **A**: microbleeds within white matter and associated myelin damage in corpus callosum and external capsule ipsilaterally (A.i) as compared to the contralateral corresponding region (A.c). **B**: Loss of thalamic white matter and myelin structures and formation of calcifications ipsilaterally (B.i) while no myelin pathology occurs contralaterally (B.c). The right side column of the figure shows an in vivo phase map (PHASE) of the same situation and demonstrates the differences in frequency shift between closely located white matter bundles (dashed square in phase map and corresponding region in myelin stained section (C), note the higher frequency shift in cingulum (Cg), dorsal hippocampal commissure (dhc), and external capsule (ec) as compared to the corpus callosum (cc) in between them). The phase contrast reflects the myelin content but depends also on the structure orientation in respect to the main magnetic field B_0 (here, B_0 orientation is through the plane). Abbreviations of indicated white matter bundles: Cg, cingulum; cc, corpus callosum; ec, external capsule; dhc, dorsal hippocampal commissure. Scale bar equals 1 mm.

Hemodynamic disturbances found in experimental models of TBI include local CBF decrease from 15 minutes to 4 hours post-injury close to the LFP site (Ginsberg et al., 1997; Muir et al. 1992; Ozawa et al., 1991) and transient hypoperfusion also in the contralateral hemisphere (Pasco et al., 2007). Perfusion deficits after traumatic impact can lead to a local ischemic state of the tissue where the oxygen and glucose delivery is so severely impaired that it causes disturbances in energy metabolism and mitochondrial function. Dietrich and collaborators report severe ischemia (i.e., mean local CBF < 25 ml/100g/min) after severe TBI (fluid percussion rat model) within lateral parietal cortex, moderate reductions in CBF throughout the traumatized hemisphere, including the frontal and occipital cortices, hippocampus, thalamus, and striatum, along with milder decreases in CBF also throughout the contralateral cerebral cortex. Their parallel histological studies report subarachnoid hemorrhage, blood-brain barrier (BBB) breakdown overlying the pial surface and superficial cortical layers of the injured hemisphere, focal leakage at the gray-white matter interface of the lateral cortex, petechial hemorrhages associated with small venules and focal platelet accumulation. (Dietrich et al., 1994a, 1994b, 1996, 1998).

Although the initiation mechanisms of TBI are more complex than those of ischemia the thresholds for tissue perfusion reductions and adjacent cellular consequences are common for both. The tissue in the ischemic core region suffers irreversible damage when the blood supply drops below a threshold level of around 10-15 ml/100g/min, corresponding to the anoxic cell depolarization (Hossmann and Schuier 1980), and is not restored immediately. The surrounding region, the ischemic penumbra, is hemodynamically compromised and suffers from protein synthesis inhibition [CBF between 35-55 ml/100g/min] and impaired glucose metabolism [CBF 22-35 ml/100g/min] which promotes anaerobic glycolysis, and lactic acidosis (Allen et al., 1993; Obrenovitch et al., 1988). Once the CBF declines below 20 ml/100g/min the adenosine triphosphate (ATP) levels reduce markedly and functional impairment (cessation of evoked potentials and electroencephalographic (EEG) activity) follows. The ischemic penumbra is hemodynamically defined as the region between this functional impairment and anoxic depolarisation, thus it is salvageable if the blood supply is restored acutely. The aforementioned perfusion thresholds are estimates and the factors such as the variability between measurement techniques, tissue types and animal species must be kept in mind (Baron 2001; Takasawa et al., 2008). Regarding the ischemic state of the tissue, perfusion findings are complemented by data from diffusion studies. Diffusion values reduce about 40-50% within hours after severe focal ischemia in experimental models, while thereafter some normalization of values precedes a gradual secondary increase in diffusion (Hoehn-Berlage et al., 1995). The diffusion-perfusion mismatch is a recognized marker for the ischemic penumbra. The mismatch means that the acute diffusion decrease indicates the ischemic core and the region of reduced perfusion extends further to include the penumbra (Baird et al., 1997; Finelli et al., 1992; Pierce et al., 1997; Roberts et al., 1993; Sorensen et al., 1996). Local ischemia can be one consequence of traumatic impact, due to the vascular damage at the primary contusion site or due to blood clots, but otherwise the role of perfusion and blood supply changes in traumatic head injury mechanisms are largely unknown.

Our recent studies in the rat LFPI rat model have highlighted region specific CBF alterations at acute, sub-acute and chronic time points post-injury. Blood flow alterations vary remarkably between perilesional, hippocampal, thalamic, and contralateral areas. Importantly, the animal data shows temporal developments of the autoregulation status that are much like those seen in TBI patients. The three observed phases of the

hemodynamic profile around the lesion are acute hypoperfusion, normalization, and secondary hypoperfusion. The CBF map series in Figures 7 and 8 display the regional perfusion patterns (Hayward et al., 2010).

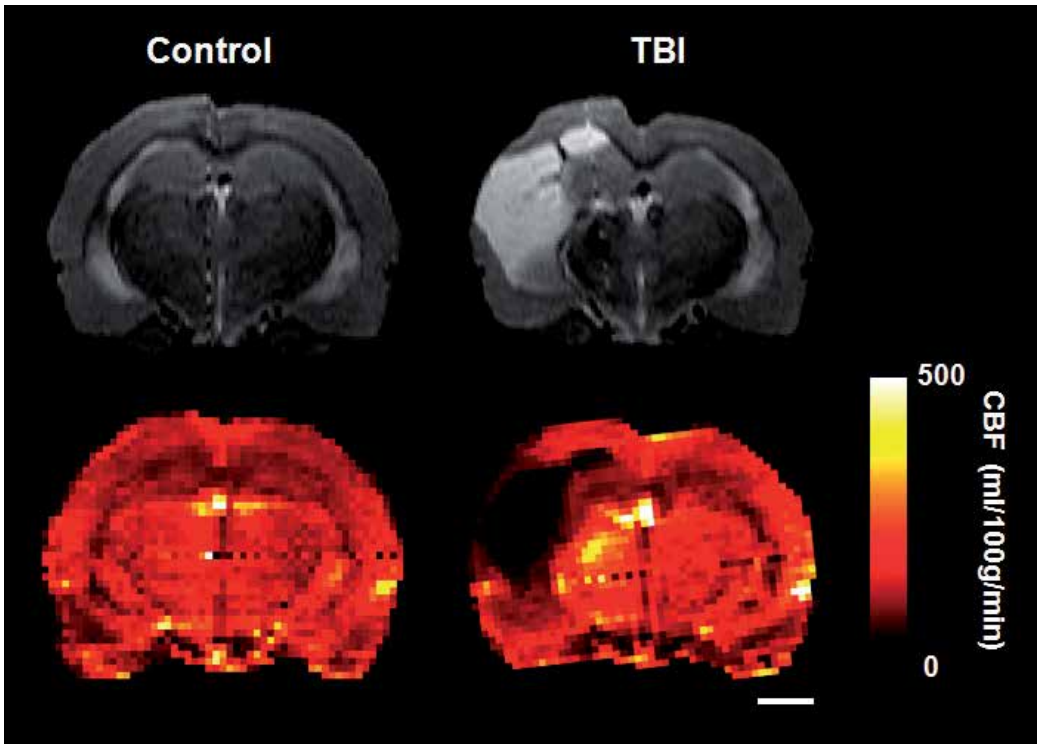


Fig. 7. T_2 weighted images (top row) and cerebral blood flow (CBF) maps measured by arterial spin labeling (bottom row) from a healthy (left) and chronic trauma animal (right, 8 months post LFPI). Note hypoperfusion in the ipsilateral cortex surrounding the cerebrospinal fluid filled cavity (lesion & hydrocephalus) and hyperperfusion in the ipsilateral thalamus in the chronic TBI rat. Scale bar equals 2 mm. (data by Hayward, see further details in Hayward et al., 2010)

It has been shown that immediately after TBI the cytotoxicity leads to increased cerebral glucose utilization while CBF remains low (Ginsberg et al, 1997; Richards et al, 2001). After TBI the decreased CBF results from vasoconstriction induced by endothelium-derived signaling (reviewed in Golding, 2002). This may explain the initial cerebral hypoperfusion we observed after experimental TBI induction. Later, cellular efforts to reestablish ionic gradients (Hovda et al, 1995) lead to hyperglycolysis (Martin et al, 1997) and this may lead to hyperemia. Furthermore, gliosis begins ~24 hours after TBI (Gehrmann et al, 1995; Graham et al, 2000), and demands energy, which may in part be the reason for the recovery of CBF observed at this time point in many regions. Thereafter, vasospasm (Martin et al, 1997) or vasoconstriction caused by delayed vasogenic edema (Rangel-Castilla et al, 2008) may lead to the second hypoperfusion phase we observed.

We compared CBF findings with the immunohistochemistry of the vascular reorganization and density alterations that may be due to post-traumatic angiogenesis (RECA-1 staining).

Each of the investigated brain areas had a unique pattern of vascular abnormalities, but they did not explain the observed MRI CBF results (Figures 7 and 8). At 8 months after TBI in the perilesional cortex, chronic hypoperfusion co-occurred with increased vascular density, while in the ipsilateral hippocampus we observed mild hypoperfusion despite no blood vessel changes. Differently again, hyperperfusion the ipsilateral thalamus was associated with a considerably increased vascular density. Although regional CBF has long been suggested to correlate positively with regional blood vessel density in healthy tissues (Gross et al., 1986), these results indicated very few links between CBF and chronic vascular reorganization after trauma. The fact that we found that the lower the CBF in the perilesional cortex, the higher the vascular density implies that cortical angiogenesis after TBI may not provide new vessels with sufficient vascular integrity to recover the chronic perfusion deficit. Due to the mixed cerebrovascular results from the thalamus and hippocampus, chronic alterations in CBF 8 months post-injury could not be attributed to changes in vascular density.

We have found even fewer links between vascular reorganization and CBF in acute and sub-acute phases after TBI. We reported an acute loss of blood vessel density and subsequent increase between 6 hours and 2 weeks after TBI in the stratum oriens and perilesional cortex. In the perilesional cortex, CBF measures did not correlate with vessel density and it is possible that newly formed vessels may not be fully functional at 2 weeks after TBI. A recent immunohistochemistry study by Park and colleagues (Park et al., 2009) demonstrated cortical blood vessel loss 24 hours after LFPI, which recovered by 2 weeks in moderately injured rats but not in severely injured rats (the TBI rats in Hayward's study all have severe injury). We also observed hypoperfusion in regions without significant vessel loss, such as the thalamus.

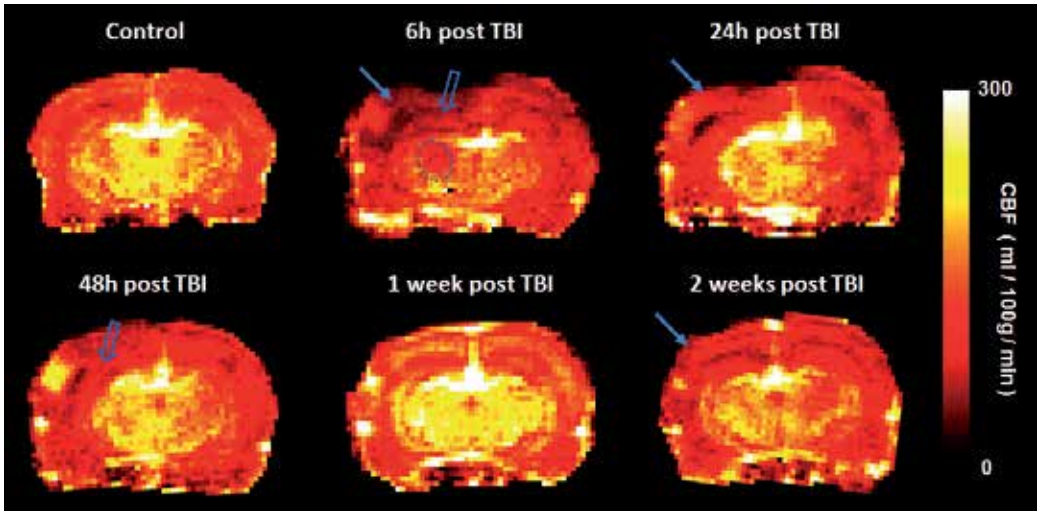


Fig. 8. CBF maps of a control rat (top left) and time series of a representative TBI rat during 2 weeks follow-up after LFPI induction. In the perilesional cortex around the lesion, the flow alterations over time have three phases: acute decrease to 54% of the CBF in controls, recovery, and secondary decrease to 75% of control measures (solid arrows). The ipsilateral hippocampal (open arrows) CBF changes show a similar pattern, but secondary hypoperfusion appears sooner at 48 h. The ipsilateral thalamus suffers from acute hypoperfusion at 6 hours after injury (dashed oval) and displays hyperperfusion 2 weeks later in its subregions. (data by Hayward, see further details in Hayward et al., 2011)

3. Correlation of MRI findings with cognitive deficits and hyperexcitability: can early MRI predict behavioral outcome?

It is of course essential to understand the cellular changes and histologically determined correlates that underlie the observed MRI abnormalities. But equally or even more important is the ability to use MRI as a direct predictor or marker for brain functionality. The morphological MRI findings, alterations in quantitative MR relaxation and diffusion properties and cerebral blood flow changes could all potentially have a role in predicting long-term TBI outcomes such as cognitive impairment, motor deficits or the development of epilepsy. The chance of head injury patients presenting with epilepsy later in life is 16% after moderate brain injury and may be as high as 53% after ballistics or blast injuries (Lowenstein 2009).

In clinical head trauma studies, intracranial and intracerebral hemorrhages have been found to be associated with poor long-term outcome, as assessed with the Glasgow Outcome Scale (Chieregato et al., 2005). Increased diffusivity of white matter structures has been reported to correlate with later impairment of learning and memory functions (Salmond et al., 2006; Sidaros et al., 2008). Magnetization transfer ratio decrease in the corpus callosum and abnormal fractional anisotropy values in several major white matter tracts (obtained by techniques called magnetization transfer imaging and diffusion tensor imaging, respectively) are used to estimate the myelin loss and axonal injury. The severity of these DAI related changes have been found to correlate with cognitive recovery in mild TBI (Belanger et al., 2007). Herniation, due to a swollen ipsilateral hemisphere can be seen as midline shift in MRI and it has been listed as a risk factor for human post traumatic epilepsy (Pitkänen et al., 2011). Metting and colleagues reviewed the early MRI findings with predictive values for chronic outcome in mild-to-moderate head injury (defined by having Glasgow Coma Score > 8) (Metting et al., 2007). The list included the number of lesions (detected by a T_2* weighted gradient echo technique), lesion size, perfusion abnormalities and reduced cerebral blood volume observed days to weeks (< 3 weeks) post-injury. These early findings could be linked to outcome at 3 to 12 months later.

In experimental research, many of the factors contributing to long term outcome after brain injury can be controlled for and monitored. Our follow-up studies in the LFPI rat model have yielded many potential MRI surrogate markers for neurodegeneration, memory deficits and epileptogenesis. As outcome measures, the final atrophy extent 6 months post-injury, spatial memory and learning ability tested in Morris Water maze 7 months post-injury, and histopathological neuronal loss 12 months post-injury were assessed. At the chronic phase after TBI the animals displayed largely expanded lesion cavities, the ipsilateral hippocampal volume was decreased by 21%, the number of hilar neurons was decreased by 63% and the hippocampus related learning and memory performance was impaired in trauma animals. Non-invasive MRI was performed frequently over the course of disease progression. The lesion volume measured and the amount of intracerebral hemorrhage in the acute (3 hours) and sub-acute (3-23 days) phases after injury correlated with the final lesion extent 6 months later. The $T_{1\rho}$ increase in the perifocal area surrounding the lesion at 9 days post-injury correlated with the final cortical lesion volume. The edema related transient increase in $T_{1\rho}$ and T_2 in the ipsilateral hippocampus 3 days post-injury correlated with the chronic volume reduction of the ipsilateral hippocampus and with the hilar neuron loss (in the dentate gyrus of the hippocampus) - and so did the $T_{1\rho}$ increase 9 days post-injury. The delayed secondary increase 23 days post-injury in $T_{1\rho}$, T_2 and D_{av} in

the ipsilateral hippocampus, which was already an indicator of the severity of the secondary injury cascades, correlated with the later hippocampal volume decrease at 6 months and with the final hilar neuron loss. This same delayed diffusion increase (in the ipsilateral hippocampus 23 days post-injury) correlated also with the long-term learning impairment 7 months post-injury. Also, the severity of hemorrhage at the sub acute phase was indicative of long-term learning impairment. See the behavioural test and correlation details in Immonen et al., 2009a. Upon assessing the risk for posttraumatic epilepsy, we have shown that acute diffusion drop 3 hours post lateral fluid percussion injury has predictive value for seizure susceptibility one year later (Kharatishvili et al., 2007).

The causal relationship between hemodynamic alterations and behavioral symptoms is still under investigation and the studies presented here, using arterial spin labeling technique for CBF mapping (Hayward et al., 2010), touched the surface by showing that enhanced seizure susceptibility post TBI was associated with reduced CBF in the ipsilateral hippocampus ($r = 0.78$, $p < 0.05$) and increased vascular density in the thalamus ($r = 0.69$, $p < 0.05$). Although the perilesional cortex may generate ictal activity after LFPI (D'Ambrosio et al., 2004, 2005, Kharatishvili et al. 2006), we did not find any association between the hypoperfusion or increased vascular density in the perilesional cortex and seizure susceptibility. This might be because injury to more caudal cortical areas associates with hyperexcitability, while the cortical regions close to the lesion may not be involved (Kharatishvili and Pitkänen 2010a). More importantly, we found that a reduction in the ipsilateral hippocampal CBF at 8 months after TBI was associated with increased seizure susceptibility at 9 months such that the lower the CBF, the higher the seizure susceptibility. Circuitry alterations and cerebrovascular responses in the hippocampus are common in experimental epilepsy and in epilepsy patients (Pitkänen and Lukasiuk 2009, Ndode-Ekane et al. 2010) and the ipsilateral hippocampus is involved in electrographic activity during spontaneous seizures after TBI (Kharatishvili et al. 2006). Indeed, previous reports have used ASL together with other neuroimaging techniques to delineate the ictogenic zone (Rougier et al. 1999, Lim et al. 2008). Further studies are needed, however, to discern whether chronic hippocampal hypoperfusion after TBI can be used as a surrogate marker for epileptogenesis.

Our study did not yield any correlations between CBF changes and memory and learning impairment, but poor performance in the Morris water maze was found to correlate with enhanced thalamic vessel density ($r = -0.81$, $p < 0.01$). The thalamus is often found to be damaged in moderate and severely injured TBI patients as well as in animal models (Pierce et al. 1998, Maxwell et al. 2004, 2006, Tollard et al. 2009, Little et al. 2010), but the role of the thalamic pathology in epileptogenesis after acquired etiologies like TBI is poorly understood (Bonilha et al. 2004, Blumenfeld et al. 2009). We also found that a high vessel density in the ipsilateral thalamus was associated with increased CBF and enhanced seizure susceptibility in injured rats. A recent clinical report measured increased thalamic CBF during secondary generalization of focal onset seizures (Blumenfeld et al. 2009). Still, one must be cautious with the interpretation of such clinical and preclinical correlations and keep in mind that in most of the cases it is still unclear whether the cerebrovascular sequelae is the cause or the consequence of abnormal neural activity.

4. Conclusions

This chapter introduced several different MRI techniques and how each of them aid in probing TBI pathology. Techniques target features at the micro- and macro size scales, gray

matter pathology, white matter injury, and hemodynamic disturbances. Non-invasive MRI has been demonstrated to be a highly sensitive and valuable tool in progressive brain disease research and care. The temporal pattern and magnitude of measured T_2, $T_{1\rho}$, D_{av}, and CBF changes varied substantially between brain regions highlighting how quantitative MRI can be used to differentiate the regions with risk of delayed secondary damage from those of continuously progressing irreversible damage or from the normal areas. Importantly, quantitative MRI can also identify how advanced the pathological processes in each region are, which is a major advantage in targeting treatments and following the treatment response.

Considering the prognostic value of early MRI findings in evaluating long term outcomes, many of the promising singular MRI findings and their correlations with outcome are lacking in specificity, and thus we must continue to search for more robust MRI markers. In particular, the multimodal approach and utilization of a combination of different MRI parameters for more sensitive and specific prediction paradigm is on the rise. A series of reviews emphasized the need for predictive markers (Belanger et al., 2007; Catroppa et al., 2008; Gallagher, Hutchinson, Pickard 2007; Kharatishvili and Pitkänen 2010b, Lewine et al., 2007; Metting et al., 2007, Pitkänen et al., 2009a, 2009b, 2011).

The MRI techniques discussed in this chapter are only a fraction of all available non-invasive MRI 'probes'. Furthermore, there is a wealth of information about the metabolic alterations in TBI accessible by variety of MR spectroscopy techniques.

5. References

Akiyama, Y., Miyata, K., Harada, K., Minamida, Y., Nonaka, T., Koyanagi, I., et al. (2009). Susceptibility-weighted magnetic resonance imaging for the detection of cerebral microhemorrhage in patients with traumatic brain injury. *Neurologia Medico-Chirurgica, 49*(3), 97-9; discussion 99.

Albensi, B. C., Knoblach, S. M., Chew, B. G., O'Reilly, M. P., Faden, A. I., & Pekar, J. J. (2000). Diffusion and high resolution MRI of traumatic brain injury in rats: Time course and correlation with histology. *Experimental Neurology, 162*(1), 61-72.

Allen, K. L., Busza, A. L., Proctor, E., King, M. D., Williams, S. R., Crockard, H. A., et al. (1993). Controllable graded cerebral ischaemia in the gerbil: Studies of cerebral blood flow and energy metabolism by hydrogen clearance and 31P NMR spectroscopy. *NMR in Biomedicine, 6*(3), 181-186.

Alsop, D. C., Murai, H., Detre, J. A., McIntosh, T. K., & Smith, D. H. (1996). Detection of acute pathologic changes following experimental traumatic brain injury using diffusion-weighted magnetic resonance imaging. *Journal of Neurotrauma, 13*(9), 515-521.

Bagley, L. J., McGowan, J. C., Grossman, R. I., Sinson, G., Kotapka, M., Lexa, F. J., et al. (2000). Magnetization transfer imaging of traumatic brain injury. *Journal of Magnetic Resonance Imaging , 11*(1), 1-8.

Baird, A. E., Benfield, A., Schlaug, G., Siewert, B., Lovblad, K. O., Edelman, R. R., et al. (1997). Enlargement of human cerebral ischemic lesion volumes measured by diffusion-weighted magnetic resonance imaging. *Annals of Neurology, 41*(5), 581-589.

Baron, J. C. (2001). Perfusion thresholds in human cerebral ischemia: Historical perspective and therapeutic implications. *Cerebrovascular Diseases , 11 Suppl 1*, 2-8.

Belanger, H. G., Vanderploeg, R. D., Curtiss, G., & Warden, D. L. (2007). Recent neuroimaging techniques in mild traumatic brain injury. *The Journal of Neuropsychiatry and Clinical Neurosciences, 19*(1), 5-20.

Blumenfeld, H., Varghese, G. I., Purcaro, M. J., Motelow, J. E., Enev, M., McNally, K. A., et al. (2009). Cortical and subcortical networks in human secondarily generalized tonic-clonic seizures. *Brain, 132*(Pt 4), 999-1012.

Bonilha, L., Rorden, C., Castellano, G., Pereira, F., Rio, P. A., Cendes, F., et al. (2004). Voxel-based morphometry reveals gray matter network atrophy in refractory medial temporal lobe epilepsy. *Archives of Neurology, 61*(9), 1379-1384.

Bonne, O., Gilboa, A., Louzoun, Y., Kempf-Sherf, O., Katz, M., Fishman, Y., et al. (2003). Cerebral blood flow in chronic symptomatic mild traumatic brain injury. *Psychiatry Research, 124*(3), 141-152.

Bramlett, H. M., Kraydieh, S., Green, E. J., & Dietrich, W. D. (1997). Temporal and regional patterns of axonal damage following traumatic brain injury: A beta-amyloid precursor protein immunocytochemical study in rats. *Journal of Neuropathology and Experimental Neurology, 56*(10), 1132-1141.

Caroli, M., Locatelli, M., Campanella, R., Balbi, S., Martinelli, F., & Arienta, C. (2001). Multiple intracranial lesions in head injury: Clinical considerations, prognostic factors, management, and results in 95 patients. *Surgical Neurology, 56*(2), 82-88.

Chieregato, A., Fainardi, E., Morselli-Labate, A. M., Antonelli, V., Compagnone, C., Targa, L., et al. (2005). Factors associated with neurological outcome and lesion progression in traumatic subarachnoid hemorrhage patients. *Neurosurgery, 56*(4), 671-80; discussion 671-80.

Conti, A. C., Raghupathi, R., Trojanowski, J. Q., & McIntosh, T. K. (1998). Experimental brain injury induces regionally distinct apoptosis during the acute and delayed post-traumatic period. *The Journal of Neuroscience, 18*(15), 5663-5672.

Cortez, S. C., McIntosh, T. K., & Noble, L. J. (1989). Experimental fluid percussion brain injury: Vascular disruption and neuronal and glial alterations. *Brain Research, 482*(2), 271-282.

D'Ambrosio, R., Fender, J. S., Fairbanks, J. P., Simon, E. A., Born, D. E., Doyle, D. L., et al. (2005). Progression from frontal-parietal to mesial-temporal epilepsy after fluid percussion injury in the rat. *Brain, 128*(Pt 1), 174-188.

D'Ambrosio, R., & Perucca, E. (2004). Epilepsy after head injury. *Current Opinion in Neurology, 17*(6), 731-735.

Dietrich, W. D., Alonso, O., Busto, R., & Ginsberg, M. D. (1994a). Widespread metabolic depression and reduced somatosensory circuit activation following traumatic brain injury in rats. *Journal of Neurotrauma, 11*(6), 629-640.

Dietrich, W. D., Alonso, O., & Halley, M. (1994b). Early microvascular and neuronal consequences of traumatic brain injury: A light and electron microscopic study in rats. *Journal of Neurotrauma, 11*(3), 289-301.

Dietrich, W. D., Alonso, O., Busto, R., Prado, R., Dewanjee, S., Dewanjee, M. K., et al. (1996). Widespread hemodynamic depression and focal platelet accumulation after fluid percussion brain injury: A double-label autoradiographic study in rats. *Journal of Cerebral Blood Flow and Metabolism, 16*(3), 481-489.

Dietrich, W. D., Alonso, O., Busto, R., Prado, R., Zhao, W., Dewanjee, M. K., et al. (1998). Posttraumatic cerebral ischemia after fluid percussion brain injury: An

autoradiographic and histopathological study in rats. *Neurosurgery, 43*(3), 585-93; discussion 593-4.

Dube, C., Boyet, S., Marescaux, C., & Nehlig, A. (2001). Relationship between neuronal loss and interictal glucose metabolism during the chronic phase of the lithium-pilocarpine model of epilepsy in the immature and adult rat. *Experimental Neurology, 167*(2), 227-241.

Dunn, J. F., Roche, M. A., Springett, R., Abajian, M., Merlis, J., Daghlian, C. P., et al. (2004). Monitoring angiogenesis in brain using steady-state quantification of DeltaR2 with MION infusion. *Magnetic Resonance in Medicine, 51*(1), 55-61.

Faden, A. I., Demediuk, P., Panter, S. S., & Vink, R. (1989). The role of excitatory amino acids and NMDA receptors in traumatic brain injury. *Science, 244*(4906), 798-800.

Finelli, D. A., Hopkins, A. L., Selman, W. R., Crumrine, R. C., Bhatti, S. U., & Lust, W. D. (1992). Evaluation of experimental early acute cerebral ischemia before the development of edema: Use of dynamic, contrast-enhanced and diffusion-weighted MR scanning. *Magnetic Resonance in Medicine, 27*(1), 189-197.

Gehrmann, J., Banati, R. B., Wiessner, C., Hossmann, K. A., & Kreutzberg, G. W. (1995). Reactive microglia in cerebral ischaemia: An early mediator of tissue damage? *Neuropathology and Applied Neurobiology, 21*(4), 277-289.

Ginsberg, M. D., Zhao, W., Alonso, O. F., Loor-Estades, J. Y., Dietrich, W. D., & Busto, R. (1997). Uncoupling of local cerebral glucose metabolism and blood flow after acute fluid-percussion injury in rats. *The American Journal of Physiology, 272*(6 Pt 2), H2859-68.

Golding, E. M. (2002). Sequelae following traumatic brain injury. the cerebrovascular perspective. *Brain Research. Brain Research Reviews, 38*(3), 377-388.

Graham, D. I., McIntosh, T. K., Maxwell, W. L., & Nicoll, J. A. (2000a). Recent advances in neurotrauma. *Journal of Neuropathology and Experimental Neurology, 59*(8), 641-651.

Graham, D. I., Raghupathi, R., Saatman, K. E., Meaney, D., & McIntosh, T. K. (2000b). Tissue tears in the white matter after lateral fluid percussion brain injury in the rat: Relevance to human brain injury. *Acta Neuropathologica, 99*(2), 117-124.

Gross, P. M., Sposito, N. M., Pettersen, S. E., & Fenstermacher, J. D. (1986). Differences in function and structure of the capillary endothelium in gray matter, white matter and a circumventricular organ of rat brain. *Blood Vessels, 23*(6), 261-270.

Haacke, E. M., Xu, Y., Cheng, Y. C., & Reichenbach, J. R. (2004). Susceptibility weighted imaging (SWI). *Magnetic Resonance in Medicine, 52*(3), 612-618.

Hallam, T. M., Floyd, C. L., Folkerts, M. M., Lee, L. L., Gong, Q. Z., Lyeth, B. G., et al. (2004). Comparison of behavioral deficits and acute neuronal degeneration in rat lateral fluid percussion and weight-drop brain injury models. *Journal of Neurotrauma, 21*(5), 521-539.

Hayward, N. M., Immonen, R., Tuunanen, P. I., Ndode-Ekane, X. E., Grohn, O., & Pitkänen, A. (2010). Association of chronic vascular changes with functional outcome after traumatic brain injury in rats. *Journal of Neurotrauma, 27*(12), 2203-2219.

Hayward, N. M., Tuunanen, P. I., Immonen, R., Ndode-Ekane, X. E., Pitkänen, A., & Grohn, O. (2011). Magnetic resonance imaging of regional hemodynamic and cerebrovascular recovery after lateral fluid-percussion brain injury in rats. *Journal of Cerebral Blood Flow and Metabolism*, *31*(1), 166-177.

Hoehn-Berlage, M., Norris, D. G., Kohno, K., Mies, G., Leibfritz, D., & Hossmann, K. A. (1995). Evolution of regional changes in apparent diffusion coefficient during focal ischemia of rat brain: The relationship of quantitative diffusion NMR imaging to reduction in cerebral blood flow and metabolic disturbances. *Journal of Cerebral Blood Flow and Metabolism, 15*(6), 1002-1011.

Hossmann, K. A., & Schuier, F. J. (1980). Experimental brain infarcts in cats. I. pathophysiological observations. *Stroke, 11*(6), 583-592.

Hovda, D. A., Lee, S. M., Smith, M. L., Von Stuck, S., Bergsneider, M., Kelly, D., et al. (1995). The neurochemical and metabolic cascade following brain injury: Moving from animal models to man. *Journal of Neurotrauma, 12*(5), 903-906.

Hunt, R. F., Scheff, S. W., & Smith, B. N. (2011). Synaptic reorganization of inhibitory hilar interneuron circuitry after traumatic brain injury in mice. *The Journal of Neuroscience, 31*(18), 6880-6890.

Immonen, R. J., Kharatishvili, I., Grohn, H., Pitkänen, A., & Grohn, O. H. (2009a). Quantitative MRI predicts long-term structural and functional outcome after experimental traumatic brain injury. *NeuroImage, 45*(1), 1-9.

Immonen, R. J., Kharatishvili, I., Niskanen, J. P., Grohn, H., Pitkänen, A., & Grohn, O. H. (2009b). Distinct MRI pattern in lesional and perilesional area after traumatic brain injury in rat--11 months follow-up. *Experimental Neurology, 215*(1), 29-40.

Iwamoto, Y., Yamaki, T., Murakami, N., Umeda, M., Tanaka, C., Higuchi, T., et al. (1997). Investigation of morphological change of lateral and midline fluid percussion injury in rats, using magnetic resonance imaging. *Neurosurgery, 40*(1), 163-167.

Kelly, D. F., Kordestani, R. K., Martin, N. A., Nguyen, T., Hovda, D. A., Bergsneider, M., et al. (1996). Hyperemia following traumatic brain injury: Relationship to intracranial hypertension and outcome. *Journal of Neurosurgery, 85*(5), 762-771.

Kelly, D. F., Martin, N. A., Kordestani, R., Counelis, G., Hovda, D. A., Bergsneider, M., et al. (1997). Cerebral blood flow as a predictor of outcome following traumatic brain injury. *Journal of Neurosurgery, 86*(4), 633-641.

Kharatishvili, I., Nissinen, J. P., McIntosh, T. K., & Pitkänen, A. (2006). A model of posttraumatic epilepsy induced by lateral fluid-percussion brain injury in rats. *Neuroscience, 140*(2), 685-697.

Kharatishvili, I., Immonen, R., Grohn, O., & Pitkänen, A. (2007). Quantitative diffusion MRI of hippocampus as a surrogate marker for post-traumatic epileptogenesis. *Brain, 130*(Pt 12), 3155-3168.

Kharatishvili, I., & Pitkänen, A. (2010a). Association of the severity of cortical damage with the occurrence of spontaneous seizures and hyperexcitability in an animal model of posttraumatic epilepsy. *Epilepsy Research, 90*(1-2), 47-59.

Kharatishvili, I., & Pitkänen, A. (2010b). Posttraumatic epilepsy. *Current Opinion in Neurology, 23*(2), 183-188.

Kurth, S. M., Bigler, E. D., & Blatter, D. D. (1994). Neuropsychological outcome and quantitative image analysis of acute haemorrhage in traumatic brain injury: Preliminary findings. *Brain Injury, 8*(6), 489-500.

Lenzlinger, P. M., Morganti-Kossmann, M. C., Laurer, H. L., & McIntosh, T. K. (2001). The duality of the inflammatory response to traumatic brain injury. *Molecular Neurobiology, 24*(1-3), 169-181.

Lim, Y. M., Cho, Y. W., Shamim, S., Solomon, J., Birn, R., Luh, W. M., et al. (2008). Usefulness of pulsed arterial spin labeling MR imaging in mesial temporal lobe epilepsy. *Epilepsy Research, 82*(2-3), 183-189.

Little, D. M., Kraus, M. F., Joseph, J., Geary, E. K., Susmaras, T., Zhou, X. J., et al. (2010). Thalamic integrity underlies executive dysfunction in traumatic brain injury. *Neurology, 74*(7), 558-564.

Lowenstein, D. H. (2009). Epilepsy after head injury: An overview. *Epilepsia, 50 Suppl 2*, 4-9.

Lowenstein, D. H., Thomas, M. J., Smith, D. H., & McIntosh, T. K. (1992). Selective vulnerability of dentate hilar neurons following traumatic brain injury: A potential mechanistic link between head trauma and disorders of the hippocampus. *The Journal of Neuroscience, 12*(12), 4846-4853.

Mac Donald, C. L., Dikranian, K., Song, S. K., Bayly, P. V., Holtzman, D. M., & Brody, D. L. (2007). Detection of traumatic axonal injury with diffusion tensor imaging in a mouse model of traumatic brain injury. *Experimental Neurology, 205*(1), 116-131.

Martin, N. A., Patwardhan, R. V., Alexander, M. J., Africk, C. Z., Lee, J. H., Shalmon, E., et al. (1997). Characterization of cerebral hemodynamic phases following severe head trauma: Hypoperfusion, hyperemia, and vasospasm. *Journal of Neurosurgery, 87*(1), 9-19.

Maxwell, W. L., Pennington, K., MacKinnon, M. A., Smith, D. H., McIntosh, T. K., Wilson, J. T., et al. (2004). Differential responses in three thalamic nuclei in moderately disabled, severely disabled and vegetative patients after blunt head injury. *Brain, 127*(Pt 11), 2470-2478.

Maxwell, W. L., MacKinnon, M. A., Smith, D. H., McIntosh, T. K., & Graham, D. I. (2006). Thalamic nuclei after human blunt head injury. *Journal of Neuropathology and Experimental Neurology, 65*(5), 478-488.

McGowan, J. C., McCormack, T. M., Grossman, R. I., Mendonca, R., Chen, X. H., Berlin, J. A., et al. (1999). Diffuse axonal pathology detected with magnetization transfer imaging following brain injury in the pig. *Magnetic Resonance in Medicine, 41*(4), 727-733.

McIntosh, T. K. (1994). Neurochemical sequelae of traumatic brain injury: Therapeutic implications. *Cerebrovascular and Brain Metabolism Reviews, 6*(2), 109-162.

McIntosh, T. K., Smith, D. H., Meaney, D. F., Kotapka, M. J., Gennarelli, T. A., & Graham, D. I. (1996). Neuropathological sequelae of traumatic brain injury: Relationship to neurochemical and biomechanical mechanisms. *Laboratory Investigation, 74*(2), 315-342.

McIntosh, T. K., Vink, R., Noble, L., Yamakami, I., Fernyak, S., Soares, H., et al. (1989). Traumatic brain injury in the rat: Characterization of a lateral fluid-percussion model. *Neuroscience, 28*(1), 233-244.

Metting, Z., Rodiger, L. A., De Keyser, J., & van der Naalt, J. (2007). Structural and functional neuroimaging in mild-to-moderate head injury. *Lancet Neurology, 6*(8), 699-710.

Morais, D. F., Spotti, A. R., Tognola, W. A., Gaia, F. F., & Andrade, A. F. (2008). Clinical application of magnetic resonance in acute traumatic brain injury. *Arquivos De Neuro-Psiquiatria, 66*(1), 53-58.

Morganti-Kossmann, M. C., Rancan, M., Stahel, P. F., & Kossmann, T. (2002). Inflammatory response in acute traumatic brain injury: A double-edged sword. *Current Opinion in Critical Care, 8*(2), 101-105.

Moseley, M. E., Cohen, Y., Mintorovitch, J., Chileuitt, L., Shimizu, H., Kucharczyk, J., et al. (1990). Early detection of regional cerebral ischemia in cats: Comparison of diffusion- and T2-weighted MRI and spectroscopy. *Magnetic Resonance in Medicine, 14*(2), 330-346.

Muir, J. K., Boerschel, M., & Ellis, E. F. (1992). Continuous monitoring of posttraumatic cerebral blood flow using laser-doppler flowmetry. *Journal of Neurotrauma, 9*(4), 355-362.

Ndode-Ekane, X. E., Hayward, N., Grohn, O., & Pitkänen, A. (2010). Vascular changes in epilepsy: Functional consequences and association with network plasticity in pilocarpine-induced experimental epilepsy. *Neuroscience, 166*(1), 312-332.

Obenaus, A., Robbins, M., Blanco, G., Galloway, N. R., Snissarenko, E., Gillard, E., et al. (2007). Multi-modal magnetic resonance imaging alterations in two rat models of mild neurotrauma. *Journal of Neurotrauma, 24*(7), 1147-1160.

Obrenovitch, T. P., Garofalo, O., Harris, R. J., Bordi, L., Ono, M., Momma, F., et al. (1988). Brain tissue concentrations of ATP, phosphocreatine, lactate, and tissue pH in relation to reduced cerebral blood flow following experimental acute middle cerebral artery occlusion. *Journal of Cerebral Blood Flow and Metabolism*, *8*(6), 866-874.

Onyszchuk, G., Al-Hafez, B., He, Y. Y., Bilgen, M., Berman, N. E., & Brooks, W. M. (2007). A mouse model of sensorimotor controlled cortical impact: Characterization using longitudinal magnetic resonance imaging, behavioral assessments and histology. *Journal of Neuroscience Methods, 160*(2), 187-196.

Ozawa, Y., Nakamura, T., Sunami, K., Kubota, M., Ito, C., Murai, H., et al. (1991). Study of regional cerebral blood flow in experimental head injury: Changes following cerebral contusion and during spreading depression. *Neurologia Medico-Chirurgica, 31*(11), 685-690.

Park, E., Bell, J. D., Siddiq, I. P., & Baker, A. J. (2009). An analysis of regional microvascular loss and recovery following two grades of fluid percussion trauma: A role for hypoxia-inducible factors in traumatic brain injury. *Journal of Cerebral Blood Flow and Metabolism, 29*(3), 575-584.

Pasco, A., Lemaire, L., Franconi, F., Lefur, Y., Noury, F., Saint-Andre, J. P., et al. (2007). Perfusional deficit and the dynamics of cerebral edemas in experimental traumatic brain injury using perfusion and diffusion-weighted magnetic resonance imaging. *Journal of Neurotrauma, 24*(8), 1321-1330.

Pierce, A. R., Lo, E. H., Mandeville, J. B., Gonzalez, R. G., Rosen, B. R., & Wolf, G. L. (1997). MRI measurements of water diffusion and cerebral perfusion: Their relationship in a rat model of focal cerebral ischemia. *Journal of Cerebral Blood Flow and Metabolism*, *17*(2), 183-190.

Pierce, J. E., Smith, D. H., Trojanowski, J. Q., & McIntosh, T. K. (1998). Enduring cognitive, neurobehavioral and histopathological changes persist for up to one year following severe experimental brain injury in rats. *Neuroscience, 87*(2), 359-369.

Pitkänen, A., Immonen, R. J., Grohn, O. H., & Kharatishvili, I. (2009a). From traumatic brain injury to posttraumatic epilepsy: What animal models tell us about the process and treatment options. *Epilepsia, 50 Suppl 2*, 21-29.

Pitkänen, A., & Lukasiuk, K. (2009b). Molecular and cellular basis of epileptogenesis in symptomatic epilepsy. *Epilepsy & Behavior, 14 Suppl 1*, 16-25.

Pitkänen, A., Bolkvadze, T., & Immonen, R. (2011). Anti-epileptogenesis in rodent post-traumatic epilepsy models. *Neuroscience Letters, 497*(3), 163-171.

Raghupathi, R., Conti, A. C., Graham, D. I., Krajewski, S., Reed, J. C., Grady, M. S., et al. (2002). Mild traumatic brain injury induces apoptotic cell death in the cortex that is preceded by decreases in cellular bcl-2 immunoreactivity. *Neuroscience, 110*(4), 605-616.

Rangel-Castilla, L., Gasco, J., Nauta, H. J., Okonkwo, D. O., & Robertson, C. S. (2008). Cerebral pressure autoregulation in traumatic brain injury. *Neurosurgical Focus, 25*(4), E7.

Richards, H. K., Simac, S., Piechnik, S., & Pickard, J. D. (2001). Uncoupling of cerebral blood flow and metabolism after cerebral contusion in the rat. *Journal of Cerebral Blood Flow and Metabolism, 21*(7), 779-781.

Rink, A., Fung, K. M., Trojanowski, J. Q., Lee, V. M., Neugebauer, E., & McIntosh, T. K. (1995). Evidence of apoptotic cell death after experimental traumatic brain injury in the rat. *The American Journal of Pathology, 147*(6), 1575-1583.

Roberts, T. P., Vexler, Z., Derugin, N., Moseley, M. E., & Kucharczyk, J. (1993). High-speed MR imaging of ischemic brain injury following stenosis of the middle cerebral artery. *Journal of Cerebral Blood Flow and Metabolism*, *13*(6), 940-946.

Rougier, A., Lurton, D., El Bahh, B., Lespinet, V., Bidabe, A. M., Guillot, M., et al. (1999). Bilateral decrease in interictal hippocampal blood flow in unilateral mesiotemporal epilepsy. *Journal of Neurosurgery, 90*(2), 282-288.

Salmond, C. H., Menon, D. K., Chatfield, D. A., Williams, G. B., Pena, A., Sahakian, B. J., et al. (2006). Diffusion tensor imaging in chronic head injury survivors: Correlations with learning and memory indices. *NeuroImage, 29*(1), 117-124.

Sato, M., Chang, E., Igarashi, T., & Noble, L. J. (2001). Neuronal injury and loss after traumatic brain injury: Time course and regional variability. *Brain Research, 917*(1), 45-54.

Scheid, R., Ott, D. V., Roth, H., Schroeter, M. L., & von Cramon, D. Y. (2007). Comparative magnetic resonance imaging at 1.5 and 3 tesla for the evaluation of traumatic microbleeds. *Journal of Neurotrauma, 24*(12), 1811-1816.

Schouten, J. W., Fulp, C. T., Royo, N. C., Saatman, K. E., Watson, D. J., Snyder, E. Y., et al. (2004). A review and rationale for the use of cellular transplantation as a therapeutic strategy for traumatic brain injury. *Journal of Neurotrauma, 21*(11), 1501-1538.

Schutz, C., Stover, J. F., Thompson, H. J., Hoover, R. C., Morales, D. M., Schouten, J. W., et al. (2006). Acute, transient hemorrhagic hypotension does not aggravate structural damage or neurologic motor deficits but delays the long-term cognitive recovery following mild to moderate traumatic brain injury. *Critical Care Medicine, 34*(2), 492-501.

Sidaros, A., Engberg, A. W., Sidaros, K., Liptrot, M. G., Herning, M., Petersen, P., et al. (2008). Diffusion tensor imaging during recovery from severe traumatic brain injury and relation to clinical outcome: A longitudinal study. *Brain, 131*(Pt 2), 559-572.

Smith, D. H., Chen, X. H., Pierce, J. E., Wolf, J. A., Trojanowski, J. Q., Graham, D. I., et al. (1997). Progressive atrophy and neuron death for one year following brain trauma in the rat. *Journal of Neurotrauma, 14*(10), 715-727.

Soares, H. D., Hicks, R. R., Smith, D., & McIntosh, T. K. (1995). Inflammatory leukocytic recruitment and diffuse neuronal degeneration are separate pathological processes resulting from traumatic brain injury. *The Journal of Neuroscience, 15*(12), 8223-8233.

Sorensen, A. G., Buonanno, F. S., Gonzalez, R. G., Schwamm, L. H., Lev, M. H., Huang-Hellinger, F. R., et al. (1996). Hyperacute stroke: Evaluation with combined multisection diffusion-weighted and hemodynamically weighted echo-planar MR imaging. *Radiology, 199*(2), 391-401.

Takasawa, M., Jones, P. S., Guadagno, J. V., Christensen, S., Fryer, T. D., Harding, S., et al. (2008). How reliable is perfusion MR in acute stroke? validation and determination of the penumbra threshold against quantitative PET. *Stroke, 39*(3), 870-877.

Thompson, H. J., Lifshitz, J., Marklund, N., Grady, M. S., Graham, D. I., Hovda, D. A., et al. (2005). Lateral fluid percussion brain injury: A 15-year review and evaluation. *Journal of Neurotrauma, 22*(1), 42-75.

Tollard, E., Galanaud, D., Perlbarg, V., Sanchez-Pena, P., Le Fur, Y., Abdennour, L., et al. (2009). Experience of diffusion tensor imaging and 1H spectroscopy for outcome prediction in severe traumatic brain injury: Preliminary results. *Critical Care Medicine, 37*(4), 1448-1455.

van de Looij, Y., Mauconduit, F., Beaumont, M., Valable, S., Farion, R., Francony, G., et al. (2011). Diffusion tensor imaging of diffuse axonal injury in a rat brain trauma model. *NMR in Biomedicine,*

Van Putten, H. P., Bouwhuis, M. G., Muizelaar, J. P., Lyeth, B. G., & Berman, R. F. (2005). Diffusion-weighted imaging of edema following traumatic brain injury in rats: Effects of secondary hypoxia. *Journal of Neurotrauma, 22*(8), 857-872.

Vink, R., Mullins, P. G., Temple, M. D., Bao, W., & Faden, A. I. (2001). Small shifts in craniotomy position in the lateral fluid percussion injury model are associated with differential lesion development. *Journal of Neurotrauma, 18*(8), 839-847.

Williams, D. S., Detre, J. A., Leigh, J. S., & Koretsky, A. P. (1992). Magnetic resonance imaging of perfusion using spin inversion of arterial water. *Proceedings of the National Academy of Sciences of the United States of America, 89*(1), 212-216.

11

Neurointensive Care Monitoring for Severe Traumatic Brain Injury

Zamzuri Idris, Muzaimi Mustapha and Jafri Malin Abdullah
Universiti Sains Malaysia
Malaysia

1. Introduction

Worldwide, traumatic brain injury is one of the leading cause of permanent disability and death. Contextually, close monitoring and immediate therapy for any accompanying abnormalities are crucial in order to reduce the rate of mortality or morbidity associated with this acquired brain injury. For this purpose, an isolated neurointensive care (NIC) specialised for brain injury is highly warranted, manned by appropriately trained staff who understand the current pathophysiology of brain injury and equipped with various modes of therapy to tackle any diagnosed abnormalities. To date, we are witnessing progress in managing severely injured brain from no specific monitoring to specific ones, and from a single intracranial pressure monitoring to future trend of multiple cranial monitoring alongside availability of various mode of therapies. The multimodal brain monitoring as it is commonly known is a concept whereby, intracranial pressure as well as various other important cerebral parameters can readily be monitored.

Monitoring alone will not alter the outcomes of severely injured brain patients. Prompt recognition of any abnormality from the monitoring and availability of therapy to correct the diagnosed abnormality are some of the factors that are recognised to influence better outcome score. However, at present, two obvious limitations in the multimodality brain monitoring are our incomplete understanding of the underlying pathophysiology of the severely injured brain and the limited availability of mode of therapy to the neurosurgeon or neurointensivist to treat abnormalities detected from the state-of-the-art monitoring. Research are ongoing in these areas and in this chapter, we discuss in details the current pathophysiology of traumatic brain injury, roles of multimodality brain monitoring in NIC and the prospects of brain hypothermia to correct commonly associated abnormal monitored-parameters.

2. Pathophysiology underlying severely injured brain

Closed brain injury processes can be divided into two phases, primary and secondary brain injury. The primary brain injury occurs during the initial insult. The severity and type of impact incurred during the initial insult will substantially influence the structural lesions that ensue. This severely injured brain is vulnerable to further insult which is known as the secondary brain injury (figure 1). In this phase, the commonly associated forms of insult can be broadly categorised into three: 1) intracranial hypertension; 2) cerebral hypoxia and

hypoperfusion which can lead to hypoxic-ischaemic damage and 3) electrographic seizures. These secondary insults can progress to three major forms of end-stage events: 1) cerebral infarct; 2) cerebral or brain swelling and 3) herniation syndromes, all of which impose a negative impact on outcome. Alternatively, pathophysiology of brain injury can be viewed consisting of three components: 1) *macroscopic* pathophysiological events or primary brain injury; 2) *microscopic* pathophysiological events which are part of secondary brain injury consisting of intracranial hypertension, cerebral ischaemia and hypoxia, and electrographic seizures and finally 3) *ultramicroscopic* events which are also part of secondary brain injury processes including cascades of glial cells and molecular events, microvascular dysfunction, blood brain barrier (BBB) disruption, inflammatory cells recruitment and reactivation, and immunological response. All of these, macroscopic, microscopic and ultramicroscopic processes or events are related to patient's outcome. The 'micro' and 'ultramicro' pathophysiological events are potentially avoidable and amenable to treatment. Currently, the primary focus in the acute management of traumatic brain injury is to prevent and ameliorate these micro and ultramicroscopic events that accentuate secondary brain injury. In practice, the multimodality brain monitoring to certain extent, bears much reflection of these complex inter-related events, and hence explains the rationale of such monitoring approach in severely injured brain as we attempt to expand our scientific understanding on this complex issue.

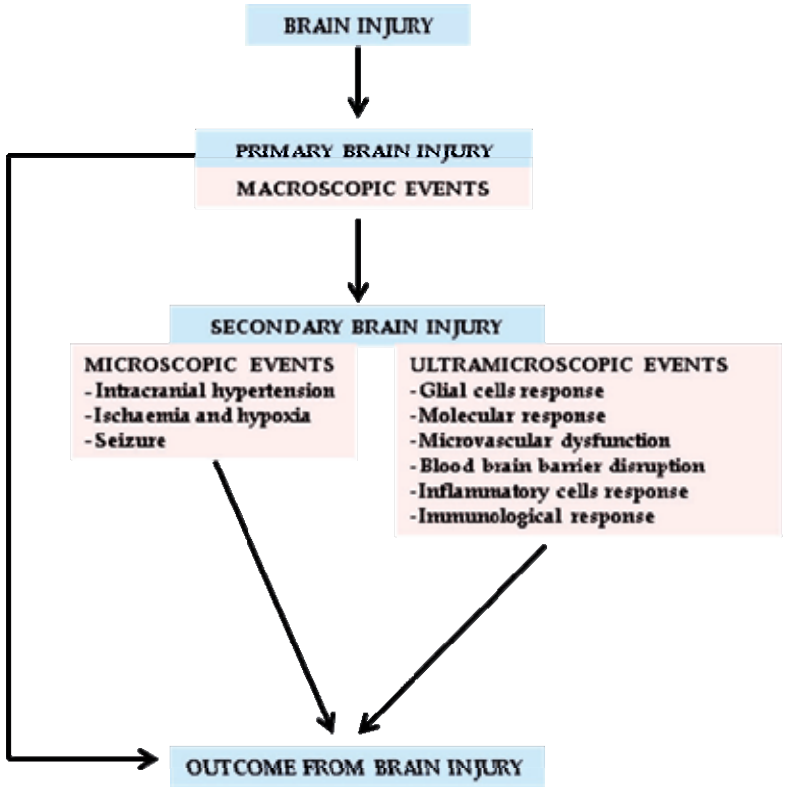


Fig. 1. Schematic flow of events in the pathophysiology of traumatic non penetrating (closed) brain injury

2.1 Primary and secondary brain injury

Primary brain injury occurs at the time of impact with an instantaneous clinical effect. Pathological classification for this form of injury is largely evident either clinically or by imaging (macroscopic events) which include focal-epidural, subdural, intracerebral haematomas, focal contusion and laceration, focal or diffused subarachnoid haemorrhage and focal or diffused axonal and vascular injuries. Surgery plays a major role to treat some of these pathologies. On the other hand, secondary brain injury or damage occurs at some stage after the initial impact and therefore can aggravate the primary brain injury. From figure 1, it is apparent that the microscopic and ultramicroscopic events contribute to the secondary brain injury pathogenesis. However, currently there are limited treatment options available in treating the secondary brain injury processes or damages. In 2002, Narayan reviewed clinical trials in head injury and concluded that most trials assessing pharmacological neuroprotective agents failed to show clinically robust efficacy (Narayan et al., 2002). Surgically, the typical available options are burr hole and cerebrospinal fluid (CSF) drainage and a wide decompressive craniectomy. The latter has regained therapeutic interest in the past years with beneficial effects shown for massive brain infarcts, brain swelling and brain herniation that constitute the end results of secondary brain injury processes as discussed above (Carandang & Krieger, 2008; Cooper et al., 2008; Eberle et al., 2010; Figaji et al., 2007). Other possible alternatives to treat secondary brain damage include therapeutic cerebral hypothermia (Dietrich & Bramlett, 2010; Gluckman et al., 2005), barbiturates therapy (Marshall et al., 2010), raising cerebral blood flow and perfusion by using pressors (Lewis et al., 1998) and rheological agents such as mannitol and hypertonic saline therapy (Berger et al., 1995; Oddo et al., 2009).

2.1.1 Ultramicroscopic events

Trauma will induce a significant and protracted inflammatory, molecular, microvascular and immunological responses. These response cascades following direct damage to the neurovascular unit which incite ischaemic-hypoxic events. Systematically, four major mechanisms may contribute to cerebral ischaemia or hypoxia: a) direct or indirect structural damage to intracerebral arteries, neurons and glial cells (neurovascular unit); b) cerebrovascular-metabolic uncoupling; c) intracranial hypertension and, d) vasospasms of cerebral artery. These mechanisms are the interrelated cascades which underlie the pathophysiological processes that propagate the brain injury (figure 2). Damage to the neurovascular unit which comprises cerebral endothelial cells, astrocytes and neurons as well as extracellular matrix plays significant role in these cascades (Dirnagl et al., 1999). The cascades involved opening of voltage dependent and agonist-gated ion and calcium channels causing intracellular calcium and sodium overload and efflux of potassium. Intracellular sodium overload causes cytotoxic oedema while efflux of potassium leads to peri-infarcted brain tissue depolarisation and seizures. Influx of calcium is further promoted by the release of large amount of excitatory amino acids particularly glutamate. Intracellular calcium and glutamate act in a vicious circle which augments the amount of intracellular calcium. Excess intracellular calcium can induce damage to the organelles such as mitochondria, intracellular second messengers, cellular membrane and activation of numerous intracellular enzymes system which finally lead to apoptosis. Apoptosis is a form of cell death in which a programmed sequence of events leads to the elimination of cells without releasing much harmful substances. When brain cells experienced an injury or episode of ischaemia, they either become dead and the area involved is better known as

infarcted area, or the cells may survive the injuries and in time recover completely or partially, or alternatively the cells enter the apoptotic pathway. Interestingly, during the early stages of injury, the process of apoptosis can still be reversed. This reversal is made possible by correcting the cellular energy metabolism or cerebrovascular metabolic coupling or by lowering the brain temperatures (Liou et al., 2003). In addition to the events described above, damage to the neurovascular unit can also cause production of free radicals. Free radicals are atomic or molecular species with unpaired electrons. They are highly reactive and unstable, therefore capable of oxidizing and damaging all cellular components when their amounts exceed the anti-oxidant defence mechanisms as seen in episode of ischaemia/reperfusion brain injury (Lewen et al., 2000). Other harmful events that occur at ultramicroscopic levels when the brain is injured are inflammatory and immunological responses. Increased levels of pro-inflammatory cytokines such as interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10) and tumour necrosis factor alpha (TNF-α) have been demonstrated in cerebrospinal fluid, serum and brain tissue following head injury (Csuka et al., 1999; Is et al., 2007; Kossmann et al., 1997). The underlying dynamics of inflammation in traumatic brain injury are complex, encompassing cytokines, chemokines, adhesion molecules, complement factors and even the free radicals. The lead triggers for inflammatory activation are multi-factorial and thought to involve factors such as extravasated blood products, intracellular components, reactive free oxygen and nitrogen radicals by brain resident microglia and astrocytes sensing perturbation of tissue homeostasis (Juliet et al., 2008; Mathew et al., 1994). These initial events fuel further release of inflammatory mediators which facilitate influx of extracerebral inflammatory cells by vascular endothelial changes and chemotaxis. Studies have also shown the role of adhesion molecules such as vascular cell adhesion molecule, P-selectin, intracellular adhesion molecule-1 and gradient of chemotactic chemokines in upregulating these events (Hausmann et al., 1998). The ensuing extracellular oedema is triggered by vascular dilatation and leakage as a result of vasoactive inflammatory molecules such as nitric oxide (NO) and vascular endothelial growth factor, as well as activations of complement system by dying cells to aggravate the initial inflammation and secondary tissue damage (Grzybicki et al., 1998; Stahel et al., 1998). Currently, monitoring of neuroinflammation is only made possible with techniques such as brain microdialysis, whilst others such as free radical imaging and monitoring remains under intensive research (Vergely et al., 2003).

2.1.2 Microscopic events

Typically in normal subjects, the cerebral blood flow (CBF) and metabolism are a coupled phenomenon. Cerebrovascular metabolic uncoupling is reported to occur in over half of brain injured patients (Obrist et al., 1984). Uncoupling, which impairs the CBF and metabolism following traumatic severe brain injury is related to unfavourable functional outcome because of the correlation with ischaemic-hypoxic events. Reduction in CBF is obviously affecting the status of cellular metabolism and hence promoting cellular death. Interestingly, uncoupling is also expected with an excessive increased in CBF (luxury perfusion or hyperaemia) which is harmful to the injured brain by causing brain swelling (congested brain) or oedema. In 2007, Menashe Zaaroor noted significant reduction in CBF within 24 hours of brain injury and then increased at the second day after the injury. Further analysis revealed patients with good outcome after head injury had stable and normal CBF after initial period of significant reduction, whereas in patients with poor outcome, the course of CBF is triphasic with a secondary decrease on the third day post trauma (Menashe

Zaaroor et al., 2007). They postulated the subsequent decrease in CBF may be due to brain swelling or persistent impairment in cerebral autoregulation with disruption in blood brain barrier (BBB). In addition, persistent ischaemic insults would subsequently progress towards destructive processes involving disintegration of cell membranes and intracellular organelles, failure of various ion pumps, development of mitochondrial dysfunction, inappropriate activation of numerous enzyme systems with cellular hyperactivity and increment in lactate levels. All these contribute to the development of intracellular acidosis and retention of carbon dioxide which can further aggravate the preceding insults which could finally lead to cerebral oedema, swelling and peri-infarct cerebral depolarisation. The ultramicroscopic and microscopic cascades as illustrated in figure 2, depict the pathophysiological mechanisms acting in vicious circle, with the consequential brain swelling and oedema that inflict intracranial hypertension and reduction in cerebral perfusion pressure (CPP). The reduction in CPP further aggravates the ischaemia and encourages local release of vasoactive mediators such as endothelin, thomboxane A_2 and prostaglandin I_2. Endothelin and thromboxane A_2 are powerful vasoconstrictor, whereas prostaglandin I_2 is a potent vasodilator. Thromboxane can also induce platelet aggregation. Thromboxane and prostaglandin I_2 play an important role in regulating local cerebral blood flow. This local homeostasis may be disrupted following an ischaemic event or direct neurovascular injury, with a relative increase in the production of thromboxane (Chen et al., 1986). The predominant increase in thromboxane can lead to vasoconstriction and hence further reduces cerebral perfusion and also promotes local thrombus formation. Aside from the mechanisms involved in cerebral hypoperfusion state in brain injury as highlighted above, cerebral vasospasm could also play a pivotal role. An obvious culprit to this is the presence of blood products such as haemoglobin that act through multiple mechanisms. Such mechanisms include decreased production of a vasodilator such as NO, increased vasoconstrictors, free radical production and lipid peroxidation of cell membranes, modification of potassium and calcium channels, direct oxidative stress on smooth muscle cells, differential up-regulation of genes and cortical spreading depolarisation. The state of cortical spreading depolarisation or depression inverses the coupling between spreading depolarisation of the cortex and CBF by increasing the extracellular potassium and endothelin concentration and decreasing NO concentration. This finally leads to microarterial spasm and spreading cerebral ischaemia which act in a vicious circle (Pluta et al., 2009). In addition, cortical spreading depolarisation arising from peri-infarcted brain tissues (peri-infarcted depolarisation) underlying an ischaemic brain can also induce subclinical or non-convulsive seizures which can worsen the brain ischaemia (Fabricius et al., 2008).

3. Neurointensive care monitoring

The neurointensive care (NIC) warded critical patients who are mostly intubated. To the clinician, the clinical status assessment or monitoring for intubated patients are difficult. Hence, various important patients' parameters must be monitored. Monitoring should therefore be focused on two categories; a) standard monitoring and b) specific monitoring. The standard NIC monitoring covers multiple vitals including the brain and other organ systems. The specific monitoring is focused mainly on the brain. Monitoring the pressure inside the cranium is regarded by most as the gold standard for severely injured brain patients in NIC (Ross & Eynon, 2005). Our latest understanding of the macroscopic, microscopic and ultramicroscopic pathophysiological events that underlie brain injury has

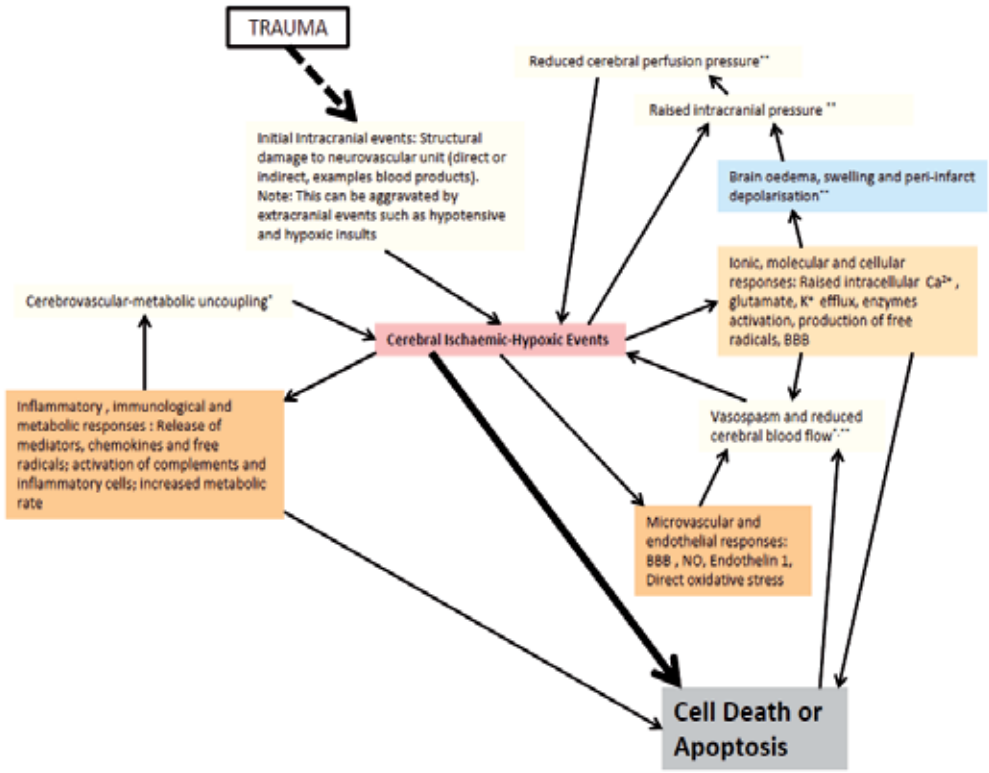


Fig. 2. A hypothetical scheme to depict post-traumatic and post-ischaemic events. ** treatment available; *monitoring only; orange boxed - no treatment yet, but under intensive research (BBB – Blood brain barrier; NO – Nitric oxide)

widened our scopes of monitoring. Although intracranial hypertension is one of the main pathophysiological processes involved in secondary brain injury, it is not the sole predictor of the patient's outcome. Notably, the observed intracranial hypertension in severely injured brain is a common end-point of the various pathophysiological cascades involved. Hence, other cerebral-specific monitoring parameters are required to assist neurointensivists detect 'earlier' secondary insults and prevent the harmful progression to three end-stage processes as mentioned earlier, i.e. a) cerebral infarct b) brain swelling and oedema and c) herniation syndromes. The 'extra' monitoring parameters include cerebral blood flow, oxygenation, metabolites, brain temperature and perfusion, or otherwise known as the multimodality monitoring.

3.1 Standard monitoring

The severely injured brain is vulnerable to further insults after sustaining the traumatic primary brain injury. The secondary insults are commonly identified as systemic hypoxia and hypotension, intracranial hypertension and cerebral hypoperfusion which can progress to cerebral ischaemia, brain swelling and herniation syndrome. These secondary insults are regarded as the survival determinants for patients receiving care in the NIC. Therefore the

aim of standard monitoring is to prevent or to recognize these potential threats which could originate from other body systems besides the brain. Head injured patients in NIC should be monitored for nine key body systems: a) central nervous system (discussed specifically under brain monitoring); b) cardiovascular system which includes parameters such as heart rate and rhythm, blood and venous pressure, stroke volume, cardiac output and total peripheral resistance; c) pulmonary system with regular monitoring of arterial blood gases, chest radiographs, and breathing and ventilator parameters; d) renal and metabolic system with regular blood, urine and metabolic profiles monitoring; e) gastrointestinal and nutritional status pertaining to body calories; f) hematological profiles; g) microbiologic and sepsis profiles; h) peripheries and skin condition and lastly i) endocrine and hormonal profiles which are frequently overlooked, except for features of diabetes insipidus or syndrome of inappropriate antidiuretic hormone hypersecretion. Recent studies have demonstrated 75% incidence of endocrine dysfunction at 6 months post trauma in children and 21% incidence of hypothalamic-pituitary dysfunction in adults (Kaulfers et al., 2010; Krahulik et al., 2010). These findings prompted debates as to whether hormonal profile evaluation should be routinely checked in all head injured patients and treated accordingly.

3.2 Specific monitoring

Since limitations exist in bedside neurological findings as a monitoring tool for various pathophysiological processes described in previous sections, specific central nervous system monitoring is needed for severe brain injured patients in the NIC. The use of serial computed tomography (CT) brain scanning protocol to predict the presence or absence of abnormality or raised ICP has not been well received in practice, partly due to poor correlations between CT findings and raised ICP, and because of undesirable effects from repeated radiation exposure and frequent mobilization to the patients (Kouvarellis et al., 2011; Miller et al., 2004). Therefore, specific brain monitoring seems necessary. Currently, the assessment of intracranial status for severely injured brain is commonly done with gold standard ICP monitoring alone. Lately, as our understanding on the complex pathophysiology underlying severely injured brain expands, new modalities of brain monitoring emerge, incorporating various brain parameters known as the multimodality monitoring (Idris et al., 2007; Isa et al., 2003; Stuart et al., 2010).

4. Multimodality monitoring

The development of new neuromonitoring techniques has been particularly important because typically standard cranial monitoring techniques such as ICP and CPP measurements seem insufficient in detecting subtle manifestations of brain injury or poor surrogates for physiologic parameters of interest. For these reasons, multimodality monitoring which monitors more brain parameters is required. Multimodality monitoring includes monitoring of the following parameters:

i. Intracranial pressure (ICP)
ii. Cerebral perfusion pressure (CPP)
iii. Cerebral blood flow (CBF parameters)
iv. Focal and global cerebral tissue oxygenation (PtiO2 and $SjVO_2$)
v. Cerebral tissue carbon dioxide tension ($PtiCO_2$)
vi. Cerebral pH

vii. Cerebral temperature
viii. Cerebral metabolic parameters (brain microdialysis)
ix. Cerebral EEG (EEG and BIS)
x. Cerebral evoked potentials (somatosensory, brainstem and visual evoked potentials: SSEP/BAEP/VEP)

Figure 3 shows the role of multimodality monitoring for the injured brain. Intracranial pressure is used to monitor the cranial pressure, Licox or Neurotrends system is used to monitor status of regional brain oxygenation, carbon dioxide tension, pH and brain temperature, while jugular bulb oximetry is used to monitor global brain oxygenation. Saber cerebral blood flow, transcranial doppler, cerebral EEG and evoked potentials can be used to monitor cerebral blood flow and therefore identifying ischaemic insults, and cerebral microdialysis can monitor the cellular events. Furthermore, continuous EEG monitoring in NIC can also diagnose the subclinical seizures which play significant role in secondary brain injury and one of the parameters that determine the prognosis (Kazibutowska et al., 1992).

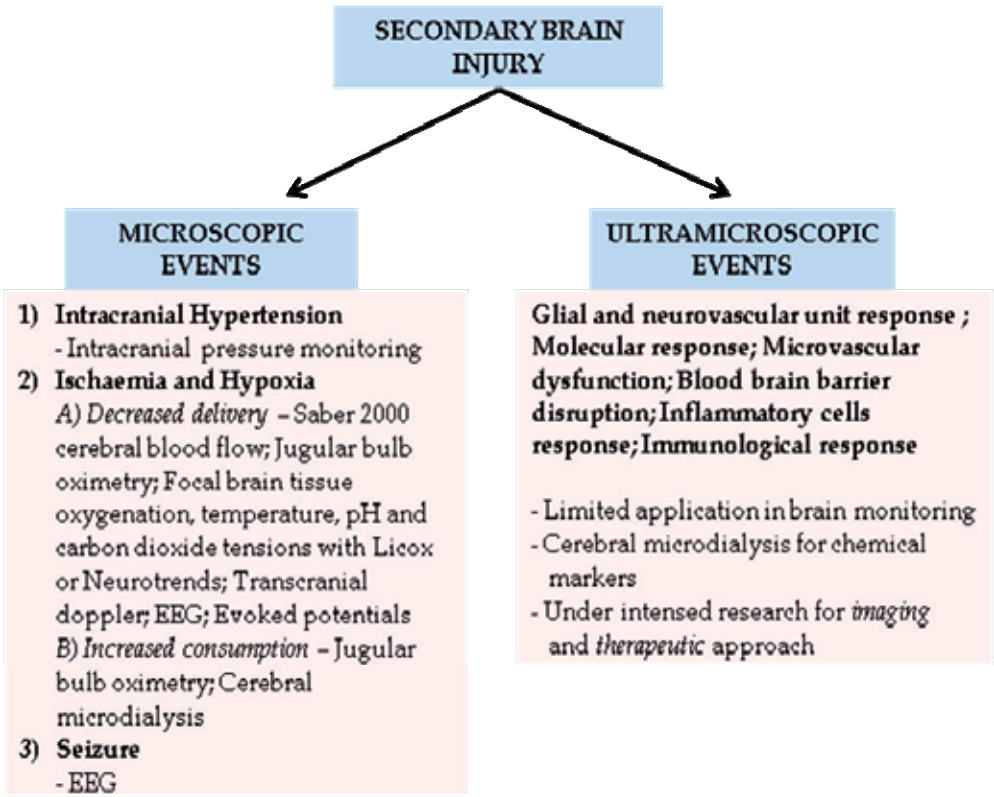


Fig. 3. Multimodality monitoring and secondary brain injury.

5. Two major parameters to consider during multimodality monitoring: ICP and CBF

Intracranial hypertension causes a reduction in cerebral perfusion pressure which can lead to ischaemia, brain swelling and ultimately infarction. In addition, persistent increased in

ICP could cause a shift in intracranial structures (midline shift or mass effect) and finally herniation syndromes. Therefore, monitoring ICP is considered a standard part of head injury management. Nonetheless, monitoring the ICP alone seems inadequate, because raised in ICP in head injured patients can be associated with both an increment and a reduction in CBF (Menashe Zaaroor, 2007). In 1992, Bouma noted about one-third of head injured patients had low CBF and therefore showed an early cerebral blood flow-metabolism mismatch (Bouma et al., 1992). Figure 2 highlights the importance of CBF monitoring to prevent or reduce the degree of cerebral ischaemic-hypoxic insults. Whilst the optimal method for measuring CBF remains to be established, there are numerous alternatives when used together can estimate the CBF. Those alternatives include: a) CPP; b) blood flow velocity by transcranial doppler; c) focal and global cerebral oxygenation with Licox or Neurotrends systems or jugular bulb oximetry; d) regional CBF with Saber 2000; e) cerebral metabolic parameters with brain microdialysis; f) cerebral perfusion imaging techniques and g) cerebral evoked potentials and EEG. These are in fact features of multimodality neuromonitoring.

The CBF and CPP are two variables that are inter-related. The CBF is proportional to CPP and inversely related to the cerebral vascular resistance (CVR). The CPP represents the pressure gradient driving CBF and hence oxygen and metabolite delivery, i.e. in other words, the CPP is the perfusion pressure gradient across cerebral vascular bed whereby the incoming systemic arterial pressure minus the opposing force from venous outflow pressure. In traumatic brain injury cases, the elevated ICP is the opposing force to the incoming mean systemic arterial pressure (MAP). Therefore, CPP in equation form can be stated as CPP = MAP – ICP. In theory, CPP is equivalent to the transmural pressure across the cerebral vessel walls. In other words, at the arteriolar level, CPP is the stimulus for autoregulatory response and at the capillary, it is the driving force for fluid exchange. The normal brain autoregulates its blood flow to provide a constant flow regardless of blood pressure by altering the CVR to obtain a maintained flow within an autoregulatory range of 60 - 160 mmHg of MAP or CPP of 50 – 150 mmHg. These homeostatic mechanisms are often lost in severely injured brain due to impairment in cerebral autoregulation, implying that cerebral autoregulation is a homeostatic mechanism that minimizes deviations in CBF when CPP or MAP changes. It acts through vasomotor effectors that control CVR. Vasomotor effectors are controlled by a) CPP; b) metabolites; c) oxygen pressure; d) carbon dioxide pressure and e) temperature. In NIC setting, the level of changes in cerebral autoregulation among patients is individualised and therefore the optimum CPP may vary considerably. Clinically, continuous methods of autoregulation tests or monitoring rely on the observation of spontaneous responses of CBF to spontaneous fluctuations in MAP or CPP (Zweifel et al., 2008), arterial partial pressure of CO_2 (Puppo et al., 2008) or metabolites (Lee et al., 2001). Study by Lee in 2001 showed that during the first 2 weeks after head injury, CO_2 reactivity remains relatively intact, pressure autoregulation is variably impaired and metabolic suppression reactivity remains severely impaired and they further noted that unlike hypotension, hypoxia or haemorrhagic brain lesions, elevated ICP appears to affect all components of vasoreactivity that were tested (Lee et al., 2001). In similar year, Czosnyka also noted that autoregulation is not only impaired when associated with a high ICP or low arterial blood pressure, but it can also be disturbed by an excessive CPP (Czosnyka et al., 2001). Studies have shown that impairment or failure of autoregulation can contribute to unfavorable outcome (Czosnyka et al., 2001; Lam et al., 1997; Smielewski et al., 1997). This could possibly be explained by a disturbance in the pressure-volume (elastance) curve

that is steeper suggesting poor compliance of the brain (figure. 4). Since elevated ICP and changes in CBF are two key factors to consider in all severely injured brain patients (figure. 2), besides cerebral autoregulation, the concept of cerebral pulsation is also thought to play a significant role in head injury pathogenesis.

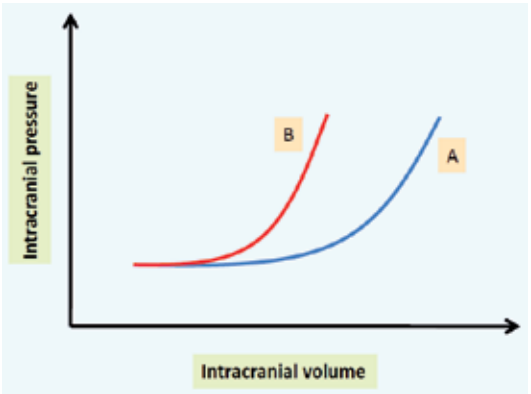


Fig. 4. Pressure-volume curves of craniospinal contents. (A) is normal pressure-volume curve of compliant system and (B) is the pressure-volume curve when brain compliance is reduced.

5.1 Monro-Kellie doctrine, cerebral autoregulation, cerebral pulsation, pulsatility and resistive index and brain compliance

According to Monro-Kellie doctrine, the sum of the intracranial volumes of blood, brain and CSF is constant and that an increase in any one component must be offset by an equal decrease in another or else ICP rises. For traumatic brain injury, a pathological increase in brain volume due to cerebral oedema or an increase in blood volume would induce an initial reduction in CSF volume response. This has been traditionally viewed as CSF being forced out into the spinal dural sac to maintain ICP. This *anatomical compensatory response* can quickly become exhausted and further rise in ICP is expected. Since raised ICP can lead to reduction in CPP and therefore compromising CBF, the body finally has to utilise the second defence mechanism, *functional compensatory response* (augment the CBF). This type of compensatory response is to ensure the brain receives an adequate blood flow and hence counteracts the effect of raised ICP. Once the body compensatory responses (anatomical and functional) become overstretched, that is the only time we note a fast increase in ICP and striking reduction in brain compliance (the steep part of the elastance curve). This decompensatory phase is related to poor brain compliance, raised ICP and reduction in CBF (figure. 5, labelled as A,B and C). Besides cerebral autoregulation, increased in intracranial vessels pulsation is one of the mechanisms that could augment the CBF during ischaemic-hypoxic events (Chan et al., 1992; Egnor et al., 2002; Muttaqin et al., 1993). Since both seem to be important, monitoring level of autoregulation and assessing the intracranial vessels pulsation are two parameters that should be done in NIC for multimodality monitoring. Cerebral autoregulation is normally tested or monitored with transcranial doppler by using static (vasopressor) or dynamic (Aaslid leg-cuff, carotid artery compression or tilt table declination) provoking test or with cerebrovascular reactivity index (PRx) whereby digitised ICPs is divided with digitised MAPs and focusing on their trends or values; positive values

imply that increases in MAP are positively associated with increases in ICP, suggesting autoregulatory impairment, whereas negative or flattened values indicate intact autoregulatory mechanism, with vasoconstriction and decreased or maintained ICPs in relation to surges in MAPs (figure 6) (Aaslid et al., 1989; Mitsis et al., 2006; Smielewski et al., 1997; Timofeev et al., 2011; Zweifel et al., 2008). Similarly, assessing the intracranial vessels pulsation is made possible using transcranial doppler (TCD) and measuring the velocity of middle cerebral artery (MCA) and quoted as pulsatility (PI) or resistive index (RI). Researchers have found strong correlation between the RI or PI with raised ICP (Bellner et al., 2004; Gura et al., 2011; Voulgaris et al., 2005).

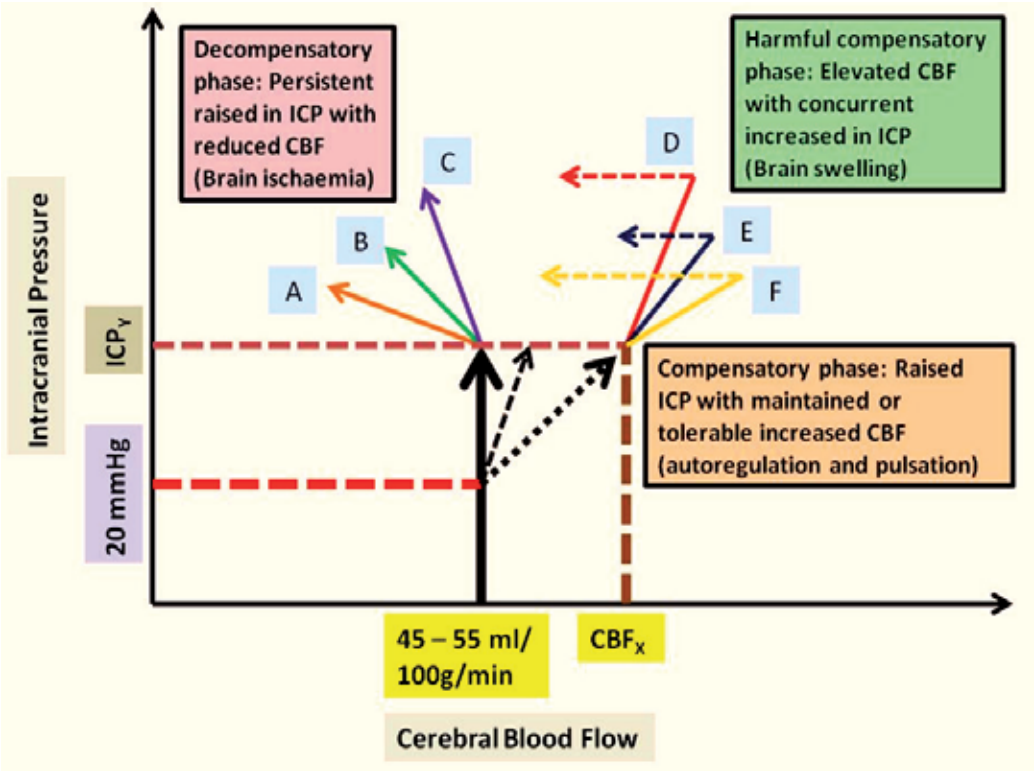


Fig. 5. The correlation between ICP and CBF. The compensatory phase exists at the beginning of raised ICP and followed by the decompensatory phase in some cases with persistently raised ICP. There exist individual variations labeled as A, B or C with different degrees of ICP increment or CBF reduction. Similarly, there is also harmful compensatory phase, labeled as D,E and F, in which increased in CBF would cause increment in ICP (commonly induced by clinicians). The role of multimodality monitoring is to identify ICP_Y and CBF_X and to identify the time-shift from compensatory to decompensatory or harmful compensatory phase.

The correlation between intracranial brain pulsations and brain compliance can also be noted on ICP waveforms. A good ICP waveform looks similar to an arterial pressure wave (Ross & Eynon, 2005). There are three peaks (figure. 7, labelled A): a) P1 is known as percussion wave and produced by systolic arterial pressure transmitted from the choroid

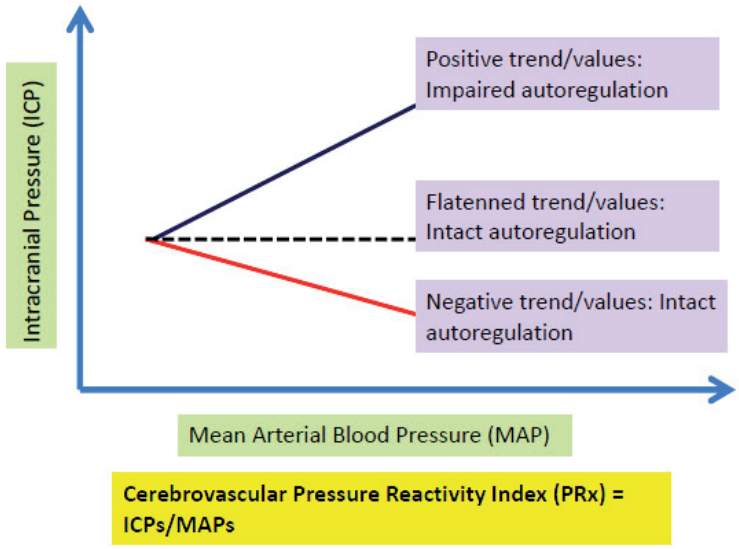


Fig. 6. Positive and negative trends for the cerebrovascular pressure reactivity index (PRx). Positive trends signify impaired cerebral autoregulation whereas, negative and flattened trends denote intact autoregulation.

plexus to the ventricle; b) P2 is the tidal wave, its shape is more variable and originated from central venous wave from the right atrium and correlated with venous volume or venous congestion, sinus pressure and brain pulsations in injured brain. P2 may also stand for overall brain compliance; c) P3 is the dicrotic wave and is due to closure of the aortic valves. In between P2 and P3, there is dicrotic notch which is also thought arising from the effect of aortic valves closure. In 1965, Lundberg described three time-dependent patterns of pressure waves in patients with raised ICP (Lundberg et al., 1965). Lundberg A or plateau waves are 50 – 100 mmHg high and last for 5 to 20 minutes. This type of pressure waves demonstrate an early functional compensatory response between raised ICP and CBF. Cerebral vasculatures start to dilate in response to intracranial hypertension and critically low CBF, however, this autoregulatory vasodilatory response can dangerously lead to further increment in ICP via increased in intracranial blood volume in some brain injured patients with impaired autoregulation (figure. 5, labelled as D,E and F). Lundberg B waves occur at frequency of 0.5 – 2/min and are up to 50 mmHg in amplitude. It is detectable in normal and some individuals with impaired cerebral autoregulation (Balestreri et al., 2004). Lundberg C waves last 4 to 5 minutes and are up to 20 mmHg in amplitude and are of little clinical significance. Studies on ICP waveforms and Lundberg patterns of pressure waves have demonstrated that in the presence of raised ICP and intact cerebral autoregulation, the brain is able to regulate or optimise its CBF through compensatory mechanisms. Maintainance of CBF can be made via autoregulatory cerebral vasculature dilatations and increased in cerebral vasculature pulsations. Since P2 indicates overall compliance of the brain, at an early stage of compensation the peak-interval between P1 and P2 is increased which signifies increased cerebral vasculature pulsations and increased brain compliance (figure.7, labelled B). In the presence of persistently raised ICP, this initial response is

followed by a gradual reduction in the peak-interval between P1 and P2 (P2 is nearly at the same level or is higher than P1) signifying decreased brain compliance as well as marked reduction in brain pulsations suggesting decreased distensibility or increased stiffness of the brain (figure.7, labelled C). This decompensatory phase starts when the body is unable to maintain an adequate CBF with simultaneous rapid increment in ICP and invariably manifests in various ways: a) reduced in any parameters that measured CBF; b) increased in RI measured with TCD; c) elevation in anaerobic metabolism parameters which commonly measured with microdialysis; d) retention of carbon dioxide, presence of hypoxia or acidosis and increased in brain temperature as measured by Neurotrends or Licox system and e) change in EEG waveforms or alterations in evoked potentials. The time-shift from compensatory to decompensatory or harmful compensatory phase is not known unless ICP and various other monitorings are implemented on patients monitored in NIC. Therefore, the roles of multimodality monitoring are as follows:

1. To identify early the potential dangerous effect of known vicious circle of ICP and CBF, therefore guiding the clinician to institute the appropriate mode of therapy (ICP or CPP targeted therapy) with closed neuromonitoring
2. To identify the time-shift from compensatory to decompensatory or harmful compensatory phase, therefore helping the clinician to proceed with end-tier therapy (decompressive craniectomy or barbiturate coma therapy) when absolutely indicated
3. To help clinician understand the complex pathophysiology of severely injured brain, therefore creating a future possibility to develop new and innovative therapy

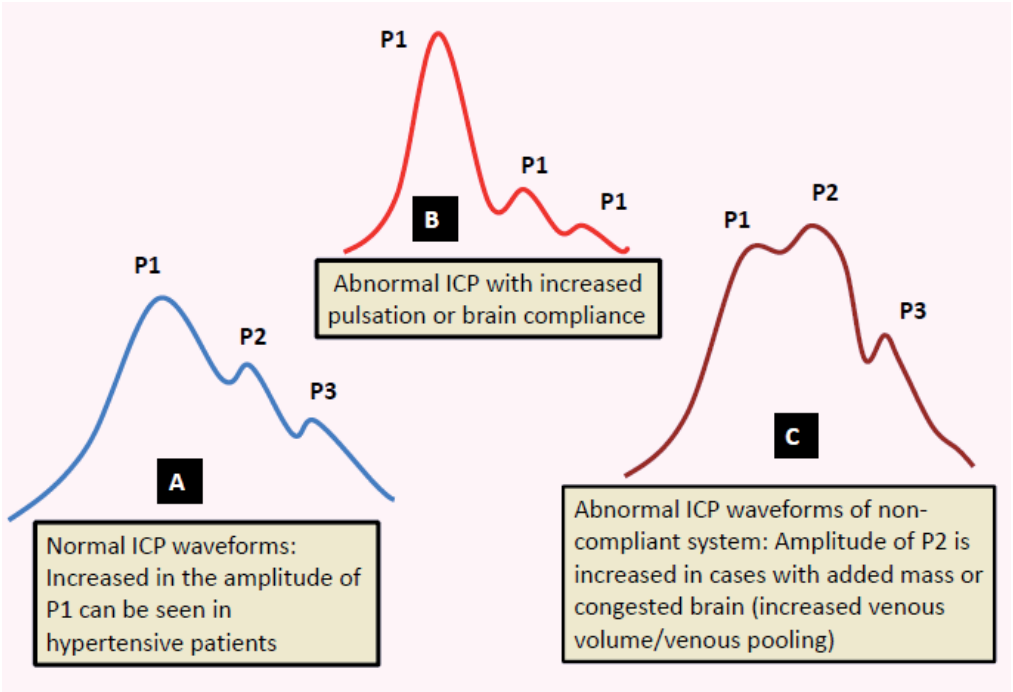


Fig. 7. Intracranial pressure waveforms. In a non-compliant system, P2 wave is exceeding P1.

5.1.1 Vicious circle of ICP and CBF: ICP and CPP targeted therapy versus multimodality monitoring

In previous sections, we recognised that an increase in ICP sets in an autoregulatory response to compensate for a reduction in CBF. Since CPP is proportional to CBF, increment in ICP would cause decrement in CPP. This is easily understood from the equation: CPP = MAP – ICP, whereby raised ICP causes reduction in CPP when MAPs are kept constant. Reduced CPP can be corrected by raising the MAP or by reducing the ICP. Reduction in ICP is normally called ICP targeted therapy, whilst CPP targeted therapy corrects the reduced CPP by raising the MAP. In 2003, Young recommended an aggressive CPP management for patients with elevated ICP (Young et al., 2003). Four patients in their study had comparatively good neurological outcome after receiving such management for extremely high ICP. Nonetheless, in some severely injured brain patients who have high CPP values or those received an aggressive CPP management appeared to develop cerebral oedema or brain swelling and had poor outcomes. This phenomenon is thought to arise from impairment in cerebral autoregulatory mechanisms (Dickman et al., 1991; Nujaimin et al., 2009; Zweifel et al., 2008). In patients with an impaired cerebral autoregulation, ICP targeted therapy seems more appropriate than CPP targeted therapy. This is mainly because of the vicious circle that can occur in patients with impaired cerebral autoregulation whereby ICP is further elevated by elevating the CPP (figure 5, labelled D,E and F). Many studies have shown a strong association between an elevated ICP and poor patient outcome in head injury (Cooper et al., 2008; Eberle et al., 2010; Idris et al., 2007). In most cases with poor outcome, both, elevated ICP and low CPP values appeared common features. Therefore it seems prudent to conclude that in such patients, the emphasis should not only on raising CPP but also on lowering the ICP to gain better outcome. In fact, the combination of ICP and CPP therapy forms the basis of the guidelines or protocols proposed by the US Brain Trauma Foundation (Bullock et al., 1996), European Brain Injury Consortium (EBIC) (Maas et al., 1997), Addenbrooke protocol from the Cambridge (Patel et al., 2002) and Lund protocol (that is still considering the importance of reducing ICP and improving the CPP) (Asgeirsson et al., 1994). In relation to figure 5, it is a daunting task to speculate which patient will benefit from CPP or ICP targeted therapy. Additional tests or monitoring are obviously needed to facilitate this difficult task in deciding which patients are likely to tolerate CPP targeted therapy. Since multimodality monitoring does monitor various cerebral parameters (ICP, CPP and CBF) and at the same time allowing cerebral autoregulation test or monitoring to be done, therefore it serves as an appropriate monitoring approach. Multimodality monitoring can detect not only further increment in ICP values but also the presence of any compromise in CBF in patients who already have elevated, extremely elevated or lethal levels of ICP and still undergoing CPP targeted therapy.

5.1.2 Shift from compensatory to decompensatory or harmful compensatory phase – Role of multimodality monitoring

Intracranial hypertension (high ICP/low CPP) and reduction in CBF are two important parameters that determine the outcome of severely injured brain patients. Figure 2 illustrates cerebral ischaemic-hypoxic insults are the core events that trigger various vicious circles, including intracranial hypertension. Therefore, CBF monitoring should be incorporated into the standard care for severely injured brain patients in the NIC. The multimodality monitoring for the ICP and CBF offers crucial information on the status of

cerebral oxygenation, nutrition and function. The combination of various monitored parameters can cross-validate one another and can give indications as to the phase the brain is in for the managing clinicians (figure 5). Patients who are in the compensatory phase with raised ICP and undergoing CPP targeted therapy may not yet be a candidate for decompressive craniectomy or barbiturate therapy as long as the ICP is kept stable and CBF parameters are within an acceptable range. However, those with deteriorating CBF parameters (figure 5, labelled A,B and C) and/or persistently raised ICP (figure 5, labelled A,B,C,D,E and F) may be considered for above mentioned end-tier therapy. In summary, besides its potential to safe guard the CPP targeted therapy, multimodality monitoring of the severely injured brain patients aids clinicians to decide on the appropriate timing for end-tier therapy, decomperessive craniectomy or barbiturate therapy.

6. Measuring the two major parameters: ICP and CBF

The multimodality monitoring can be viewed as way of monitoring two important parameters that play significant roles in the pathogenesis of ischaemic-hypoxic events, namely, the ICP and CBF.

6.1 Intracranial pressure

The ICP is normally monitored until an acceptable upper limit of 20 mmHg (Balestreri et al., 2006). This is attained by using either ventricular catheter if the ventricles are opened or a compliance monitor (Spiegelberg GmbH & Co., Hamburg, Germany) or an intraparenchymal ICP, using Codman (Codman, Randolph, MA, USA) or Camino transducers (Camino Laboratories, San Diego, CA)(Idris et al., 2007; Isa et al., 2003). If the ICP values persistently read more than 20 mmHg and/or direct calculation of cerebral perfusion pressure (CPP) appears less than 50 mmHg, draining of CSF is warranted. If the ICP remains high, other modes of therapy such as adding muscle relaxants, giving mannitol or hypertonic saline, temporary hyperventilation, hypothermia with cooling blanket and fans are usually considered. At this stage, further investigation with CT brain is justifiable provided that these additonal steps fail to reduce the ICP values. The subsequent treatment should be based on the new imaging and monitored parameters. Generally, if the CT brain revealed no new surgical lesion but the brain appeared swollen, the ICP values remained high (or CPP values remained low) and/or presence of abnormal cerebral blood flow parameters, the patient should then be treated with either one of the following:

1. Bifrontal decompressive craniectomy and dura augmentation for diffuse brain swelling without midline shift, or
2. Unilateral wide decompressive craniectomy and dura augmentation for diffuse brain swelling and presence of midline shift, or
3. Barbiturate therapy until burst suppression attained on continuous EEG monitoring. Nonetheless, if the ICP values remain persistently high, decompressive craniectomy can still be considered.

For patients with persistently low CPP values without a corresponding raised ICP, decompressive craniectomy surgery or barbiturate therapy is not yet indicated. Instead, these patients should be treated with inotropes and/or hypervolumic therapy to increase CPP values. If by implementing CPP targeted therapy and the ICP started to increase, then the end-tier therapy should now be considered.

6.2 Cerebral blood flow

CBF can be measured in various ways. Measuring CBF can be classified into 4 ways:

a. Direct measurement of CBF
b. Indirect measurement of CBF
c. Quantitative measurement of CBF
d. Qualitative measurement of CBF

Quantitative and qualitative measurements are employed mainly for research purposes and hardly used during monitoring of severely brain injured patients in NIC. Examples include using radioisotope or tracer to measure CBF with imaging modality such as PET or SPECT scan.

A) Direct measurement of CBF

The direct measurement of CBF can be made by means of thermal diffusion techniques (Saber 2000) and LASER Doppler Flowmetry (LDF) which measure the local or regional CBF.

1. Thermal diffusion technique (Saber 2000)

The thermal diffusion measurement of cortical CBF is based on the thermal conductivity of cortical tissue (Carter et al., 1982; Dickman et al., 1991). The Saber 2000 regional cerebral blood flow (rCBF) sensor (flowtronics, Phoenix, AZ) consists of two small flat gold plates, one of which is neutral and the other heated, is placed through a burr hole or craniotomy on a cortical region of interest. The rCBF is estimated from the temperature difference between these two plates. Then, a microprocessor continuously converts the temperature difference to CBF in ml/100g/min. In practice, the sensor measures cortical area of approximately 1 cm deep in the cerebral hemisphere and 3 cm by implication. Its sensor plates are 24-k gold, whereas its wires are solid silver and teflon-coated. Although rCBF is not representative of blood flow in the whole hemisphere or in deeper regions of the brain, continuous measurement of relative changes in days after severe head injury may be of great value in guiding clinical management as an indirect measurement of perfusion status of the brain. Normal rCBF measured by Saber 2000 should be ≥ 30 ml/100g/min.

Fig. 8. Saber 2000 measures regional cerebral blood flow

2. LASER Doppler Flowmetry (LDF)

LDF measures continuously the local microcirculatory blood flow. The method is based on the principle of doppler shift. Monochromatic LASER light reflected from stationary tissue remains unchanged in frequency, but when it is reflected from moving blood cells, it

undergoes a frequency shift and therefore light reflected from the microcirculation measures the movements of red blood cells (Meyerson et al., 1991). The light is carried by a fibreoptic probe and the reflected light is converted into an electrical signal from which several values are derived. The flux (flow unit) represents the movement and concentrations of blood cells through the microvasculature. It is derived from the concentration of moving blood cells (CMBC) in the measured volume multiplied by the mean velocity. All three values, flux, CMBC and velocity are continuously recorded and displayed by the computer (Bolognese et al., 1993). LDF measures the local flow within a very small sample volume of approximately 1 mm^3. LDF can also be used to measure the state of cerebral autoregulation via CO_2 reactivity.

B) Indirect measurement of CBF

The indirect measurement of CBF consists of near infrared spectroscopy (NIRS), jugular bulb venous oximetry ($SjVO_2$), transcranial doppler sonography (TCD), cerebral interstitial tissue oxygen ($PtiO_2$) and carbon dioxide tension ($PtiCO_2$), brain pH and temperature, intracerebral microdialysis and electrical function monitoring.

1. Near infrared spectroscopy (NIRS)

It is a non invasive monitoring that detects changes in brain oxygenation, CBF and cerebral blood volume. NIRS depends upon the relative transparency of biological tissue to light in the near-infrared spectrum. The absorption of oxyhemoglobin, deoxyhemoglobin and oxidized cytochrome can be detected transcranially. In adults, only certain brain areas can be monitored because of bone thickness produces a scattering of the light spectrum (Elwell et al., 1993). This made NIRS less accurate and only focal measurement is feasible.

2. Jugular bulb venous oximetry ($SjVO_2$)

A 5G fibreoptic oxygen saturation catheter is inserted percutaneously in a retrograde direction into the internal jugular vein. The tip of the catheter must be placed within the dominant jugular bulb which is determined by separate test compression on each internal jugular vein and note an increment in ICP values. Prior to monitoring, its position must be verified by the mastoid x-ray. $SjVO_2$ can be used to calculate arteriovenous difference or cerebral oxygen extraction ($AVDO_2$) and to monitor cerebral oxygenation. Both determine the status of cerebral metabolic rate for oxygen ($CMRO_2$) and CBF which are coupled when under physiological conditions. The relationships between CBF, $CMRO_2$ and $AVDO_2$ can be summarised by the formula $CMRO_2 = CBF \times AVDO_2$. The normal values for $AVDO_2$ is approximately 6.5 ml O_2/100 ml blood, for $CMRO_2$ is 3.2 ml O_2/100g/min and for cerebral oxygenation ranges between 50% - 75% (Idris et al., 2007; Muizelaar & Schroder, 1994). Ischaemic brain with low CBF exhibits low $SjVO_2$ reading of less than 50%, whereas for the hyperaemic brain, the reading is more than 75%. Similarly, in brain dead patient where no uptake of oxygen occurred, the $SjVO_2$ would also be higher than 75%.

Fig. 9. Jugular bulb venous oximetry monitor

3. Transcranial doppler sonography (TCD)

Transcranial doppler provides a mean of measuring relative changes in CBF by observing blood flow velocity (FV) in basal cerebral arteries, mostly on middle cerebral artery (MCA) or anterior cerebral artery (ACA) (Muttaqin et al., 1993). It uses doppler shift as a basic principle. This shift is monitored intermittently by using a 2 MHz pulsed TCD ultrasonography probe (model MultiDop 2; DWL Electronishe System GmbH, Sipplingen, Germany) inserted at depths of between 4 to 6 cm. TCD measures the velocity of red blood cell moving within a vessel, quoted in cm/sec. In contrast, CBF is a three dimensional measure of volume of blood delivered per unit of cerebral tissue per unit of time, quoted as ml/100g/min. As the two parameters are quantitatively different, the correlation between absolute values of FV and CBF is poor. However, in normal individuals, the cross-sectional area of the insonated blood vessels remains constant, hence, the CBF and FV should vary directly with one another. In traumatic brain injury, the blood vessel diameter may be altered by changes in ICP (altered autoregulation and vascular pulsation) and/or the possible effect of vasospasm, reducing the reliability of the relationship between relative CBF and FV. This variable relationship between the absolute FV and CBF is a clear disadvantage, as reflected by the normal variation in MCA FV of between 35 – 90 cm/sec in the awake resting state. Thus caution is needed in interpreting TCD data in its estimate of CBF. Abnormally low (ischaemic brain) or high (vasospasm or hyperaemic brain) FV must be interpreted carefully and preferably in conjunction with other CBF monitoring parameters (Idris et al., 2007; Isa et al., 2003).

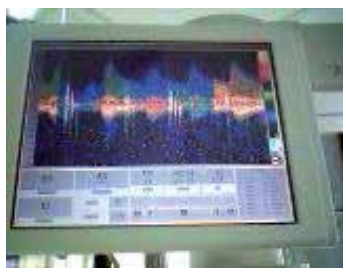


Fig. 10. Continuous transcranial doppler sonography monitoring in neurointensive care

4. Cerebral interstitial tissue oxygen tension ($PtiO_2$), carbon dioxide ($PtiCO_2$), pH and temperature measured by Licox or Neurotrend/Paratrend system.

Neurotrend (old system called Paratrend 7™) system (Codman, Johnson & Johnson, Raynham, MA, USA) is an effective method of measuring tissue cerebral oxygen tension ($PtiO_2$), along with carbon dioxide tissue levels ($PtiCO_2$), brain pH and brain temperature (Charbel et al., 1997). On the other hand, Licox system (GMS, Kiel-Mielkendorf, Germany) is capable in measuring only PtiO2 and brain temperature. The importance of CBF to maintain neuronal viability is well known. In brain tissue, this can be defined as maintaining tissue oxygenation and a metabolic environment for oxidative energy production and therefore for optimal neuronal function. Patients with brain ischaemia secondary to head injury are thought to have changes in brain tissue partial pressure for oxygen (Po_2) and carbon dioxide (Pco_2), pH and temperature. During ischaemia, the Po_2 decreases due to increased consumption, Pco_2 increases due to decreased clearance and the interstitial tissue environment is acidic with falling pH and accumulation of toxic metabolites. The system can provide continuous quantitative measurements of regional brain. Normally, the sensor is

inserted into peri-contusional, peri-infarted, peri-haemorrhagic or peri-lacerated brain areas. The purpose of measuring these parameters is to safeguard the ischaemic or penumbra areas of brain which are compromised and the measured abnormal parameters could reflect the globally threatened CBF. Normal values for these parameters have been acquired experimentally, whereby in normal individuals $PtiO_2$ ranges between 37 to 48 mmHg. However, hypoxia of brain tissue has been reported to occur when $PtiO_2$ is < 15 mmHg (Hoffman et al., 1996; van Santbrink et al., 1996). $PtiCO_2$ normal values is within the range of 49 to 59 mmHg, normal brain pH is 7 to 7.16 and normal brain temperature is 0.3 to 0.5 higher than the normal range of core body temperature which is within 36 to 39°C (the ideal core body temperature is the midpoint of this range, that is 37.7°C) (Hoffman et al., 1996; Sakai et al., 2011; Zauner et al., 1995). Brain temperature of healthy person denotes the balance between two processes: cerebral metabolism and CBF. Under normal conditions, the brain metabolises glucose via the Krebs cycle to carbon dioxide, water and adenosine triphosphate (ATP). Cells in the brain use ATP as energy resource and consequently generate heat. $CMRO_2$ and cerebral metabolic rate for glucose (CMRglu) are the factors that directly related to heat production in the brain. Conversely, the heat is removed by CBF. Therefore, in presence of ischaemia (reduction in CBF), the brain temperature is higher than normal (Sakai et al., 2011).

Fig. 11. A: Neurotrend and B: Licox system. Neurotrend is capable of measuring brain temperature, brain pH, $PtiO_2$, $PtiCO_2$ and HCO_3 whereas Licox only measures $PtiO_2$ and brain temperature.

5. Intracerebral microdialysis

Intracerebral microdialysis (CMA70 and CMA 71 catheters and CMA600 and ISCUS analyser; CMA Microdialysis AB, Solna, Sweden) enables endogenous substances in the extracellular fluid of the brain to be retrieved. Excitatory amino acids, neurotransmitter, electrolytes and energy-related metabolites are released in abnormal quantities after severe head injury. There would be an increase in glutamate, glycerol, aspartate, K^+ and lactate/pyruvate ratio and a decrease in glucose level in head injured patient with cerebral ischaemia (Timofeev et al., 2011). Their continuous measurements via dialysis principle can help the neurointensivist to correlate their findings with imaging of the brain, other monitored parameters and patient conditions to decide on the appropriate management.

6) Electrical function monitoring and bispectral index (BIS)

Electrophysiological monitoring is a measurement of brain electrical function which is regarded as vital in nervous communication. The electroencephalogram (EEG) and evoked potentials such as visual evoked potential (VEP), brainstem auditory evoked potential (BAEP) and somatosensory evoked potential (SSEP) can give valuable information

regarding the status of CBF and metabolism. The normal CBF is 45 to 55 ml/100g/min, and the electrical function is impaired (isoelectric silence) if CBF is < 23 ml/100g/min with irreversible damage occurs if CBF is < 8 ml/100g/min. Deterioration in their values correspond to reduced levels of transcranial oxygen extraction and hence CBF (Jordan, 1993; Procaccio et al., 2001). Besides, continuous bedside EEG monitoring is the best method for detecting non-convulsive seizures and presence of significant amount of background slowing frequencies informs the neurointensivist regarding the depth of coma and prognosis of the severely injured brain patients.

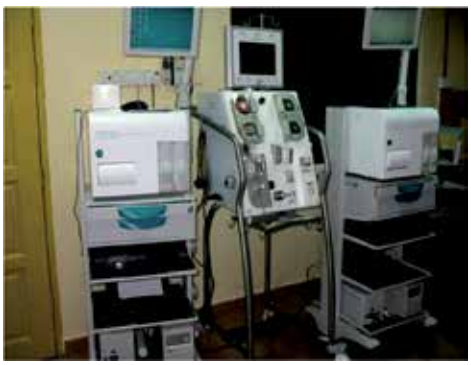


Fig. 12. Intracerebral microdialysis.

The bispectral index (BIS) of the electroencephalogram is a weighted sum of electroencephalographic subparameters containing time domain, frequency domain and higher-order spectral information, which are optimized to correlate maximally with clinical signs of hypnosis (Fabregas et al., 2004) and CBF (Myles, 2009). BIS system is patched onto the forehead of patients. The aim is to keep the general anaesthesia effect or adequate hypnotic effect at 40 – 60% (Gan et al., 1997). The targeted values indicate adequacy of muscle relaxants and sedation to reduce the cerebral metabolic rate and ICP. Inadequacy (higher values) indicates higher titrating dose of sedative medications is required and equally important is to realise that an abnormally low or presence of sudden deterioration in BIS values should alert the neurointensivist for possible CBF compromise. However, this should be interpreted carefully with other cerebral monitoring to realise its importance.

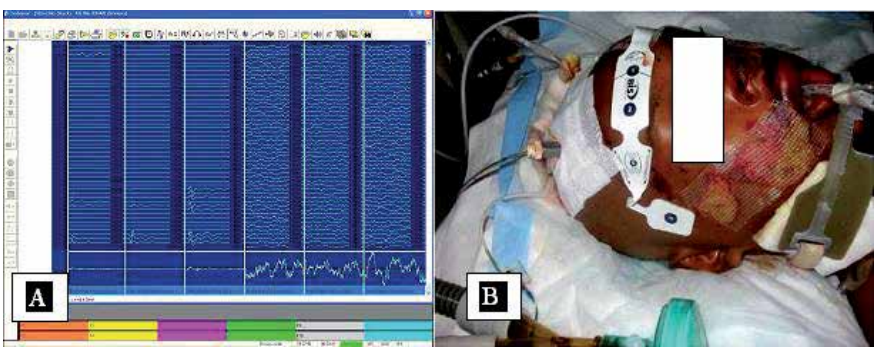


Fig. 13. A: Electrical function monitoring and B: The BIS system was patched onto the patient's forehead.

7. Interpretation of data and management steps

In general, two parameters are monitored during multimodality monitoring for severe head injury: ICP and CBF (Idris et al., 2007). Combination of these two important parameters help neurointensivist to know the status of brain autoregulation, either impaired or intact (cerebrovascular reactivity index or PRx)(Smielewski et al., 1997). Therefore, based upon this observation, the clinician can opt for ICP or CPP targeted therapy. ICP targeted therapy aims to reach ICP < 20 mmHg and CPP targeted therapy aims CPP > 50 mmHg. The commonly targeted CPP is 60 - 70 mmHg, but when managing patients with impaired cerebral autoregulation, the targeted CPP should be kept < 50 – 60 mmHg. Recently, Johnson studied status of cerebral autoregulation, targeted CPP and clinical outcome for trauma patients and found that in patients with impaired cerebrovascular autoregulation, the favorable outcome was noted when CPP kept less than 50 - 60 mmHg (Johnson et al., 2011). Besides ICP (ICP is a factor in pathophysiology of midline shift, mass effect, herniation syndromes), monitoring CBF (CBF is a factor in pathophysiology of ischaemic-hypoxic damage or cerebral infarction) is also important and plays significant role in determining patient's outcome as depicted in figure 2 and discussed in details above. Since currently there is still no optimal and practical method to be used in NIC for measuring CBF, other methods of monitoring (besides ICP) in 'multimodality monitoring' are trying to estimate the CBF values for either the whole brain (CPP, $SjVO_2$, TCD, electrical function, EEG and BIS) or for only at certain region of the brain (Saber 2000, LDF, NIRS, Neurotrends and cerebral microdialysis). The main goal of these modalities can be viewed as to identify early and at a reversible stage either intracranial hypertension or ischaemic-hypoxic insults (other goal is to identify subclinical seizures as discussed above).

In term of CBF (management for ICP or CPP has been discussed previously), the clinician effort consists of several aims: to optimize CBF to all regions of the brain whilst to prevent expansion of intracranial haematoma or contusion and to restore perfusion of the penumbra area. Regional CBF (rCBF) can be measured with many methods as mentioned earlier. The Saber 2000 which can be laid onto the area of interest seems more appropriate when the clinician wants to safeguard the peri-contusional, peri-haematoma or peri-ischaemic areas. The combination of Saber 2000 rCBF monitoring with microdialysis, Licox or Neurotrends system would certainly be a helpful approach. Reduction or abnormality in almost all parameters would validate one another and hint corrective measures for the clinician to take. Similarly, the availability of information on the more globalised CBF (gCBF) monitorings such as $SjVO_2$, would indicate any compromise to CBF on a more global basis to instigate appropriate corrective actions to prevent ischaemia from inflicting more areas of the brain. During ischaemic insults, if both rCBF (Saber 2000 CBF < 30 ml/100g/min; $PtiO_2$ < 15 mmHg; $PtiCO_2$ > 59 mmHg, hyperthermia, acidotic profiles on pH or microdialysis) and gCBF ($SjVO_2$ of decreasing trends or persistent values of less than 50%, abnormal EEGs, low value of TCDs) are abnormal, cerebral hypoperfusion is the likeliest suspicion. Further actions are needed to try and correct these abnormalities, especially for correctable causes. For instance, CBF may be low because of low in mean arterial pressure (MAP) due to the new onset cardiac abnormalities such as abnormal rhythms or abnormal stroke volume. Other related possibilities include overdosage or side effects of drugs such as anti-epileptics or overzealous use of inotropic drugs, new onset pneumothorax, cerebral or cardiac embolisation or cerebral vessels vasospasm. TCD monitoring of MCAs, ACAs and other major intracranial or neck vessels can readily diagnose vasospasm. Contradictory values

between TCDs and the stated parameters discussed in earlier sections would alert the clinician towards cerebral vasospasm as the aetiology for cerebral hypoperfusion (high TCD but reduce CBF parameters). Triple-H therapy (includes expanding the plasma volume or hypervolemia, hemodilution and importantly hypertension), nimodipine or urgent interventional angiography for vasospasm should then be attempted to treat the identified problem. If only rCBF is impaired, steps should be taken to augment blood flow to those compromised areas only. However, currently, there is limited choice available for clinician to optimize rCBF. Most of the corrective measures for rCBF are in fact lending to optimize the gCBF. By augmenting the gCBF, the clinician may face notorious effect of 'hyperaemic insult' which in cases with markedly impaired cerebral autoregulation can be associated with brain swelling or odema and further elevation in ICPs that can aggravate the ischaemic-hypoxic circle. Therefore, multimodality monitoring offers more parameters to be closely considered when practicing 'CPP targeted therapy'. By pushing the CPP, the clinician should look at $SjVO_2$ which can become more than 75% suggesting cerebral hyperaemia and patterns of TCDs values which can become abnormally high. The aggressive CPP targeted therapy should not stop unless there is simultaneous elevation in ICPs, especially with parallel gradual emergence of deterioration in other monitored parameters (especially from Licox or Neurotrends and cerebral microdialysis), suggesting presence of markedly impaired cerebral autoregulation and evolving brain swelling which finally ends up with reduction in CBF in 'that particular patient'. If ICPs become elevated (with other abnormal parameters suggesting/confirming reliable ICPs), the CPP targeted therapy should be stopped and measures such as lowering the MAP, reduction in plasma volume or inducing diuresis (with mannitol or diuretics), ICP lowering measures (such as mannitol or hypertonic saline, draining of CSF, adding or increasing sedative agent or muscle relaxant) should be taken. Decompressive craniectomy or barbiturate therapy should be reserved for patients who failed to respond to the initial measures and displayed concurrent features of time shift from compensatory to harmful compensatory or decompensatory phase.

8. Malaysia experience in multimodality monitoring

Our initial study published in 2003 on outcome of severe traumatic brain injury comparing the 3 monitoring approaches (multimodality, ICP alone and no cranial monitoring) revealed a statistically significant difference in the proportions of good outcomes between the multimodality group compared with the group of patients that underwent a single intracranial-based monitoring method and the group that received no monitoring ($p = 0.003$) based on a disability rating scale after a follow up of 12 months. Death was the focus of outcome in this study in which the multimodality approach to monitoring had superior results (Isa et al., 2003). The subsequent studies done in our centre were prognostic and cost effectiveness analysis studies of using different monitoring modalities in treating severe traumatic brain injury which were completed in 2004 and published in 2007 (Ibrahim et al., 2007; Idris et al., 2007).

8.1 Prognostic and cost effectiveness analysis studies of using different monitoring modalities in treating severe traumatic brain injury

Between June 2002 and March 2004, 52 adult patients with severe traumatic non penetrating head injury were recruited into our prospective randomised study at our University

Hospital, Universiti Sains Malaysia. The aim of the study was to investigate whether multimodality monitoring in severe traumatic brain injury would alter the outcome score. All adult patients, age of more than 14 years old with traumatic non penetrating head injury who had GCS of less than 9 and CT brain without infratentorial pathology were included. We excluded those who had a unilateral or bilateral fixed and dilated pupils resulted from an on-going herniation, brain dead patient and patient known to have any condition that lowering his or her functional status score. The randomisation process was made to allocate to either multimodality monitoring or to standard ICP monitoring. In the multimodality group, we monitored basic intensive care parameters and multiple cerebral parameters. In the standard ICP monitoring group, only ICP was monitored together with basic intensive care parameters. Monitoring proceeded for at least 3 days if uneventful. We recorded the outcome at 6 months post treatment using the Barthel index score.

The sixth month's outcome between those groups (26 patients in each group) was not statistically significant ($p < 0.48$). However, the percentage of cases independent at 6 months was higher in the multimodality group compared with single modality group, 21.2% and 17.3% respectively. The multimodality monitoring group had also lower percentage of dependent cases at 6 months in comparison to the single ICP modality group, 28.8% and 32.7% respectively. The univariate analysis revealed, age ($p < 0.03$), GCS on arrival ($p < 0.01$), 24 hours fluid balance at day 2 of monitoring ($p < 0.01$), serum sodium ($p < 0.03$) and intracranial pressure at day 3 of monitoring ($p < 0.01$) were correlated with the outcomes. The trends of the parameters disclosed younger, higher GCS, lower injury severity score and Marshall grade would have a higher chance to be independent at 6 months post trauma. Tachycardic, hyperthermic, hypo- and hyper-volumic patients during the first 3 days of monitoring tend to be dependent at 6 months post injury. The dependent patients also tend to be acidotic or alkalotic for their arterial blood gases, have P_aco_2 of < 20 mmHg or P_aco_2 > 45 mmHg, blood haemoglobin level of < 8 g/dl, high blood urea and sodium, have persistently high ICP of more than 20 mmHg, CPP of < 55 mmHg, $SjVO_2$ of < 50% or > 75%, rCBF < 35 ml/100g/min, TCD MCA flow velocity of < 35 cm/sec and $PtiO_2$ of < 15 mmHg. Despite no significant statistical difference between the multimodality and single modality group, a possiblity of better outcome obtained with multimodality monitoring technique remains because of higher cases with independent status observed in that group. Obviously, this requires larger sample size for future study (Idris et al., 2007). We also did a study on cost effectiveness analysis of using multiple neuromodalities in treating severe traumatic brain injury. Despite a higher cost in multimodality monitoring, the application of multimodality monitoring for severe traumatic brain injury was more cost-effective (considering the cost of the equipment and the clinical outcome of the studied patients) than baseline neuromonitoring ($p < 0.001$)(Ibrahim et al., 2007).

8.2 Limitation of multimodality monitoring

Despite various available cerebral monitoring, the current limitation in managing severely injured brain is the therapy to optimize all monitored parameters. Except for high ICP, we are yet to have any accepted standard for treating abnormalities detected by CBF/CPP monitoring. Future works should focus on improving these diagnostic monitoring tools as well as ways to treat or correct those detected abnormalities. Research on therapy is continuing and one of the potential therapies is cerebral hypothermia.

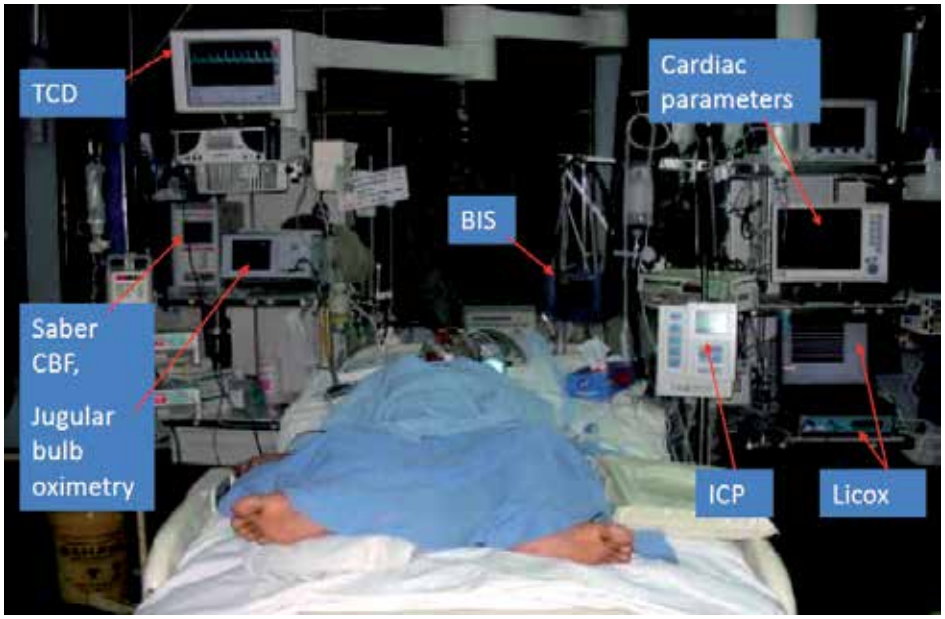


Fig. 14. Multimodality monitoring for a patient with severe traumatic brain injury.

9. The future of monitoring and managing the severely injured brain – multimodality monitoring and cerebral hypothermia

Figure 2 illustrates various pathways that promote cerebral ischaemic-hypoxic events which can lead to cell death or apoptosis. Interestingly, one can take note that these pathways are mostly temperature dependent and therefore can be mitigated with cerebral hypothermia. In 2006, Vincent and Polderman reviewed the neuroprotective mechanisms underlying hypothermia and listed out various benefits of hypothermic therapy (Vincent & Polderman, 2006). Hypothermia can inhibit the activation of caspase enzymes, prevents or mitigates mitochondrial dysfunction, decrease the metabolism as well as decrease the overload of excitatory neurotransmitters such as glutamate and free oxygen radicals, modify the cellular disorders of intracellular ion concentrations, suppress the inflammatory and immunological responses and epileptic activity, reduce the disruption in blood brain barrier, vascular permeability and oedema, improve the microcirculatory circuits and intra- and extra-cellular acidosis, correct the hyperthemia after brain injury, influence the local secretion of various vasoactive mediators secreted by the endothelium and enhance expression of immediate early genes and cold shock proteins. Building on the vast benefits of cerebral hypothermia, currently underway is a locally adapted approach to induce hypothermia in severely injured brain patients using a direct method and study their outcomes.

9.1 Our current study - Direct brain hypothermia for trauma patient with Glasgow coma score of 6 and 7

The objective

To determine the effect of direct focal brain cooling on outcomes of severely injured brain patients.

Materials and Methods

This is a randomized controlled study following an approval by the research and ethics committee of Universiti Sains Malaysia (USM). All adult patients with severe traumatic brain injury with GCS score of 6 - 7 and require decompressive craniectomy (DC) will be screened for eligibility to be recruited into this study. Once included, the patient will be randomized to either group A or B. Group A (treatment group) patients will receive therapy with direct focal brain cooling and group B is a control group, consists of patients who do not have direct focal brain cooling therapy. All patients will have ICP, Licox, BIS, EEGs and cardiac parameters monitoring during cooling period, and bloods will be drawn for immunological parameters prior to surgery and after local cooling therapy to the brain. This mode of multimodality monitoring and therapy will last for 24 - 48 hours, or longer if intracranial pressure parameters stays abnormal. The neurosurgical DC is a standard operation. A unilateral DC is planned for patients with the presence of ipsilateral pathology with midline shift and a bifrontal DC for brain swelling in patients with or without frontal pathology but in the absence of midline shift. The monitoring and therapy given after the surgery are the standard therapy for severely injured brain.

Direct focal brain cooling method is achieved through continual irrigation of the brain with cold Hartmann's solution in which the temperature of the infused fluid is divided into 2 groups as follows: 1. deep cooling (Hartmann's temperature of 20 - 29 degree celcius) and 2. mild cooling (Hartmann's temperature of 30 - 36 degree celcius). The cold Hartmann's solution is infused via neurojaf external ventricular drainage (EVD) with multiple modified-extra holes catheter which is placed superior to the dura flap and in contact with the surface of the brain. It acts like rain flushing through the surface of the swollen brain, and with pulsation of the brain, the cold fluid is expected to be distributed to other areas of the brain. The infusion rate is targeted at 500 mls of Hartmann's solution within 7 hours (70 mls/hr). Because of the position of the head in NIC, the second draining tube will be inserted at the lower part of the craniectomy flap outside the dura which at some areas, the closure is purposely made loose to drain the excess fluid with low suction pressure. The temperature of the infused Hartmann's solution is always checked via the three way connector, draining the fluid out to the collection port for temperature assessment. If temperature reading is under or above the intended value, new solution with correct intended temperature will replace the previous one. All patients will have CT scan done if the ICP shows persistently raised values despite standard therapies being given. This is important to exclude any new surgical lesions and to exclude the retention of infused solution as a cause of raised ICP. If the ICPs show normal values, the CT scan of the brain will be done after 48 hours of therapy prior to removal of the EVD tube to document the location of the EVD tip. The outcome assessment for this study is the Glasgow Outcome Score at discharge and at 6 months after trauma and requirement for tracheostomy together with number of days ventilated in NIC.

Preliminary Results

Based on the preliminary recruitment of 7 patients in each group, there was significant difference in outcome upon discharge with better outcome score for the cooling group ($p < 0.02$). In the cooling group, five patients had good GOS of 4 [independent status] and one patient attained dependent status with GOS of 3 [dependent]. The remaining one patient in this group who has an initial GOS of 3 had sudden onset aspiration pneumonia and died (GOS upon discharge scored as 1). On the contrary, five patients had GOS of 3 and two patients with GOS of 2 [all were dependent status] in the non cooling-control group. Furthermore, there were marked reductions in the ICP and brain temperature, and

improvement in $PtiO_2$ and CPP of patients who had received cooling treatment. There was also faster ventilator weaning in the cooling group with median duration of 7 days compared to the control group of 9 days duration. Detailed analysis is yet performed because this study is still ongoing.

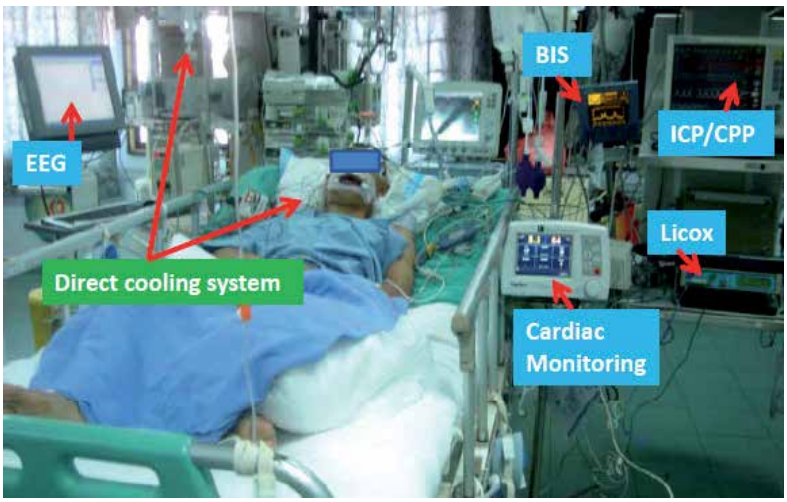


Fig. 15. The typical multimodality monitoring set up for the randomized controlled trial involving the management of the severely injured brain with hypothermia at our NIC, University Hospital, Universiti Sains Malaysia

10. Conclusion

The pathophysiology of the severe non-penetrating traumatic brain injury involves complex chain of events at molecular, cellular or vascular levels. These pathophysiological processes extend beyond the confine of the brain as they also involve other body systems such as the cardiopulmonary system. Understanding these complex mechanisms is vital in our efforts to expand the usefulness of multimodality monitoring for the severely injured brain patients. However monitoring alone will not greatly alter the outcome, therefore future research should also focus on the therapeutic aspects of ICP and CBF monitored parameters. Currently, multimodality monitoring of the brain together with therapeutic cerebral hypothermia hold leading promises in the monitoring and treatment of the severely injured brain patients.

11. References

Aaslid, R., Lindegaard, K. F., Sorteberg, W., & Nornes, H. (1989). Cerebral autoregulation dynamics in humans. *Stroke., 20*(1), 45-52.

Asgeirsson, B., Grande, P. O., & Nordstrom, C. H. (1994). A new therapy of post-trauma brain oedema based on haemodynamic principles for brain volume regulation. *Intensive Care Med., 20*(4), 260-267.

Balestreri, M., Czosnyka, M., Hutchinson, P., Steiner, L. A., Hiler, M., Smielewski, P., et al. (2006). Impact of intracranial pressure and cerebral perfusion pressure on severe disability and mortality after head injury. *Neurocrit Care., 4*(1), 8-13.

Balestreri, M., Czosnyka, M., Steiner, L. A., Schmidt, E., Smielewski, P., Matta, B., et al. (2004). Intracranial hypertension: what additional information can be derived from ICP waveform after head injury? *Acta Neurochir (Wien). 146*(2), 131-141. Epub 2004 Feb 2002.

Bellner, J., Romner, B., Reinstrup, P., Kristiansson, K. A., Ryding, E., & Brandt, L. (2004). Transcranial Doppler sonography pulsatility index (PI) reflects intracranial pressure (ICP). *Surg Neurol., 62*(1), 45-51; discussion 51.

Berger, S., Schurer, L., Hartl, R., Messmer, K., & Baethmann, A. (1995). Reduction of post-traumatic intracranial hypertension by hypertonic/hyperoncotic saline/dextran and hypertonic mannitol. *Neurosurgery., 37*(1), 98-107; discussion 107-108.

Bolognese, P., Miller, J. I., Heger, I. M., & Milhorat, T. H. (1993). Laser-Doppler flowmetry in neurosurgery. *J Neurosurg Anesthesiol., 5*(3), 151-158.

Bouma, G. J., Muizelaar, J. P., Stringer, W. A., Choi, S. C., Fatouros, P., & Young, H. F. (1992). Ultra-early evaluation of regional cerebral blood flow in severely head-injured patients using xenon-enhanced computerized tomography. *J Neurosurg., 77*(3), 360-368.

Bullock R, C. R., Clifton C,, Ghajar J, M. D., Narayan RK,, Newell DW, P. L., Rosner MJ,, & JW, W. (1996). Guidelines for the management of severe head injury. Brain Trauma Foundation, American Association of Neurological Surgeons, Joint Section on Neurotrauma and Critical Care. *J Neurotrauma., 13*(11), 641-734.

Carandang, R., & Krieger, D. (2008). Decompressive Hemicraniectomy and Durotomy for Malignant Middle Cerebral Artery Infarction. *Neurocritical Care, 8*(2), 286-289.

Carter, L. P., Erspamer, R., White, W. L., & Yamagata, S. (1982). Cortical blood flow during craniotomy for aneurysms. *Surg Neurol., 17*(3), 204-208.

Chan, K. H., Miller, J. D., Dearden, N. M., Andrews, P. J., & Midgley, S. (1992). The effect of changes in cerebral perfusion pressure upon middle cerebral artery blood flow velocity and jugular bulb venous oxygen saturation after severe brain injury. *J Neurosurg., 77*(1), 55-61.

Charbel, F. T., Hoffman, W. E., Misra, M., Hannigan, K., & Ausman, J. I. (1997). Cerebral interstitial tissue oxygen tension, pH, HCO3, CO2. *Surg Neurol., 48*(4), 414-417.

Chen, S. T., Hsu, C. Y., Hogan, E. L., Halushka, P. V., Linet, O. I., & Yatsu, F. M. (1986). Thromboxane, prostacyclin, and leukotrienes in cerebral ischemia. *Neurology., 36*(4), 466-470.

Cooper, D. J., Rosenfeld, J. V., Murray, L., Wolfe, R., Ponsford, J., Davies, A., et al. (2008). Early decompressive craniectomy for patients with severe traumatic brain injury and refractory intracranial hypertension--A pilot randomized trial. *Journal of Critical Care, 23*(3), 387-393.

Csuka, E., Morganti-Kossmann, M. C., Lenzlinger, P. M., Joller, H., Trentz, O., & Kossmann, T. (1999). IL-10 levels in cerebrospinal fluid and serum of patients with severe traumatic brain injury: relationship to IL-6, TNF-alpha, TGF-beta1 and blood-brain barrier function. *J Neuroimmunol., 101*(2), 211-221.

Czosnyka, M., Smielewski, P., Piechnik, S., Steiner, L. A., & Pickard, J. D. (2001). Cerebral autoregulation following head injury. *J Neurosurg., 95*(5), 756-763.

Dickman, C. A., Carter, L. P., Baldwin, H. Z., Harrington, T., & Tallman, D. (1991). Continuous regional cerebral blood flow monitoring in acute craniocerebral trauma. *Neurosurgery., 28*(3), 467-472.

Dietrich, W., & Bramlett, H. (2010). The evidence for hypothermia as a neuroprotectant in traumatic brain injury. *Neurotherapeutics, 7*(1), 43-50.

Dirnagl, U., Iadecola, C., & Moskowitz, M. A. (1999). Pathobiology of ischaemic stroke: an integrated view. *Trends in Neurosciences, 22*(9), 391-397.

Eberle, B. M., Schnüriger, B., Inaba, K., Peter Gruen, J., Demetriades, D., & Belzberg, H. (2010). Decompressive craniectomy: Surgical control of traumatic intracranial hypertension may improve outcome. *Injury, 41*(9), 894-898.

Egnor, M., Zheng, L., Rosiello, A., Gutman, F., & Davis, R. (2002). A model of pulsations in communicating hydrocephalus. *Pediatr Neurosurg., 36*(6), 281-303.

Elwell, C. E., Owen-Reece, H., Cope, M., Wyatt, J. S., Edwards, A. D., Delpy, D. T., et al. (1993). Measurement of adult cerebral haemodynamics using near infrared spectroscopy. *Acta Neurochir Suppl (Wien). 59*, 74-80.

Fabregas, N., Gambus, P. L., Valero, R., Carrero, E. J., Salvador, L., Zavala, E., et al. (2004). Can bispectral index monitoring predict recovery of consciousness in patients with severe brain injury? *Anesthesiology., 101*(1), 43-51.

Fabricius, M., Fuhr, S., Willumsen, L., Dreier, J. P., Bhatia, R., Boutelle, M. G., et al. (2008). Association of seizures with cortical spreading depression and peri-infarct depolarisations in the acutely injured human brain. *Clin Neurophysiol., 119*(9), 1973-1984. Epub 2008 Jul 1914.

Figaji, A., Fieggen, A., Sandler, S., Argent, A., Le Roux, P., & Peter, J. (2007). Intracranial pressure and cerebral oxygenation changes after decompressive craniectomy in a child with traumatic brain swelling. *Child's Nervous System, 23*(11), 1331-1335.

Gan, T. J., Glass, P. S., Windsor, A., Payne, F., Rosow, C., Sebel, P., et al. (1997). Bispectral index monitoring allows faster emergence and improved recovery from propofol, alfentanil, and nitrous oxide anesthesia. BIS Utility Study Group. *Anesthesiology., 87*(4), 808-815.

Gluckman, P. D., Wyatt, J. S., Azzopardi, D., Ballard, R., Edwards, A. D., Ferriero, D. M., et al. (2005). Selective head cooling with mild systemic hypothermia after neonatal encephalopathy: multicentre randomised trial. *The Lancet, 365*(9460), 663-670.

Grzybicki, D., Moore, S. A., Schelper, R., Glabinski, A. R., Ransohoff, R. M., & Murphy, S. (1998). Expression of monocyte chemoattractant protein (MCP-1) and nitric oxide synthase-2 following cerebral trauma. *Acta Neuropathol., 95*(1), 98-103.

Gura, M., Elmaci, I., Sari, R., & Coskun, N. (2011). Correlation of pulsatility index with intracranial pressure in traumatic brain injury. *Turk Neurosurg, 21*(2), 210-215.

Hausmann, E. H., Berman, N. E., Wang, Y. Y., Meara, J. B., Wood, G. W., & Klein, R. M. (1998). Selective chemokine mRNA expression following brain injury. *Brain Res., 788*(1-2), 49-59.

Hoffman, W. E., Charbel, F. T., & Edelman, G. (1996). Brain tissue oxygen, carbon dioxide, and pH in neurosurgical patients at risk for ischemia. *Anesth Analg., 82*(3), 582-586.

Ibrahim, M. I., Abdullah, M., Naing, L., Abdullah, J. M., Idris, Z., & Aljunid, S. M. (2007). Cost effectiveness analysis of using multiple neuromodalities in treating severe traumatic brain injury in a developing country like Malaysia. *Asian J Surg., 30*(4), 261-266.

Idris, Z., Ghani, R. I., Musa, K. I., Ibrahim, M. I., Abdullah, M., Nyi, N. N., et al. (2007). Prognostic Study of Using Different Monitoring Modalities in Treating Severe Traumatic Brain Injury. *Asian Journal of Surgery, 30*(3), 200-208.

Is, M., Coskun, A., Sanus, G. Z., Tanriverdi, T., Kafadar, A. M., Hanimoglu, H., et al. (2007). High-sensitivity C-reactive protein levels in cerebrospinal fluid and serum in severe head injury: relationship to tumor necrosis factor-alpha and interleukin-6. *J Clin Neurosci., 14*(12), 1163-1171. Epub 2007 Sep 1119.

Isa, R., Wan Adnan, W. A., Ghazali, G., Idris, Z., Ghani, A. R., Sayuthi, S., et al. (2003). Outcome of severe traumatic brain injury: comparison of three monitoring approaches. *Neurosurg Focus., 15*(6), E1.

Johnson, U., Nilsson, P., Ronne-Engstrom, E., Howells, T., & Enblad, P. (2011). Favorable outcome in traumatic brain injury patients with impaired cerebral pressure autoregulation when treated at low cerebral perfusion pressure levels. *Neurosurgery., 68*(3), 714-721; discussion 721-712.

Jordan, K. G. (1993). Continuous EEG and evoked potential monitoring in the neuroscience intensive care unit. *J Clin Neurophysiol., 10*(4), 445-475.

Juliet, P. A., Mao, X., & Del Bigio, M. R. (2008). Proinflammatory cytokine production by cultured neonatal rat microglia after exposure to blood products. *Brain Res., 1210*, 230-239. Epub 2008 Mar 2018.

Kaulfers, A. M., Backeljauw, P. F., Reifschneider, K., Blum, S., Michaud, L., Weiss, M., et al. (2010). Endocrine dysfunction following traumatic brain injury in children. *J Pediatr, 157*(6), 894-899. Epub 2010 Aug 2024.

Kazibutowska, Z., Stelmach-Wawrzyczek, M., & Majchrzak, R. (1992). [Results of prospective 24-hour EEG studies of patients after cranio-cerebral injuries]. *Neurol Neurochir Pol., 26*(3), 304-310.

Kossmann, T., Stahel, P. F., Lenzlinger, P. M., Redl, H., Dubs, R. W., Trentz, O., et al. (1997). Interleukin-8 released into the cerebrospinal fluid after brain injury is associated with blood-brain barrier dysfunction and nerve growth factor production. *J Cereb Blood Flow Metab., 17*(3), 280-289.

Kouvarellis, A. J., Rohlwink, U. K., Sood, V., Van Breda, D., Gowen, M. J., & Figaji, A. A. (2011). The relationship between basal cisterns on CT and time-linked intracranial pressure in paediatric head injury. *Childs Nerv Syst, 27 (7)*, 1139-1144.

Krahulik, D., Zapletalova, J., Frysak, Z., & Vaverka, M. (2010). Dysfunction of hypothalamic-hypophysial axis after traumatic brain injury in adults. *J.Neurosurg, 113*(3), 581-584.

Lam, J. M., Hsiang, J. N., & Poon, W. S. (1997). Monitoring of autoregulation using laser Doppler flowmetry in patients with head injury. *J Neurosurg., 86*(3), 438-445.

Lee, J. H., Kelly, D. F., Oertel, M., McArthur, D. L., Glenn, T. C., Vespa, P., et al. (2001). Carbon dioxide reactivity, pressure autoregulation, and metabolic suppression reactivity after head injury: a transcranial Doppler study. *J Neurosurg., 95*(2), 222-232.

Lewen, A., Matz, P., & Chan, P. H. (2000). Free radical pathways in CNS injury. *J Neurotrauma., 17*(10), 871-890.

Lewis, S., Wong, M., Myburgh, J., & Reilly, P. (1998). Determining cerebral perfusion pressure thresholds in severe head trauma. *Acta Neurochir Suppl., 71*, 174-176.

Liou, A. K., Clark, R. S., Henshall, D. C., Yin, X. M., & Chen, J. (2003). To die or not to die for neurons in ischemia, traumatic brain injury and epilepsy: a review on the stress-

activated signaling pathways and apoptotic pathways. *Prog Neurobiol., 69*(2), 103-142.

Lundberg, N., Troupp, H., & Lorin, H. (1965). Continuous recording of the ventricular-fluid pressure in patients with severe acute traumatic brain injury. A preliminary report. *J Neurosurg., 22*(6), 581-590.

Maas, A. I., Dearden, M., Teasdale, G. M., Braakman, R., Cohadon, F., Iannotti, F., et al. (1997). EBIC-guidelines for management of severe head injury in adults. European Brain Injury Consortium. *Acta Neurochir (Wien). 139*(4), 286-294.

Marshall, G. T., James, R. F., Landman, M. P., O'Neill, P. J., Cotton, B. A., Hansen, E. N., et al. (2010). Pentobarbital coma for refractory intra-cranial hypertension after severe traumatic brain injury: mortality predictions and one-year outcomes in 55 patients. *J, 69*(2), 275-283.

Mathew, P., Graham, D. I., Bullock, R., Maxwell, W., McCulloch, J., & Teasdale, G. (1994). Focal brain injury: histological evidence of delayed inflammatory response in a new rodent model of focal cortical injury. *Acta Neurochir Suppl (Wien). 60*, 428-430.

Menashe Zaaroor, E. M., Venyamin Shik, Jean F Soustiel. (2007). Course of cerebral blood flow and metabolism following severe brain injury. Correlation with neurological function and outcome. *Indian Journal of Neurotrauma (IJNT) 4*, 25-29.

Meyerson, B. A., Gunasekera, L., Linderoth, B., & Gazelius, B. (1991). Bedside monitoring of regional cortical blood flow in comatose patients using laser Doppler flowmetry. *Neurosurgery., 29*(5), 750-755.

Miller, M. T., Pasquale, M., Kurek, S., White, J., Martin, P., Bannon, K., et al. (2004). Initial head computed tomographic scan characteristics have a linear relationship with initial intracranial pressure after trauma. *J Trauma., 56*(5), 967-972; discussion 972-963.

Mitsis, G. D., Zhang, R., Levine, B. D., & Marmarelis, V. Z. (2006). Cerebral hemodynamics during orthostatic stress assessed by nonlinear modeling. *J Appl Physiol., 101*(1), 354-366. Epub 2006 Mar 2002.

Muizelaar, J. P., & Schroder, M. L. (1994). Overview of monitoring of cerebral blood flow and metabolism after severe head injury. *Can J Neurol Sci., 21*(2), S6-11.

Muttaqin, Z., Uozumi, T., Kuwabara, S., Arita, K., Kurisu, K., Ohba, S., et al. (1993). Hyperaemia prior to acute cerebral swelling in severe head injuries: the role of transcranial Doppler monitoring. *Acta Neurochir (Wien). 123*(1-2), 76-81.

Myles, P. S. (2009). Bispectral index monitoring in ischemic-hypoxic brain injury. *J Extra Corpor Technol., 41*(1), P15-19.

Narayan, R. K., Michel, M. E., Ansell, B., Baethmann, A., Biegon, A., Bracken, M. B., et al. (2002). Clinical trials in head injury. *J Neurotrauma, 19*(5), 503-557.

Nujaimin, U., Saufi, A., Rahman, A. G., Badrisyah, I., Sani, S., Zamzuri, I., et al. (2009). Post traumatic cerebral oedema in severe head injury is related to intracranial pressure and cerebral perfusion pressure but not to cerebral compliance. *Asian J Surg., 32*(3), 157-162.

Obrist, W. D., Langfitt, T. W., Jaggi, J. L., Cruz, J., & Gennarelli, T. A. (1984). Cerebral blood flow and metabolism in comatose patients with acute head injury. Relationship to intracranial hypertension. *J Neurosurg., 61*(2), 241-253.

Oddo, M., Levine, J. M., Frangos, S., Carrera, E., Maloney-Wilensky, E., Pascual, J. L., et al. (2009). Effect of mannitol and hypertonic saline on cerebral oxygenation in patients

with severe traumatic brain injury and refractory intracranial hypertension. *J Neurol Neurosurg Psychiatry., 80*(8), 916-920. Epub 2009 Mar 2016.

Patel, H. C., Menon, D. K., Tebbs, S., Hawker, R., Hutchinson, P. J., & Kirkpatrick, P. J. (2002). Specialist neurocritical care and outcome from head injury. *Intensive Care Med., 28*(5), 547-553. Epub 2002 Feb 2014.

Pluta, R. M., Hansen-Schwartz, J., Dreier, J., Vajkoczy, P., Macdonald, R. L., Nishizawa, S., et al. (2009). Cerebral vasospasm following subarachnoid hemorrhage: time for a new world of thought. *Neurol Res., 31*(2), 151-158.

Procaccio, F., Polo, A., Lanteri, P., & Sala, F. (2001). Electrophysiologic monitoring in neurointensive care. *Curr Opin Crit Care., 7*(2), 74-80.

Puppo, C., Farina, G., Lopez, F. L., Caragna, E., & Biestro, A. (2008). Cerebral CO2 reactivity in severe head injury. A transcranial Doppler study. *Acta Neurochir Suppl., 102*, 171-175.

Ross, N., & Eynon, C. A. (2005). Intracranial pressure monitoring. *Current Anaesthesia & Critical Care, 16*(4), 255-261.

Sakai, K., Yamada, K., Mori, S., Sugimoto, N., & Nishimura, T. (2011). Age-dependent brain temperature decline assessed by diffusion-weighted imaging thermometry. *NMR Biomed, 28*(Jan).

Smielewski, P., Czosnyka, M., Kirkpatrick, P., & Pickard, J. D. (1997). Evaluation of the transient hyperemic response test in head-injured patients. *J Neurosurg., 86*(5), 773-778.

Stahel, P. F., Morganti-Kossmann, M. C., & Kossmann, T. (1998). The role of the complement system in traumatic brain injury. *Brain Res Brain Res Rev., 27*(3), 243-256.

Stuart, R. M., Schmidt, M., Kurtz, P., Waziri, A., Helbok, R., Mayer, S. A., et al. (2010). Intracranial multimodal monitoring for acute brain injury: a single institution review of current practices. *Neurocrit, 12*(2), 188-198.

Timofeev, I., Carpenter, K. L., Nortje, J., Al-Rawi, P. G., O'Connell, M. T., Czosnyka, M., et al. (2011). Cerebral extracellular chemistry and outcome following traumatic brain injury: a microdialysis study of 223 patients. *Brain., 134*(Pt 2), 484-494. Epub 2011 Jan 2018.

van Santbrink, H., Maas, A. I., & Avezaat, C. J. (1996). Continuous monitoring of partial pressure of brain tissue oxygen in patients with severe head injury. *Neurosurgery., 38*(1), 21-31.

Vergely, C., Maupoil, V., Clermont, G., Bril, A., & Rochette, L. (2003). Identification and quantification of free radicals during myocardial ischemia and reperfusion using electron paramagnetic resonance spectroscopy. *Arch Biochem Biophys., 420*(2), 209-216.

Vincent, J.-L., & Polderman, K. H. (2006). Induced Hypothermia for Neuroprotection: Understanding the Underlying Mechanisms. In *Intensive Care Medicine* (pp. 328-346): Springer New York.

Voulgaris, S. G., Partheni, M., Kaliora, H., Haftouras, N., Pessach, I. S., & Polyzoidis, K. S. (2005). Early cerebral monitoring using the transcranial Doppler pulsatility index in patients with severe brain trauma. *Med Sci Monit., 11*(2), CR49-52.

Young, J. S., Blow, O., Turrentine, F., Claridge, J. A., & Schulman, A. (2003). Is there an upper limit of intracranial pressure in patients with severe head injury if cerebral perfusion pressure is maintained? *Neurosurg Focus., 15*(6), E2.

Zauner, A., Bullock, R., Di, X., & Young, H. F. (1995). Brain oxygen, CO2, pH, and temperature monitoring: evaluation in the feline brain. *Neurosurgery., 37*(6), 1168-1176; discussion 1176-1167.

Zweifel, C., Lavinio, A., Steiner, L. A., Radolovich, D., Smielewski, P., Timofeev, I., et al. (2008). Continuous monitoring of cerebrovascular pressure reactivity in patients with head injury. *Neurosurg Focus., 25*(4), E2.

12

The Dynamic Visualization Technology in Brain Deceleration Injury Research

Zhiyong Yin[1], Shengxiong Liu[2], Daiqin Tao[1] and Hui Zhao[1]
[1]*Chongqing Key Laboratory of Vehicle, Biological Crash Security, Department 4, Institute of Surgery Research, Daping Hospital, Third Military Medical University, Chongqing*
[2]*Department of Biomedical Engineering, School of Pharmacy & Bioengineering Chongqing University of Technology, Chongqing*
China

1. Introduction

Road traffic injuriy (RTI) is a serious threat to human life and health. Currently, there are about 130 million people died and about 200 to 500 million people injured in road traffic accidents worldwide each year [1]. In the developing country, for example, in China, there are 10 million people died and 50 million people injured in road traffic accidents each year [2]. With social progress and development of medicine, many diseases (such as some infectious diseases) have been effectively controlled, but the road traffic injury is increasing [3].

The biomechanics of head impact injury has become a research focus in the impact injury biomechanics. The study aims to understand the mechanical response of brain tissue to the impact, and to determine the dose-effect relationship between the brain injury and the mechanical load. It can clarify the mechanism of brain injury and provide a theoretical basis for the development of head injury protection and injury indicators.

Besides brain tissue outside the body wrapped in the scalp, there is other non-transparent tissue covering the skull. And brain tissue itself is non-transparent organization. Therefore, it is extremely difficult to research the stress-strain for brain tissue under deceleration impact. In this regard, scholars of various countries have done a lot of meaningful exploration and research.

In order to observe the impact stress wave propagation of the skull and brain tissue, Yanping Jiang [4], has developed a brain static and dynamic photoelastic model (Fig.1). As the model deformed by the collision, with the light reflectivity and angle changing accordingly, and the formation of photoelastic fringes, the changes can be calculated by the stripes of the force of brain tissue, for photoelastic fringe dense area with larger force. However, for the mechanical properties of the model and the response calibration and other reasons, it is difficult to conduct quantitative research on stress and strain.

In order to produce the same size with the real head of the head model, in 2005, Johnson and Young [5] applied rapid prototyping technology to produce a true copy of the skull (Fig.2). It has been used in the research of the brain impact response, confirming that the effective impact time decreases with the decrease of hammer quality. The model is made with opaque material. However, because of it, it can be only used for the skull study, and not suitable for the force characteristics of brain tissue.

In 2007, there was a very classic corpse occipital head impact experiment finished by Wayne State University and other research organizations (Fig.3) [6,7]. Before the experiment, markers had been made by metal balls and thin-walled tubes whose density was close to the brain tissue. During the impact, markers had been placed in the brain model as well as mini markers planted into the skull, then the high-speed X-ray machine (250 frames/sec) was used to record the impact process. After analyzing for the captured images, it concluded the "8" shaped trajectory (Fig.4) of markers relative to the skull and its laws. It is a new means of measurement and it has provided valuable raw data for the study of injury mechanism of injury provide valuable raw data.

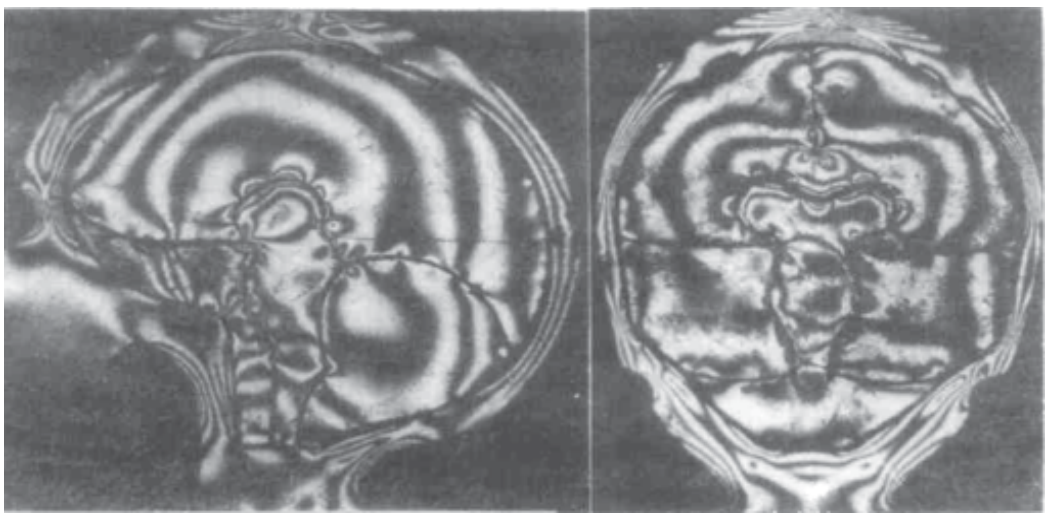


Fig. 1. Stripes of the sagittal photoelastic model in the simulation of collision

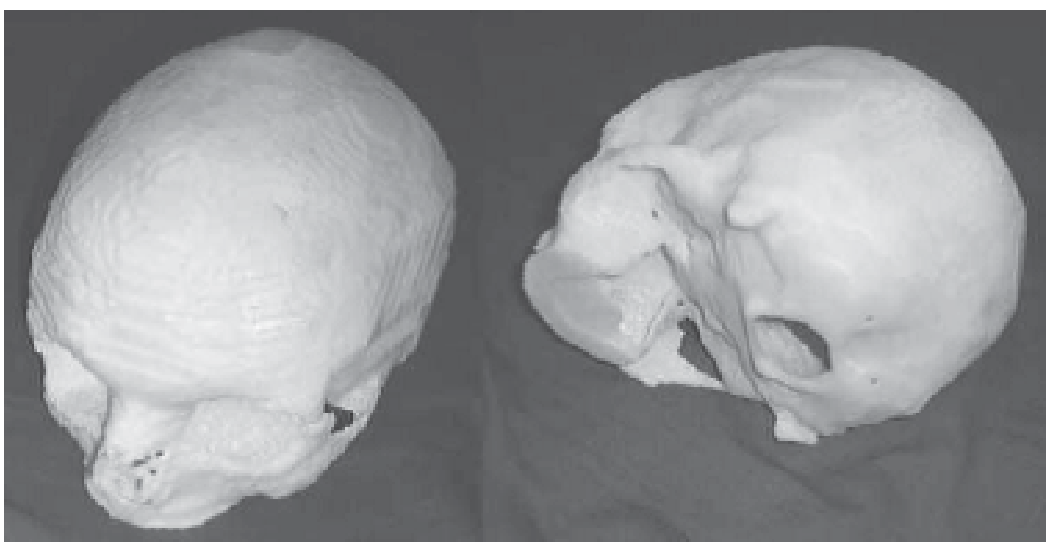


Fig. 2. The rapid prototyping skull model

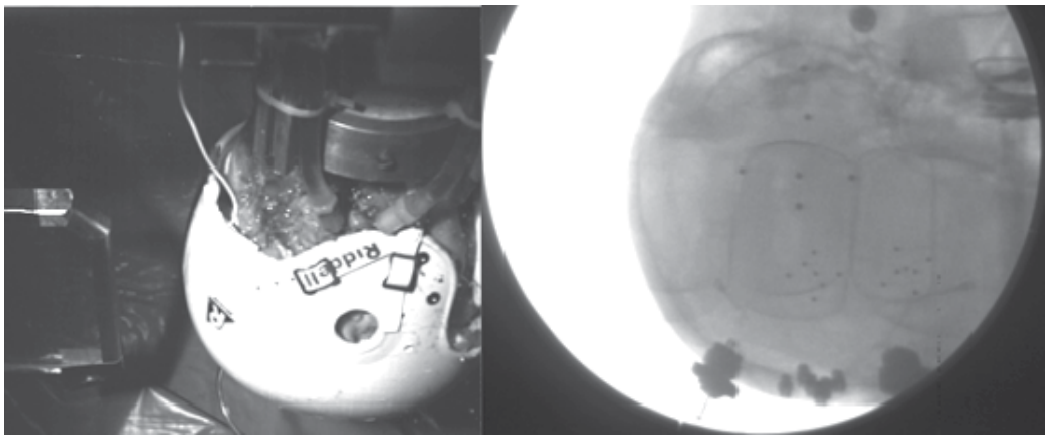


Fig. 3. Shooting of the impact process for the corpse head by the high-speed X-ray device [6]

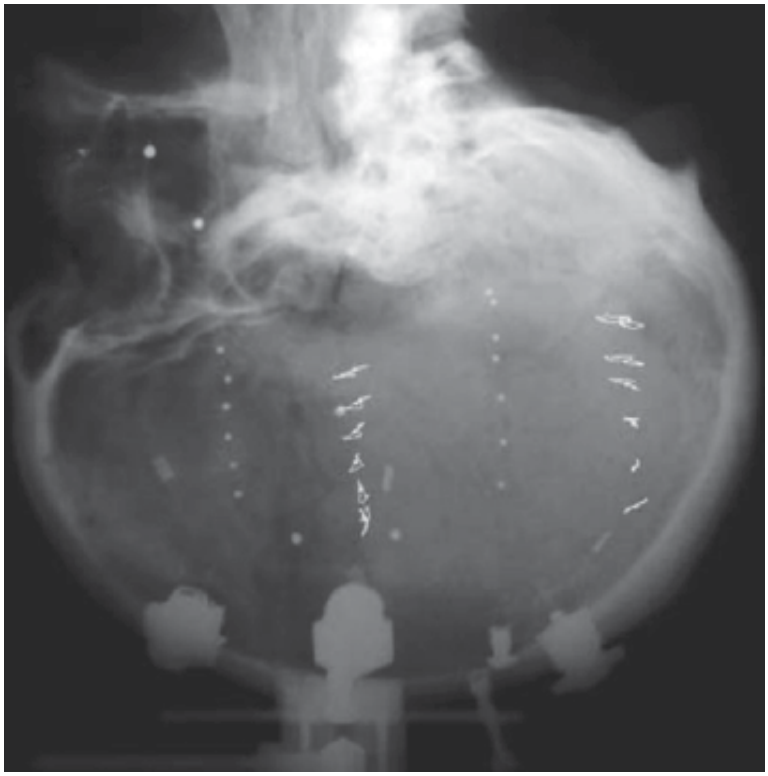


Fig. 4. The relative "8" shaped trajectory of the markers in the skull model [7]

The high-speed X-ray machine can obtain the displacement and even the strain information of markers in brain tissue, but it cannot detect intracranial stress.

To this end, a visual model of the physical brain containing bubbles has been developed (Shengxiong Liu, 2010)[8] for brain injury mechanism research. This visual model can effectively reflect the brain impact stress response. The study provides an intuitive non-

invasive intracranial stress wave detection and analysis method. It is a non-destructive and non-contact testing method, as an innovation test concept. The following will introduce the visualize brain model, the experimental device and the stress wave detection experiments.
In addition, it provides conditions for the body digital model and the study of brain injury by the development of electronics technology, computer science, and computational mathematics. Finite element (FE) method is a numerical analysis method developed from the analysis of engineering structures for solving problems of continuum mechanics. In 1969, Fridenbery applied finite element theory to the medical field for the first time, which has provided new methods and theories for human biomechanics research. FE model of human first started from the local model, and the earliest FE model is the human head model. By the head finite element model, it has greatly promoted the study of head injury biomechanics. With the increasing development of science and technology, computer simulation is more and more likely to replace the traditional biomechanical studies on the biological impact test. Finite element model of the skull has been built [9] to study the intracranial stress and strain distribution of the brain during the deceleration impact, as well as the reproduction of traumatic brain injury.

2. The dynamic visualization technology of head injury based on the transparent physical model

2.1 Physical model

2.1.1 Screening of brain tissue substitute materials

It can generate pure bubbles anywhere within the model with a syringe by plant hydro gel to simulate brain tissue before gel solution. During the collision process, the bubble locations are fixed without interfere movement. Bubble deformations are uniform that it is helpful for calculating the bubble volume changes.

2.1.2 Screening of skull alternative materials

The glass and PC resins are all optional alternative materials to the skull. The glass is of good transparency and drawing easy, which is easy to observe the morphological changes of markers in the brain impact. PC resin is of good impact resistance, good transparency and low water absorption, that its mechanical properties are relatively close to the skull.

2.1.3 Model development

In this study, the transparent skull physical model (Fig.5) has been produced with the real skull and the PC resin by the molds technology. Molds technology ensures the high degree of geometric similarity to the real skull. PC resin ensures the transparency of the model that it has good impact resistance and mechanical properties. It reaches the same level of magnitude to the real skull with low water absorption as the research foundation.
The brain model is of well transparency for the markers observation that it can get a clear bubble deformation by high-speed camera during the impact course. Since the model has similar geometry to the real skull, it is a very effective way for quantitative analysis of the deceleration injury mechanism.

2.2 Deceleration impact device

It needs firstly to solve about the initial velocity of the skull model for the deceleration injury impact experiments, then the model fixation during the experiments. If the subjects

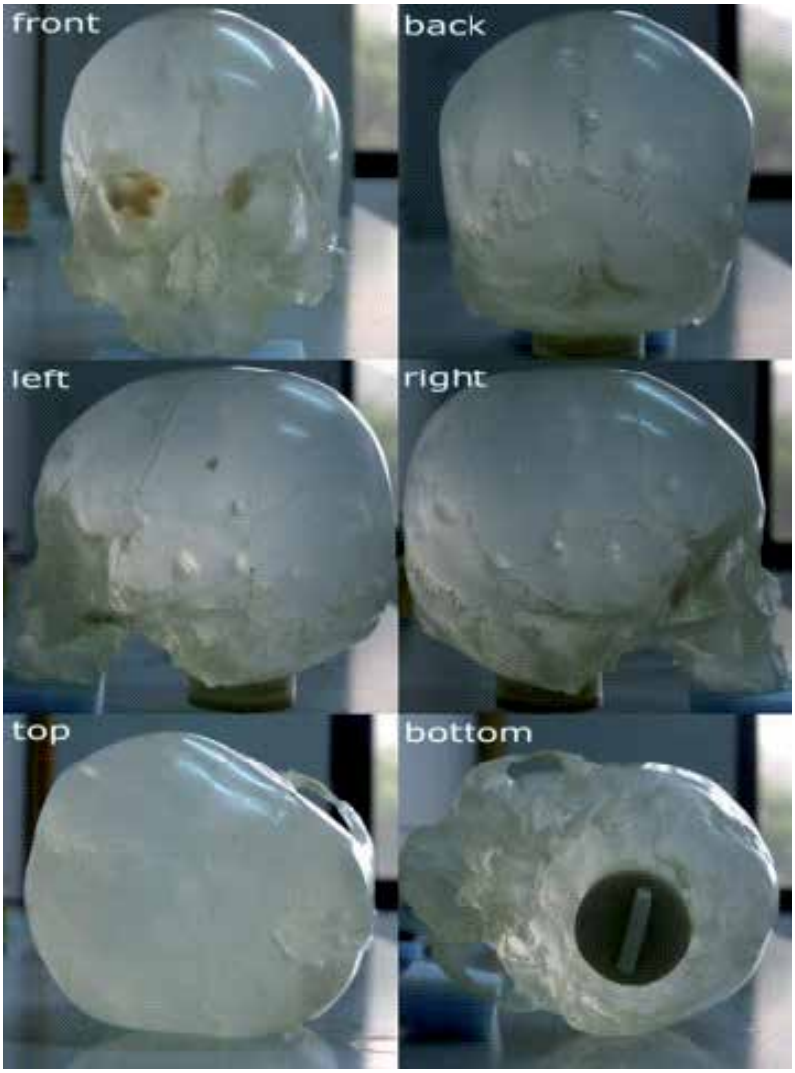


Fig. 5. The visualization skull model by the PC resin on the molds technology [8]

were fixed on the pulley, then let the pulley "free" fall along the track, its final fall speed (test speed) can be controlled by the fall height (h). The experimental principle is shown in Figure 6, and the specific principles can be derived based on Newton's laws of motion.
According to Newton's laws of motion it has the following formula:

$$v_t = \sqrt{v_0^2 + 2as} \tag{1}$$

Where:
v_t as the speed for experimental subjects falling from the height h;
v_0 as the initial velocity for subjects before falling;
s as the acceleration distance, where calculated by the fall height h;
a as the acceleration, here as the acceleration of gravity g;

Since the subject falling from height h with initial velocity (0m/s), its immediate speed is:

$$v_t = \sqrt{2gh} \tag{2}$$

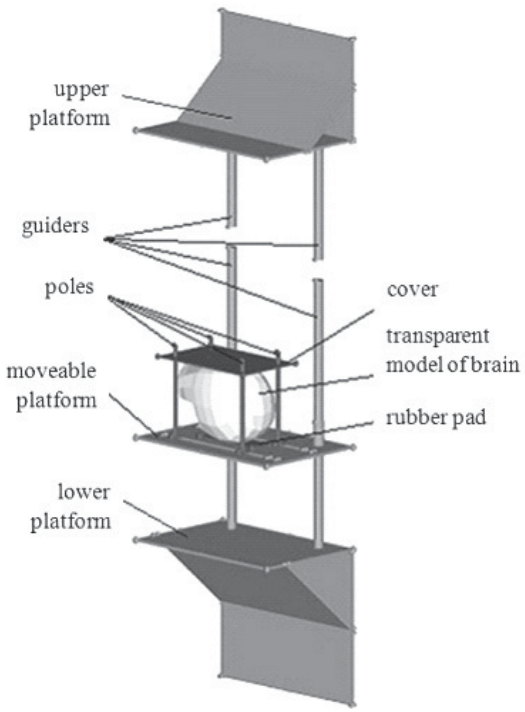


Fig. 6. The schematic diagram of deceleration injury simulation device

It can be seen from Figure 6 that the pulley is located between the upper platform and the lower platform where is the falling space for the pulley. The pulley rolling along the guiders which are two vertical stainless guide rails parallelled to each other are set up in the space. Their upper ends are fixed to the upper platform while their lower ends to the lower platform. Tightness of guiders can be adjusted as needed to prevent the obstruction disturbances during the experiment. In addition, the four poles are connected with the movable platform by the groove mode. The spacing among the poles can be adjusted to adapt to different sizes and shapes for the skull model.

2.3 Intracranial stress wave detection experiments

2.3.1 Experimental platform

Experimental platform as shown below (Fig.7), the device made by a transparent head model of the PC resin material geometrically consisted with the real brain and a deceleration impact test device.

2.3.2 Experiment preparation

First, the warm plant hydrogel was put into the model waiting for cool. During the cool process, three bubbles were injected into the semi-liquid and -solid gel by the special syringe

at positions of the impact, the neutral and the hedge points. After standing, due to thermal expansion and contraction, there should be small gaps at the junction of the gel and the container. Gaps were filled with some water, which could simulate the structural components of cerebrospinal fluid to some extent. The model was sealed by the rubber plug with a small needle connecting the gel and the external environment, which could simulate the connectivity of the foramen magnum. Then the brain model was fixed with sensors accessing to the data acquisition system.

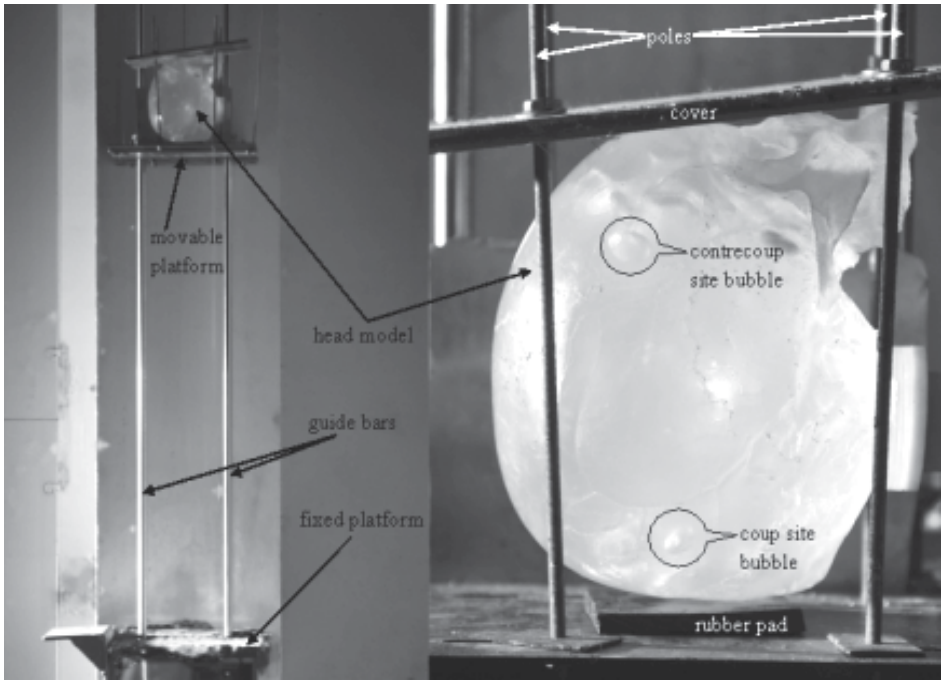


Fig. 7. The deceleration impacting system for the PC transparent resin skull model [8]

2.3.3 Test steps

At the beginning, the model together with the pulley are upgraded to the position about 0.60±0.01m away from the lower platform to simulate the moderate impact of the brain injury. Then the tackle was released and free fell along the guiders, hitting the lower platform, which triggered the sensor and the high speed camera system to record the whole impact process.

2.3.4 Stress wave analysis

By high-speed video analysis, the axial length of the bubbles were extracted from the photo. Volume changes of the bubble were analysed to obtain the changing curves of the average pressure for each bubble. Then the stress and the changing information of the brain tissue at the corresponding location were analysed.

The bubble volume during the impact can be calculated by the ellipsoid formula. According to formula (3), it is based on the short axis (a) and long axis (b) (the other axis is equivalent to the short axis (b)) as:

$$V_n = \frac{4}{3}\pi a_n {b_n}^2 \tag{3}$$

Here, V_n is the volume of the bubble at the frame of n; a_n is the short axis of the bubble at the frame of n; and b_n is the long axis of the bubble at the frame of n.
Therefore, the mean stress for the brain tissue, that is the average bubble pressure, can be calculated by the following formula (4):

$$P_n = \frac{P_0 V_0}{V_n} \tag{4}$$

Here, P_0 is the initial pressure of the bubble before the collision, which is approximately equal to a standard atmosphere pressure, in order to make P_n easy to calculate and compare with each other. V_0 is the initial volume of the bubble before the collision; V_n is the volume of the bubble at the frame of n during the collision; and P_n is the average pressure of the bubble at the frame of n.

2.3.5 Results

There were 28 photographs extracted from the video from the crash on the first frame to bubbles returning to its initial size. Then the average pressures of each bubble were calculated and compared with the average stresses of the two bubbles at the impact point and the hedging point.

2.3.5.1 Overview of photograph series

Figure 8 shows the sequence of 28 photographs about bubbles from the crash on the first frame (0 ms) to bubbles back to its original volume (5.4 ms).

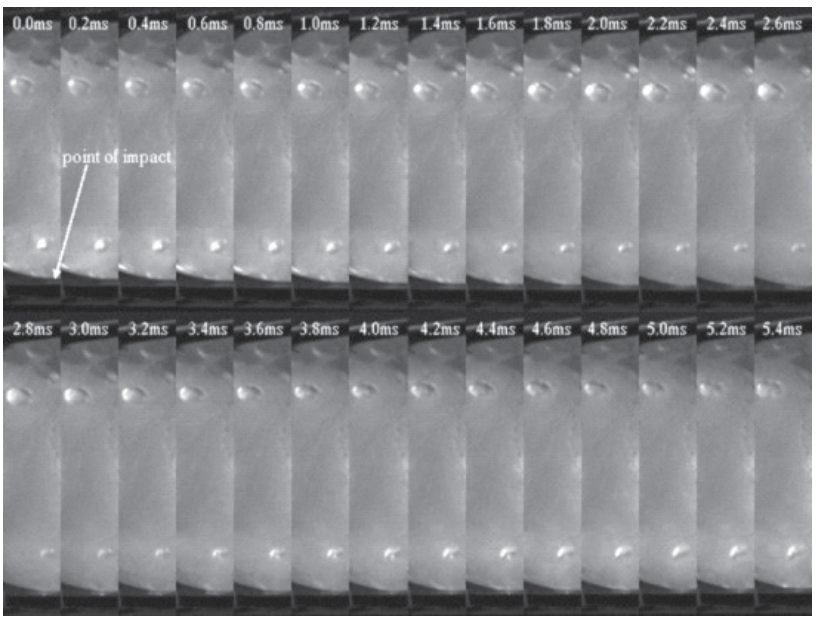


Fig. 8. Twenty eight serial frames from the onset frame of the impact to the frame in which the air bubbles came near to their original volumes [8]

As can be seen from Figure 8, in the upper pictures, the volume of small bubbles at the hedge points is increasing; while in the lower pictures, it is decreasing. In contrast, in the upper pictures, the volume of small bubbles at the impact points is decreasing; while in the lower pictures, it is increasing. It illustrates the different changing trends of the average stress for the hedging point and bubbles during the impact.

2.3.5.2 The average pressure and transmission characteristics for the hedging point and bubbles

Figure 9 shows the details of the average pressure, the changing process and the transmission characteristics for the hedging point and the bubble pressure:

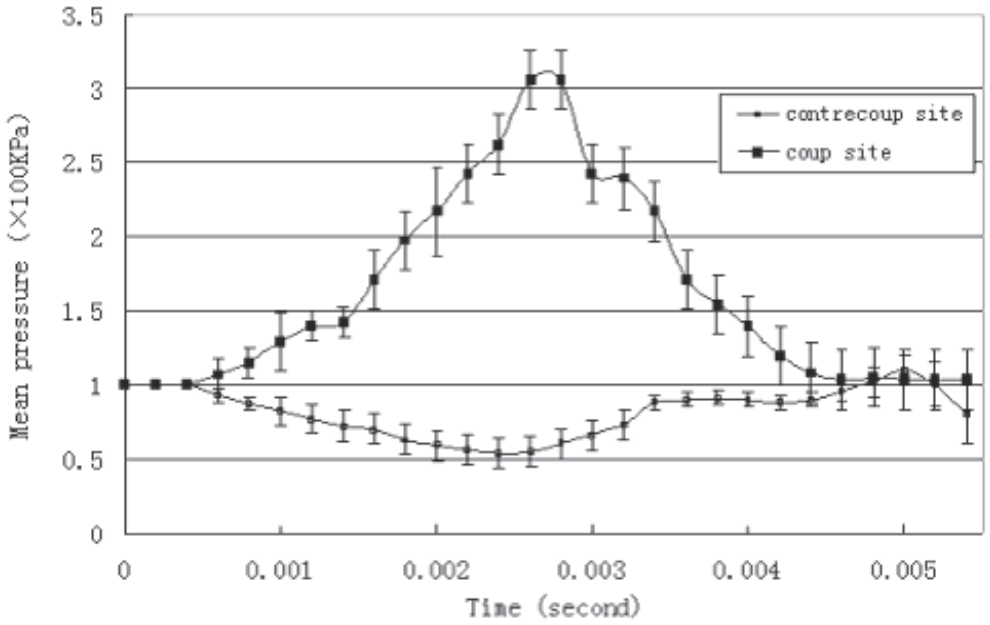


Fig. 9. The mean pressure changes and the transmission characteristics of the bubbles in coup and contrecoup points from frame 1 to frame 28 [8]

In Figure 9, the average pressure of bubbles from the first frame to the 14th frame is increasing; while from the 15th to the 28th, it is decreasing, in which the largest average pressure is 3.06×100K Pa. It demonstrates that the brain tissue at the impact locations must suffer in a larger positive pressure which produces compressive stress and results into compression injury. Similarly, it can be seen from Figure 9 that the average pressure is decreasing based on the bubbles at the hedge points from the first frame to the 13th frame; while from the 14th frame to the 28th frame, the average pressure is increasing, in which the smallest average pressure is 0.54×100K Pa. It indicates that the brain tissue in the hedge location must suffer in a continuous negative pressure in the impact process. Although the intensity of the negative pressure to the hedging point is lower than the intensity of the positive pressure to the impact point, the hedge position of the brain tissue will be injured by the tension stress and its stretch for the tensile resistant strength is lower than the pressure resistant strength.

3. The dynamic visualization technology based on the skull FE model

3.1 Establishment of FE model

3.1.1 Data sources and working platform

The data is from the second case in the CVH data. It has taken the from the 1049th to the 1791st level of the continuous thin layer of cross-sectional images from the registration data set. The layer spacing is 0.25mm and the resolution is 3072 x 2048 pixels. The image format is the uncompressed ". tiff" format. Choose the singular images as the objects in total of 372 layers to do image process in the HP xw9300 graphics workstation of high performance.

The following FE skull model was built on the platforms of the 64-bit WindowsXP operating system, three-dimensional image reconstruction software Amira 4.1 (TGS French company), Photoshop (Adobe, USA) and Hypermesh8.0 (U.S. Altair's).

3.1.2 The finite element modeling of the skull

It was identified and done segmentation on thesuccessive layers of the cross-section images on the brain, ventricular system, brain stem, cerebellum, falx, tentorium, sinus, calvarial, facial bone and mandible and other parts of the relevant structures through Photoshop (CS4, USA) software. layers of the same structure was filled with the same RGB color values (red, green and blue). Any two structures should not be in the same RGB color value and the image background was removed.

Different gray values were given to each structuire of the segmentations which was then changed into the grayscale. These images were input into the HP image workstations. The software of Amira 4.1 was applied to do the 3D reconstruction of the skull.

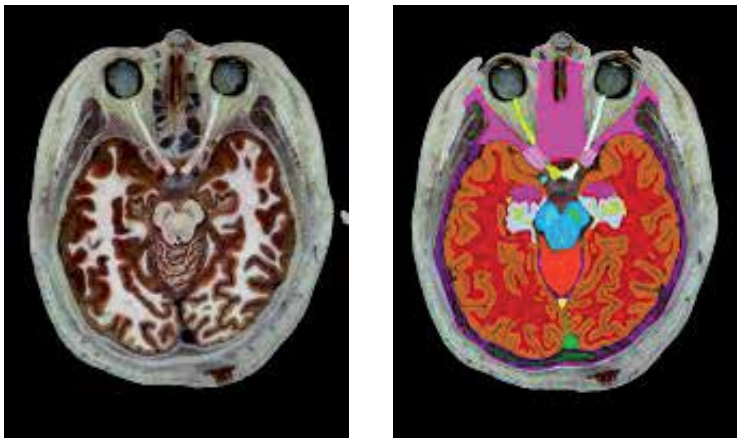
The trias unit was used to fit the anatomy of the 3D skull reconstruction. The model was simplified and smoothed according to the complexity of the anatomical structure of the model. The various parts of the shell head unit were stored as the hmascii format. The face was reconstructed by the semi-automatic way and it was by the closed surface for the brain, the cerebellum, the brain stem and other brain structures.

The surface triangulation of the skull structure was splited according to the radius of curvature of the anatomy. Then the 2D grids were splited on these surfaces. At last, the 3D meshes were splited from the 2D surfaces. They were checked on the skewness (Skew), Warp (Warpage), Jacoby number (Jacobian), aspect ratio (Aspect), the grid unit length (length) and other grid qualities.

The penetration inspection was done on the grid belonging to different structures. The self-contact inspection was done on the single structures. Ignore penetrations were set to 0. The penetrating grids were zoomed or adjusted in the penetrating direction until all the self-exposure structures were eliminated.

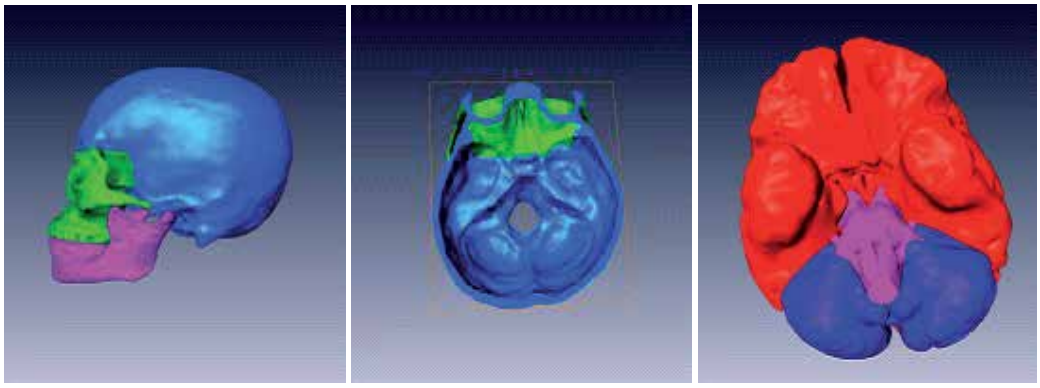
3.1.3 Skull FE model

The partition structure can be automatically extracted by the specified gray value through the thresholding function without segmentation leakage and fault. In the software of Amira, the 3D images of each partition structure can be clearly distinguished after the 3D reconstruction of the extracted segmentation information. These images are smooth and lifelike in which the small structure can be observed completely like the maxillofacial and skull base (Fig. 11). Can be constructed based on the observed need to rotate images at any angle. Image angels can be rotated arbitrarily by the observing need.



(a) (b)

Fig. 10. Slice Image of Head
(a) Raw CVH slice image; (b) Segmented CVH slice image



(a) (b) (c)

Fig. 11. 3D Reconstruction of Head
(a) skull; (b) fundus cranii; (c) brain

The solid skull model reconstructed from 3D images is of good correspondence with the anatomy especially in facial and skull base parts. From three-dimensional image reconstruction of the skull and three-dimensional image corresponding to the solid model is good, no distortion. Solid models and surface subdivisions of skull and brain are shown in Figure 12.

The quality tests results of Skewness (Skew), Warp (Warpage) and Jacoby number (Jacobian) for the finite element grid meet the engineering requirements (Table 1) with uniform grid cell size mainly in the 1.5 to 4.5. The nodes and elements numbers of the FE model are 31,223 and 119,911. All elements are solid elements. Head finite element model of the number of nodes and elements are 31,223 and 119,911. There is no contact between the skull and the brain. There is grid contact between the brain and the brain stem without Penetration.

(a) (b) (c)

Fig. 12. Reconstruction of Solid Model and Partition
(a) skull; (b) inferior maxilla; (c) cerebrum

Checking items	Engineering requirements	Actual values
Nodes	——	31223
Elements	——	119911
Length	Distribution	1.5~4.5
Skew	$<60^0$	$<50^0$
Warpage	$<50^0$	$<50^0$
Jacobian	>0.7	>0.95
Aspect	<5.0	<2.8

Table 1. Mesh Quality Check for Finite Element Model of Head

The finite element mesh of the skull includes two parts as skull and brain. The skull includes three parts as the cranium, the facial bones and the mandible bones. The FE grid of the cranium contains the frontal skull, the temporal bone, the occipital bone, the sphenoid, the ethmoid, the parietal bone and the maxillofacial bone and other anatomical structures in detail. Fine structure of the skull FE model has been carved out in detail as shown in Figure 13.

3.2 The brain deceleration injury research based on the finite element (FE) model

3.2.1 A case for the brain deceleration injury

A male of 19 years old, height 1.70 m and weight 55 kg fell accidentally in the work from 4.0 m to the cement floor grounded with right occipital and immediately coma for 3.5 hours with cerebrospinal fluid rhinorrhea not accompanied by incontinence and no vomiting. He had been head injured and in disturbance of consciousness for 1 day and waked severe headache with vomiting 2 times. Specialist examination: inspection cooperation, a right occipital palpable size of about 1 × 2 cm scalp hematoma, lethargy, poor time orientation, memory loss, calculated power loss and hallucinations. GCS score: Opening eyes responses 4, language 5, movement 6, total scores 15.

Brain CT images after injury: the right occipital scalp hematoma, linear fracture of the right occipital whose fracture line extended to the right parietal bone and the left occipital, right anterior and middle cranial fossa fracture, bilateral frontal lobe contusion with a small

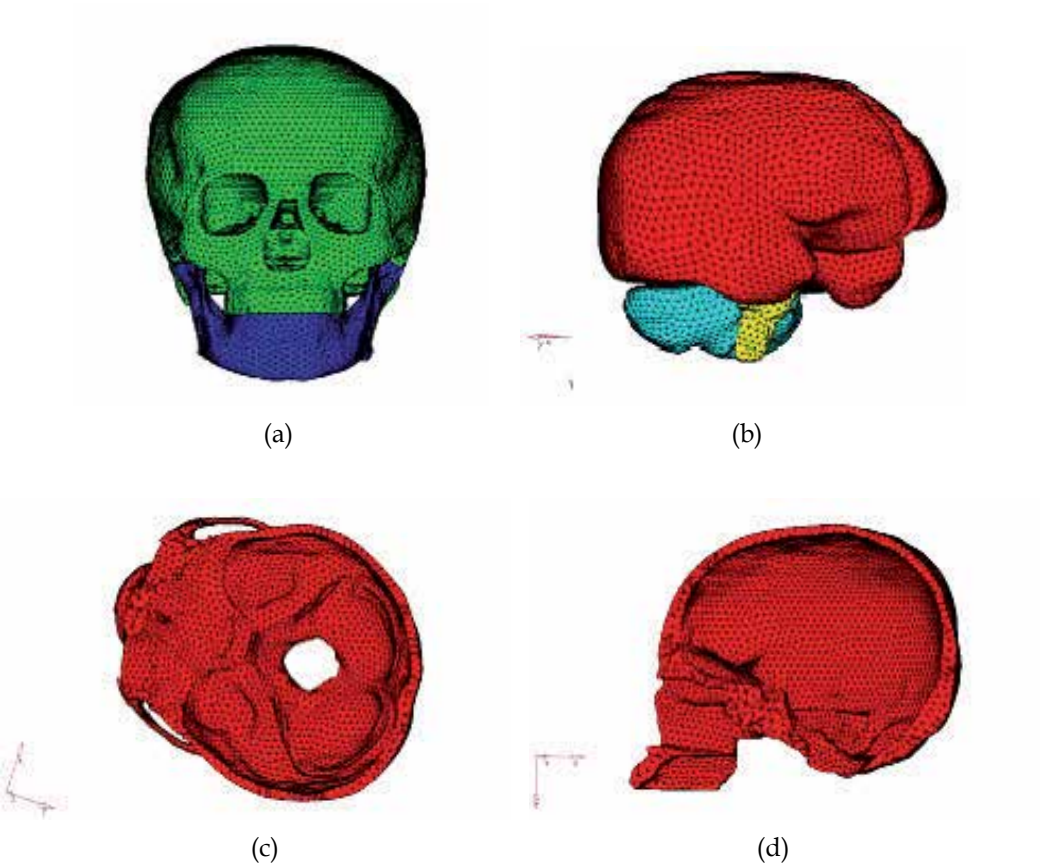


Fig. 13. Finite Element Model of Chinese Head
(a) skull; (b) brain; (c) transverse plane of fundus cranii; (d) sagittal plane of skull

hematoma (dual frontal pole and the inner side and the right amount of bottom), bilateral temporal lobe (temporal pole) contusion with a small hematoma, left temporal and occipital subdural hematoma, subarachnoid hemorrhage (around tentorial) (Fig.14-15).

3.2.2 Computer simulation of damage process

The 3D FE model was input into the software of LS-DYNA to do simulation and analysis for reconstruction of the occipital brain deceleration injury. The skull is defined as a general elastic material, and the brain as a viscoelastic material. The material mechanical parameters of the skull FE model are directly referred to the literature data [10,11]. The skull and the brain contact with each by the coupling gap as friction coefficient Fs (mean value as 0.08). It was established a finite element model of the flat impactor to simulate the stationary blunt hard objects of great quality. The flat impactor limited at the base part was defined by the Solid164 elements with E as 15000GPa and μ as 0.3. It simulated the brain deceleration injury through the hitting of the flat impactor and the right occipital (Fig.16) based on the formula $v_{02} = 2gh$ where the initial velocity of the skull was 9m/s. The software of Hyperview was applied to do analysis of the simulation and the load step brain Von Mises stress was output by the graphics form.

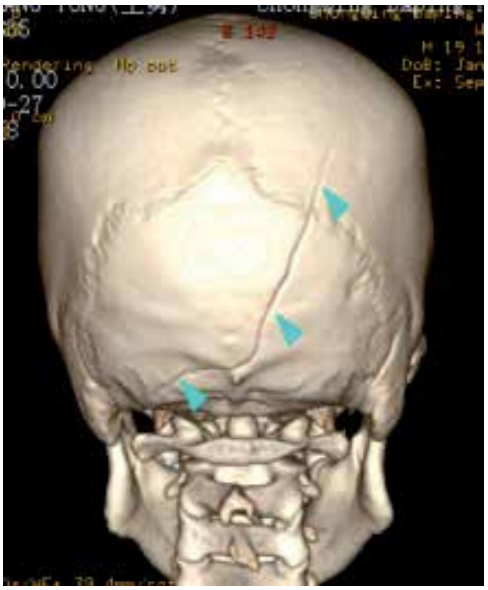


Fig. 14. A linear fracture of the right occipital, which extends to the right parietal bone and the left occipital.

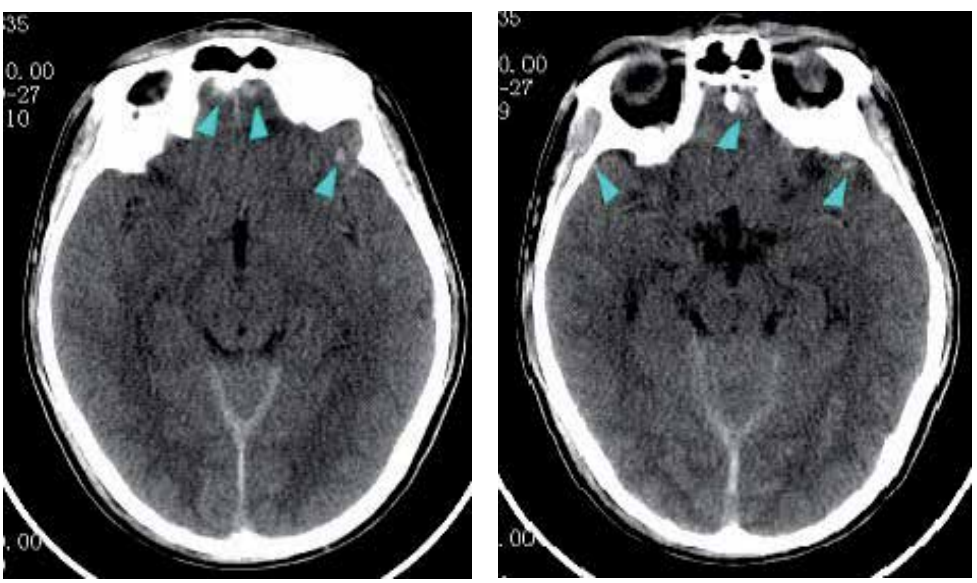


Fig. 15. Bilateral frontal and temporal lobe contusion with a small hematoma

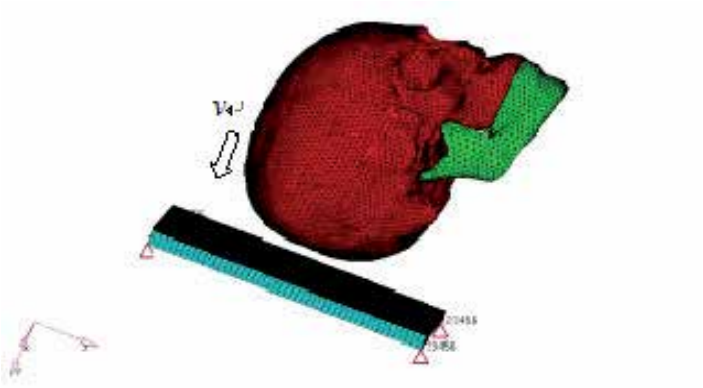


Fig. 16. A simulation model of brain deceleration injury in the flat hard board on the right occipital.

3.2.3 The intracranial stress and strain distribution of brain deceleration injury

Stress of brain tissue changes firstly in the bottom of the right occipital (the focus), then extending along the adjacent skull and the skull front and base. Von-Mises stress peaks at the right occipital. The stress wave gradually spreads from the collision site to the adjacent skull in radiating form. Stress changes include the left occipital bone, bilateral parietal and right temporal bone with high stress concentration (yellow). Stress wave moves forward involving the bilateral temporal bone, zygomatic arch and the left frontal bone, but the Von-Mises stress distribution valuesare low (light blue). Stress wave in the skull base moves from back to front and gradually expands to the bilateral posterior fossa of the foramen magnum rear, bilateral anterior and middle cranial fossa with a stress concentration (yellow) , and the right part is of more obvious changes (Fig.17-18).

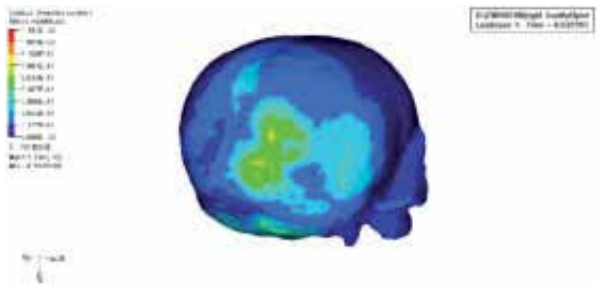


Fig. 17. The stress distribution on the back of the skull

Stress of brain tissue changes firstly in the bottom of the right occipital lobe (the focus), then diffusing along the bottom of the brain and brain surface. Von-Mises stress peaks at the right occipital lobe. In the brain surface, the stress wave spreads gradually around to the right occipital lobe in radiating form. Ranges of stress changes include the right parietal lobe, the temporal lobe, the cerebellum and the left occipital lobe, but the distribution of Von-Mises stress values are low (light blue). Stress wave gradually extended from back to front along the bottom of a gradient of brain. The changing scopes include the corpus callosum, bilateral cerebral peduncle back, bilateral temporal lobe

(temporal pole, bottom and outer side) and bilateral frontal lobe (frontal pole, bottom and inner side). In , there is a stress concentration (yellow) at the back of the corpus callosum and the bilateral cerebral peduncle. Then, there is stress concentrations (yellow) (Figure 19-20) in the bilateral temporal pole, bilateral medial frontal pole and the right and frontal underside of the lobe.

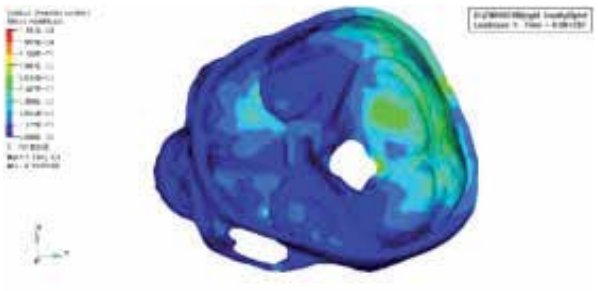


Fig. 18. The stress distribution inside the skull bottom.

Fig. 19. The stress distribution behind the brain

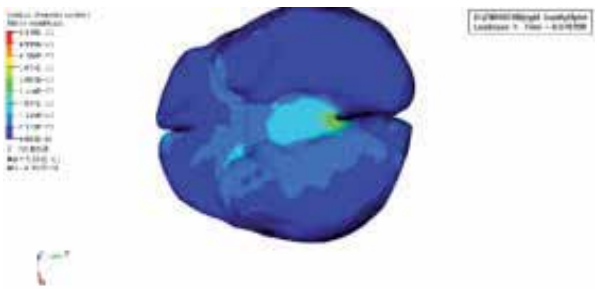


Fig. 20. The stress distribution at the bottom of the brain

3.2.4 The comparison of brain stress response results and clinical CT images

The comparison of the brain stress concentration showed by the FE model and clinical CT images is shown in Table 2. From the Table, locations of clinical skull fracture, brain contusion, subarachnoid hemorrhage are consistent with the stress concentration locations of DE simulation, but the changing locations in the FE calculation is not necessarily for the brain injury (such as the right occipital lobe).

Skull	FE anlaysis	Stress concentration	Right occipital, right parietal bone, left occipital, bilateral posterior fossa of the foramen magnum rear, bilateral anterior and middle cranial fossa
	CT performance	Skull fracture	Right occipital, right parietal bone, left occipital, right anterior and middle cranial fossa
		Subarachnoid hemorrhage	Around the posterior fossa transtentorial
Brain tissue	FE anlaysis	Stress concentration	Right occipital lobe, corpus callosum, bilateral cerebral peduncle back, bilateral temporal pole, bilateral medial frontal pole and the right frontal lobe underside
	CT performance	Brain contusion	Bilateral temporal pole, bilateral medial frontal pole and the right frontal lobe underside

Table 2. The comparison of the stress concentration calculated by the FE simulation and the clinical head CT images

4. Discussion

4.1 The dynamic visualization technology of head injury based on the transparent physical model

In the classic experiment (Nahum, 1977) [12], a columnar load unit with a circular contact surface has been used to hit the forehead of the body head. Although the experiment is limited to the acceleration impact, it is more similar to the bubble experiments than other known experiments. From both the experimental data, their results are consistent. The absolute value of head acceleration and the HIC value in the bubble experiment are slightly larger than in the Nahum experiment (experiment 43). These differences lead to the more severity of the pressure values for the impact point and the hedging point in the bubble experiments than in the Nahum experiment (experiment 43). Through these two experiments and their results, it has compared and further demonstrated the feasibility of the bubble model.

4.2 Dynamic visualization technology of brain injury based on FE model

4.2.1 Skull FE model based on the Chinese visual figure set

Since the early 1990s, a new research has begin by Ruan, et al [13], to establish the FE model of head injury. The model simulates a 50-year-old male head totally weighted 5.059 kg. Structural organization of the head included: the scalp, the skull (including the plate, in plate and intermediate layers), the meninges (including the dural membrane and sickle), the cerebrospinal fluid, the left and right cerebral hemispheres, the cerebellum, the midbrain and the brain stem. Other series of head models established based on Ruan's have achieved a lot of progress in the application, such as Zhou [14], Zhang [15], Prasad [16] and so on. FE researches on the brain injury have also made some progress in China, such as Lai Man[17], Yang Ji-hong [18] and Nguyen [19,20] and so on.

The accuracy and the reasoning of the structure directly affects the accuracy of FE analysis. So it is one of the conditions that it has the similar geometry of the FE skull model with the object. The constructed FE skull models are taken the CT or MRI slice images as the primary data sources to build the FE model that they are not of good accuracy in details because of the blurred scanning boundary limitations of CT/MRI.

In recent years, the human visualization technology has become a high tech integrated of anatomy, computers and virtual reality. Digitized visible human (DVH) relyies on the computer 3D technology showing the structure of real human organs. Professor Zhang Shaoxiang (Third Military Medical University, China) has completed the CVH image data acquisition after other researchers in the United States and South Korea [21,22]. The FE skull modelin this study has selected data from the second case of CVH. The data set selected was of no organic disease and defects in the human body; the entire specimen cross-section was of no segmental defects; the layer spacing was 0.25mm; the resolution were 3072 × 2048 pixels; the uncompressed digital image resolution were cross-section 6.3 million pixels; and each section of the image file size was 36MB. The detail results were more accurate and reliable. Therefore, it has more advantages in data integrity, and accuracy of representation than the currently reported FE skull model.

4.2.2 Brain deceleration injury research based on the head finite element model

It has successfully simulated and reproduced the distribution and characteristics of skull and brain tissue in the occipital deceleration shock by the brain 3D FE model materialized with CVH to hit the plate impactor by the right occipital in falls-style head deceleration injury. From the finite element simulation, it can be found that the stress wave pass in all directions along the skull and brain tissue at the focal point. In the path of the stress wave, the brain tissue may be damaged. The stress wave passes along two ways in the brain at the same time: one way is from the impact side to the contra-side along the skull; the other way is from the impact side to the contra-side along the brain tissue. In the occipital deceleration shock, in addition to impact parts of the skull and brain tissue under stress concentration, the hedge position is also apparent stress concentration area. For the distribution of stress concentration, it is not only of good correlation of clinical CT images, but also significant for the common clinical brain injuries.

5. Conclusion

With the achievements of materials science and computer science, researches on the head deceleration injury has achieved the dynamic visualization in the biomechanics research. Through dynamic visualization techniques, it can be visually observed and analysed for the intracranial pressure distribution in the brain and the transmission process of the stress wave. It is also an important supplement for the modern testing technology. It is a non-destructive and non-contact testing method which can reveal the mechanism of the brain deceleration injury from a different angle, and to provide biomechanical basis for its prevention and treatment.

6. References

Editorial. A 10-year plan to reduce road-traffic accidents. The Lancet, 2010; 375(9718): 866.

Zhenjun Luo,Ruipeng Fu.Road Traffic Safety and Motor Vehicle Insurance Analysis and Countermeasures in China.Insurance Studies,2008; 5: 38-43.

Zhengguo Wang. Road traffic injury research and reflection. Chinese Medical Sciences Journal, 2007; 29(4): 455-458.

Yanping Jiang，Baosong Liu，Zhengguo Wang, etc. 1999. Simulate brain injury when hit by the intracranial stress photoelastic method for the determination.Chinese Journal of Physical Medicine and Rehabilitation, 21(4): 233-236.

E.A.C. Johnson., P.G. Young. On the use of a patient-specific rapid-prototyped model to simulate the response of the human head to impact and comparison with analytical and finite element models. Journal of Biomechanics, 2005; 38: 39-45.

Albert I. King. Impact Biomechanics. The Bridge, 2004; 34(3): 11-16.

Hardy WN., Foster CD., Mason MJ., Yang KH., King AI., Tashman S. Investigation of Head Injury Mechanisms Using Neutral Density Technology and High-Speed Biplanar X-ray. Stapp Car Crash J, 2001; 45: 337-368.

Shengxiong Liu., Zhiyong Yin., Hui Zhao., Guangyu Yang. Investigation of the cavitation and pressure change of brain tissue based on a transparent head model in its decelerating impact. Journal of Mechanics in Medicine and Biology, 2010, 10(2): 361-372.

Alan M Nahum, Randall Smith, Carley Ward. Intracranial pressure dynamics during head impact. In: Proceedings of the 21st Stapp Car Crash Conference, SAE Paper No. 770922. Society of Automotive Engineers. 1977.

Editorial. A 10-year plan to reduce road-traffic accidents. The Lancet, 2010; 375(9718): 866.

Zhenjun Luo,Ruipeng Fu.Road Traffic Safety and Motor Vehicle Insurance Analysis and Countermeasures in China.Insurance Studies,2008; 5: 38-43.

Zhengguo Wang. Road traffic injury research and reflection. Chinese Medical Sciences Journal, 2007; 29(4): 455-458.

Yanping Jiang，Baosong Liu，Zhengguo Wang, etc. 1999. Simulate brain injury when hit by the intracranial stress photoelastic method for the determination.Chinese Journal of Physical Medicine and Rehabilitation, 21(4): 233-236.

E.A.C. Johnson., P.G. Young. On the use of a patient-specific rapid-prototyped model to simulate the response of the human head to impact and comparison with analytical and finite element models. Journal of Biomechanics, 2005; 38: 39-45.

Albert I. King. Impact Biomechanics. The Bridge, 2004; 34(3): 11-16.

Albert I.King, King H. Yang, Warren N.Hardy. Recent firsts in cadaveric impact biomechanics research. Clinical anatomy, 2011, 24: 294-308.

Shengxiong Liu., Zhiyong Yin., Hui Zhao., Guangyu Yang. Investigation of the cavitation and pressure change of brain tissue based on a transparent head model in its decelerating impact. Journal of Mechanics in Medicine and Biology, 2010, 10(2): 361-372.

Zhao Hui, Chen Rong, Yin Zhiyong, Zhang Shaoxiang, Wang Zhengguo. Development of the first Head Finite Element Model Based on Chinese Visible Human Data. Journal of Biomedical Engineering, 2010, 27(4): 882-886.

Thomson R, Lovsund P, Norin H. Brain injuries in real world accidents-a multidisciplinary investigation, Proceedings of the IRCOBI Conference, Isle of Man, 2001.

Doorly MC, Gilchrist MD. The use of accident reconstruction for the analysis of traumatic brain injury due to head impacts arising from falls. Comput Methods Biomech Biomed Engin, 2006, 9(6):371-377.

Alan M Nahum, Randall Smith, Carley Ward. Intracranial pressure dynamics during head impact. In: Proceedings of the 21st Stapp Car Crash Conference, SAE Paper No. 770922. Society of Automotive Engineers. 1977.

Jesse Ruan, T. Khalil, King AI. Finite Element Modeling of Direct Head Impact. Proc. 37th STAPP Car Crash Conf.,1993.

Zhou C, Khalil TB, King AI. A new model comparing impact response of the homogeneous and inhomogeneous human brain[C]. Proc. 39th STAPP Car Crash Conf.,1995.

Zhang L, Hardy W, Omori K, et al. Recent advances in brain injury research: a new human head model development and validation. Stapp Car Crash Journal, 2001, 45.

Jesse Ruan, Prasad. The effects of skull thickness variations on human head dynamic impact response[J]. Stapp Car Crash Journal, 2001, 45.

Limin He, Yicheng Lu, Jianguo Wu, et al. Validation of a three-dimensional finite element skull model. Biomedical Engineering and Clinical Medicine, 2005, 9(6): 320-325.

YANG Ji-kuang, XU Wei, WAN Xin-ming. Development and Validation of a Head-Neck Finite Element Model for the Study of Neck Dynamic Responses in Car Impacts. Journal of Hunan University(Natural Sciences)，2005, 32(2): 6-12.

Shijie Ruan, Haiyan Li, Xuekui Wang, et al. A New Exploration of the Applicability of the Head Injury Criterion. Journal of Biomedical Engineering, 2007, 24(6):1373-1377.

RUAN Shi-Jie, HE Pei, LU Jun-Peng, et al. Simulation Based Investigation on Injury Biomechanics of the Human Head by Finite Element Method. Chnese Journal of Biomedical Engineering, 2007, 26(1): 99-104.

Shaoxiang Zhang, Pingan Wang, Zhengjin Liu, et al. Number one of Chinese digitized visible human completed. ACTA Academiae Medicinae Militaris Tertiae，2003，25(7): 563-565.

Zhang SX, Heng PA, Liu ZJ. Chinese Visible Human Project[J]. Clinical Anatomy, 2006, 19:204-215.

13

The Experimental Technology on the Brain Impact Injuries

Zhiyong Yin[1], Hui Zhao[1], Daiqin Tao[1] and Shengxiong Liu[2]
[1]*Chongqing Key Laboratory of Vehicle, Biological Crash Security, Department 4, Institute of Surgery Research, Daping Hospital, Third Military Medical University, Chongqing*
[2]*Department of Biomedical Engineering, School of Pharmacy & Bioengineering Chongqing University of Technology, Chongqing*
China

1. Introduction

The traffic injury research originated during World War I. An American pilot named H. De Haven found the seat belt buckle and other cockpit parts of the fighter usually caused damage in crash accidents.Fifteen years later, he had confirmed the rubber windshield wipers could effectively prevent the metal penetrating injury caused by the forehead and facial scratches. Shortly after World War II, a mechanist J. P. Stapp led a preliminary study on the damage tolerance through accelerating pulley crash tests. Moseley (1970) tied a 5.1cm thick foam rubber pad to the right chest armpit of a dog, and fixed a steel of 7.6cm diameter, with a pistol firmly against the steel plate firing through the device to trigger shooting at the end of the spontaneous breathing. The model could cause the extensive parenchymal contusion, with the classical X-ray photographs performance and cardiopulmonary function changes. Anatomy of a few hours after the animal could be extensive lung hemorrhage, and there was often blood in the right bronchus.

2. Series devices for biological impact

In late 1960s, a simple gravity or gas-driven hammer impact animal unit has been appeared; as well as a model of liquid impact in nerve trauma study used, namely the liquid through the closed pipeline to transmit the hammer force to the brain tissue to cause damage. With the deepening of the study, it had been noted that the force changing on organisms and the precise control were critical for the study of dynamic effects. It needed to establish an ideal experimental device or model on the impact injury studies, that a series of bio-shock devices had been developed.

1) Vertical biological impact machine

1. Controllable biological impact systems

In 1974 the Swedish scholar successfully developed a controllable biological impact system to do impact injury experiments. It composed of the tower and the hit platform. The tower was equipped with a vertical pipe and cable guide. The hammer could vertically drop along the guide under the action of gravity acceleration. The impact platform included the steel

frame, the second hammer, the second hammer stopper and animal movement supporting frames. The impact machine works by: The hammer from different heights along the guide rail whereabouts -- drove down the second hammer impact sports -- animals -- injury. It was by adjusting the height (0.1 ~ 25m) and the quality (2 ~ 10kg) of the hammer to get different impact velocities (1 ~ 20m / s) and impact energy; by adjusting the height of animal backstop and the relative position of the second hammer to change the compression degree; by replacing the second hammer head to get different impact shapes and areas. In addition, there were mechanical power sliding displacement sensors and accelerometers which could respectively recorded in three parameters of time curves for the second hammer as the motion displacement, the displacement of the body wall deformation and the impact acceleration. The force sensor on the animal backstop could record the pressure pulses delivered to the body. The hammer was of a great range of quality that it was suitable for large animal impact injury experiments, but of a lower impact velocity for the drive limit.

2. Vertical air/hydraulic biological impact machine

For different driver ways, it has derived a variety of types of vertical biological impact machines. In addition to the type of free-fall impact machine, the vertical impact machine driven by pressure has been widely used. Because of power-driven, the hammer can provide higher impact velocity without falling from high altitude, which reduced the machine size greatly. Ways includes the gas and the liquid drive that two machines are basically of the same structure and working principle. These machines are mainly formed by the gas/liquid access, the sealed high-pressure piston, the impact hammer (piston), the test equipment and animal injuries units. The piston piston cylinder is divided into upper and lower chambers. The high-pressure gas or liquid goes into the chamber, pushing the piston downward to impact to the animal. The gas or the liquid in the lower champer discharges through the pipeline. In structure, the impact hammer and the piston has been integrated. The head of the piston becomes the hitting side, and can be made shapes of the steering wheel or others as needed, in order to directly simulate the whole process of traffic injuries caused by different parts of motor vehicle. There are energy-absorbing cushions under the piston cylinder head side to prevent downward movement of the piston to adjust the shock compression rate. The device is set on the rear chamber to monitor the moving process of the piston. Sensors are put under the animal injury platform to record the pressure pulses of the loading process of the body and the body's output during the impact.

Because gas has compressibility, the high pressure gas makes piston move forward quickly at the early stage of piston movement, and then it turns into a uniform motion. While driving the piston with liquid, the piston impacts animals with constant speed. The range of impact velocity of perpendicular type gas, liquid biology is commonly between 5m/s and 20 m/s.

3. Biological hit machine of horizontal type

Biological hit machine of horizontal type always moves as the high pressure air drives impact hammer. In 1978, the Viano etc. in motor vehicles research lab of the United States Michigan state biological medical department used hit machine of gas driving type. The main part of machine is the bubble similar to the above-mentioned high pressure cylinder, when launching trigger device, high pressure gas in the high-pressure chamber drives piston to do high speed forward movement and hit secondary hammer which hits animals or other simulative contents. The forward movement distance of piston is 10 cm, the weight of secondary hammer is 23.4 kg hung by guide ropes, the forward movement distance is 20 cm. Compared to vertical hit machine, animal injury frame has made a big improvement;

first, it can slide forward with fixed animal car when hitting animal; second, the pulley placed animal is hung on the framework which can slide on the rail, when hitting framework, animal and sliding framework do the reverse movement because of inertance, and it leads to damage when hitting simulative steering wheel fixed on framework.

4. Several domestic biological hit machines

The third military medical university institute of surgical field first successfully developed biological hit machine series of type BIM-I, II and III in 1990 in China, it provided a good experimental method for research of injury by collision.

1) Vertical biological hit machine of type BIM-I

This machine is composed of the impacting tower, cushion and animal injury frame with about six tons of total weight. The impacting tower is welded from angel steel, it forms closed structure with outsourcing wind sheet. The weight of impact hammer gradually increases 0.5 kg every time and its heaviest weight is 50 kg, the drop height can be controlled in 0.5 ~ 36.5 m. Impact hammer can trigger buffering devices automatically when rising to a predetermined height, and it can drop along with vertical orientation steel wire to hit secondary hammer.

Cushion consists of about a ton of table-board, pillar, buffer block and secondary hammer, its main function is to control impact range and absorb great energy produced by impact. The fastest impact speed of this machine is 26 m/s, and the instant impact speed simulated is 90 km/h.

Animal hurt frame can fix animals flexibly, according to the adjustment of horizontal and vertical position; it can precisely control impact site and the impact amplitude of the compression.

2) Horizontal biological hit machine of type BIM- II

This machine mainly consists of high-speed air cannon, secondary hammer, animal hurt frame, sliding rail system and velocity measurement system with the total weight of about two tons. The pressure of gas storage tank in high-speed gas cannon is 25 MPa, but the practical working pressure is no more than 10 MPa, the opening time of pneumatic valve is about 2ms, diameter and length of bore is 50 mm and 1300mm, the fastest initial speed of bore can reach to 90 m/s, it can simulate crash condition that the bus speed is no more than 320 km/h. It can accurately control impact amplitude of the compression through the secondary hammer, different impact area and geometric shape can be chosen when hitting head. Using secondary hammer seat as limiting stopper to avoid bullet breaking away from bore, and avoid the damage happened when bullet impacts with other objects, bullet can be used again. In order to adapt to research needs of different kinds of animal injury by collision, both bullet and secondary hammer have various standards of length and quality.

Velocity measurement system consists of speed measurement table, laser generator, shock tube beam splitter, guiding light implement, photosensitive components and signal amplification receiving circuit, the error of measuring secondary hammer speed is no more than 0.01 mm/s, it fully meet the requirement of speed measuring precision in the biological experiment.

Impact can be divided into standard static state and dynamic according to the difference of animal hurt frame and ways of fixation. Impact way of standard static state adopts fixed animal hurt frame, then animals cannot move forward when hitting. In the biomechanical testing experiments, it is need to put some sensors on the animal body, therefore, impact

way of standard static state is mostly adopted. The major structure of dynamic hitting way is sliding rail system, which is composed of horizontal sliding rail, holder and pulley etc., the effective length is 3.5m and height is 1.9m. Fixing the animal on the pulley, then using secondary hammer to impact, it can move ahead with pulley after getting impact. If installing baffle in front of sliding rail, animal can strike baffle after moving, it can lead to the animal model of acceleration injury accompanying with deceleration injury. This kind of hurting way can simulate the impacting conditions that pedestrian collides with road or other objects after hitting and throwing.

3) Portable biological hit machine of type BIM-III

The structure of this machine is simple, it is easy to install and remove with the total weight of about 11 kg, and the volume is about 70 x 20 x 10 cm3 after remove. Its working principle is to hit animals relying on impact hammer dropping from different heights; it can carry out fixed or unfixed impacts using turning institution of animal frame. It is suitable for the impact injury research on mice, rats, and guinea pig head, chest and abdomen.

3. Injury device of deceleration injury

The kinetic object impacts the still experimental object to make it accelerate along with external force, and the injury happens when impacting the experimental one. Deceleration damage means the damage happened with the sudden deceleration of kinetic experimental object when it impacts the still one. Because of buffer distance, the accelerated damage is relatively light, which is often limited to stress side; while the decelerated damage is relatively heavy, the damage can happen both on stress side and offside, and the offside damage is heavier. The first purpose of developing injury model of deceleration device is to make the experiment object accept certain initial velocity, and then solve the fixed problems of experimental object. Tan Yuanfu has designed a device for craniocerebral deceleration injury research with the power provision of rubber band, and with this device, he has developed rabbit craniocerebral deceleration injury model. This device consists of turn board(A), axis of rotation(O), elastic belt(F) and holder(B), The two-thirds of the turn board is fixed to the table through the axis of rotation, turn board can rotate 360 °, the groove(40 mm x 200 mm) in the front of turn board can be put animal head. There are many 600mm long elastic belts at the end of turn board. the elastic belt is flabby under horizontal position, the center of turn board front end is put on the cushion (E) to make it on the horizontal position, the 35 mm in diameter bump anvil(C) on the holder protrudes into groove 5mm. When turn board is on a vertical position, the elongate elastic belt is at a high tension state, and position of turn board is fixed through a pull hook (G). Turn board return to horizontal position with the help of elasticity after loosening pull hook, at this time the rabbit head in the groove of turn board gets craniocerebral deceleration injury because of collision with bump anvil, and the degree of damage can be adjusted through the number of elastic belts. Moments after impact, turn board rebounds because of the function of cushion, at this time, the hands need to hold turn board quickly to avoid the second damage.

Author has developed an injury device of slow injury according to the transforming principle between potential energy and kinetic energy, initial velocity of experimental subject is controlled by height of fall. This device is composed of turn board (A), animal fixed trolley (E), foundation bed (G), chopping block(C) and holder(B)(Figure 2). Sliding guide rail is installed in fixed bases, which consists of four steel wires, the degree of

tightness is regulated by the prestressing steel wire below foundation bed. The distance that animal fixed trolley slides along with guide rail is 7.0m, the height is marked with scale plate(D). The rolling bearing installed around that car can make it slide freely along with the sliding guide. The animal is fixed on trolley and its head is held by holder to make its position oppose to collision anvil. Bumper block is placed between animal fixed trolley and foundation bed, and the adjustment of deceleration coming from collision between trolley and foundation bed is based on bumper block features such as elasticity coefficient and thickness, it also can protect the trolley. Relying on the device, besides a series of rabbit cerebral injuries of deceleration injury tests, we still do the injuries of deceleration injury tests of physical model.

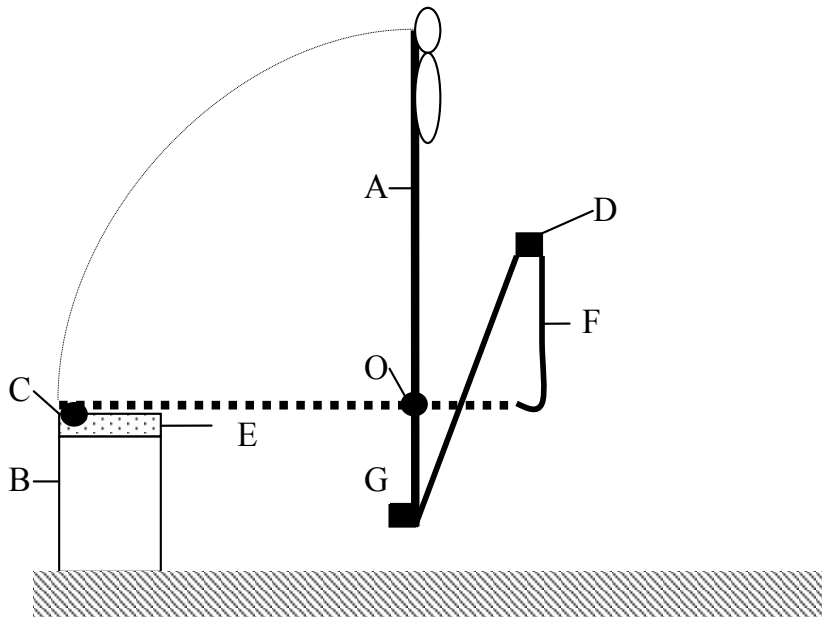


Fig. 1. Injury device of deceleration injury: A . turn board, B. holder, C. bump anvil, D. elastic belt fixed place, E. cushion, F. elastic belt, G. fixed pull hook, O. axis of rotation

4. Collision experiment of real car

In the experimental research of large traffic accident/injury, the foreign country regard real cars as injury tools, animals or dummy devices as received subjects, and it realistically imitates mechanical force that people get when cars collide with pedestrians, cars and other fixations, then multidimensional high-speed camera and all kinds of sensors will do detailed records and analysis. Many domestic organizations also have the condition of real car collision experiment, and have already conducted a large number of real car crash tests. The data and authenticity of real car crash tests is reliable, it is reliable mean to check and accept protective equipment and evaluate the safety standards. But the deficiency is that it needs larger venues, and cost is expensive, so it cannot be used to carry out a lot of repetitive biological experiments.

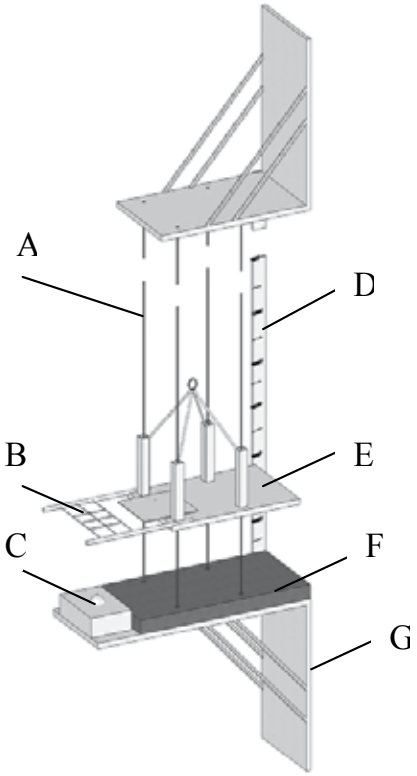


Fig. 2. Injury device of slow injury: A. guide rail, B. holder, C. chopping block, D. scale plate, E. animal fixed trolley, F. bumper block, G. foundation bed

5. Biological impact test platform of rail type

In fact, the occurrence of traffic injury is a dynamic process, which involves the hurting of the whole experimental objects, while the previous road traffic injury researches are mainly on the local impact tests, which cannot completely simulate the whole and dynamic engineering of traffic injury occurrence and development. Some foreign research institutes have established collision equipment for traffic medical research. And in recent years, China has also established collision laboratories in Beijing, Tianjin, Hubei and Shanghai, and they are mainly used for the real car crash tests and the test of safety performance of automobile parts; its purpose is to test the security of the car. But these researches are mainly based on engineering, and its purpose is to provide the basis and theoretical guidance for car improvement and protection of people, which combine less with medicine, especially with clinical medicine.

In view of the above reasons, the administrative office where the author is decides to establish a biological collision laboratory of track type, which gives priority to biological trolley and gives consideration to the car crash tests, its purpose is to realistically simulate occurrence process of various traffic injuries, then doo further research on trauma occurred mechanism, protection and treatment, the research laboratory began to construct in 2003, and the biological collision laboratory of track type was put into use in 2004.

This lab can carry out car crash test and collision experiment (including front collision, angle collision etc.); Crash conditions correspond to the basic requirements of related laws and regulations of the American FMVSS, European EEC and Chinese CMVDR294 etc, when collision speed is ≤64km/h, the speed control precision is ≤±2%, the maximum traction acceleration is not more than 0.5g, the average traction acceleration is not more than 0.3g; the fastest collision speed can reach to 120km/h。The biological impact test platform of rail type is composed of ground equipment, rail system, motor traction and control system, test system, lighting system and biological car etc.

1) Facility

The length of lab and acceleration road is about 88 meters and 75 meters, the width and length of collision hall is about 20 meters and 30 meters, and the total construction area is 1400 square meters. The main equipments of facility include the fixed counterguard and track beam to support collision of experimental vehicles. The counterguard is composed of reinforced concrete construction, and the impact surface is edged with a T shape channel steel plate, which can prevent it from movement and damage when the 2500 kg trolley impacts with the speed of 80 km/h. There are good vibration isolation measures between counterguard and surrounding, which can ensure that the collision experiment will not cause the vibration of the workshop and damage of surrounding ground. Rail beam is made of reinforced concrete (78m 2.4m 1.0m), which is used to support trolley rail, traction guides and parking buffer, etc. The shape of track girder is regular to make sure that the rupture, deformation or displacement will not appear in using process. According to the test requirements, corresponding facilities can be set up in the area to complete particular test content.

2) Rail system

Rail system includes trolley rail, traction guide rail, rail cover plate, track girders, etc. Two trolley rails are installed in both sides of traction rail symmetrically; the experimental vehicles can accelerate through trolley rail or rail cover plate, and reach the setting speed in the prescribed distance, then complete collision experiment in collision hall.

1. Trolley track The trolley track has two rails of 82m total length with center distance of 1.80m in east-west direction. The two rails are fastened symmetrically to the track beam by the fastener with an interval of 1.25m.

The fine finishing heavy rail of 50kgf/m has been welded into the seamless track. The distance between the weld and the anchor fasteners is 0.30m. Each rail has seven welds. The south rail is selected as the main rail while the north rail as the vice rail. The length of the main rail should be no more than ±0.5mm. The error of the vice rail relative to the main rail in the vertical and horizontal position should be no more than ±0.5mm. The deviation of the two tracks distance should be no more than ±1mm. The elevation of the upper plane of the rail is -0.010m with deviation of less than ±1mm.

2. Traction rail The traction rails are the running tracks of the traction car which is used to connect the traction rope and the experimental trolley for acceleration. The traction rails is a double structure with a small span. The track center distance is 0.235m. The rail is of the 25b channel. The vertical and horizontal of the upper plane of the traction rails should be in level and the elevation should be no more than ±2mm. The spacing of the edge above the guide rails is 150^{+1}_{-0} mm and the total length of the vertical and horizontal straight line should be no more than ±2mm.

3. Rail cover The rail cover is a key component both for trolley and real vehicle experiments. It Requires that when carrying out real car crash tests, it should ensure smooth movement of vehicles and the "pavement" friction coefficient should be not less than 0.75.

3) The traction motor and control system

The traction power is from the two 225kw DC. The accurate closed-loop feedback control is from the DC speed controller to achieve precise control of pre-collision speed. The test data show that the motor is running smooth, with qwll synchronization of two-motor and load capability.

4) The hydraulic tensioning and control system

A mechanical tensioning device is placed in the test preparation room mainly for adjusting elastic rope seasonally. The hydraulic tensioning device is placed in the collision hall. For each test, it needs to do tension work. The hydraulic tensioning device is arranged behind the rear-wheel drive drum. After tensioning action, the wire rope is firmly pressed in the trough of the drum rope in order to pass the torque of the motor to the wire rope.

There are two frameworks with rectangular slide on the side of the rack panel. The T-slot on the base plate and the iron plate is linked by special screws which can resist to four tons of tension. The two rope tensioners moves straightly by two hydraulic cylinders, making the rope in tension or relaxation. The tension wheel assembly runs independentlyin the rectangular slide. The tensioning assembly includes a curved file block to prevent the rope relaxed state of the slide-out round of the slot. The tensioning wheel has a diameter of 400. The cylinder of the stainless steel tube of SMC (Japan) has magnetic limit switches in the front and rear of the two extreme positions. It transmits the signal to ban the motor running in the front and rear of the two extreme positions. The hydraulic system provides a stable and adjustable pressure for the cylinder and braking system of the hydraulic tensioning device. Pumping station has a perfect control system to contact with the upper total control system.

5) lighting control system with hydraulic tensioner

For shooting the dynamic changing process of test objects in real trolley/vehicle experiments, it needs to ensure adequate lighting illumination that it can guarantee 5000lux illumination in front of 5m×2m range of the barrier and that it must meet the spot uniformity, light color stable, high energy efficiency, color reproduction and good requirements. The lighting system installs on the movable three-dimensional light shelves which can meet the lighting needs of any location in the collision hall with mobile stands for light adding.

6) The biological trolley

The previous bilogical impact experiments are usually on real cars with animals. But all know that the real vehicle collision tests are of high costs with poor reproducibility between different vehicles, the long preparing time as well as the space limitations for animal numbers. This shows that it is not suitable for biological impact tests by real vehicles with animals. The large number of biological traffic crash tests is conducted to the study for traffic medicine. It is necessary to develop the biological trolley on the orbital biological impact platform for replicating the traffic injury animal models.

To do biological trolley experiments, it needs to solve the problems like the power, the animal fixing and the energy-absorbing. The traffic injury trolley for small animals has been developed based on the orbital biological impact laboratory. The biological trolley of small animal injury is an important part of the platform including the powered trolley and the animal fixable trolley. The trolley system is directly tracked by the guide rails and the wire ropes from the motor traction system in the lab. The car is free to slide on the traction rail through the pulley (Figure 3). The powered of the car is from the mechanical hook of the fastening wire ropes. When it gets the expected speed, they leave from each other to keep the constant speed. Different types of small animals can be fixed on the animal trolley for traffic injury collision experiments. Two types of trolleys can be applied as needed to simulate the different mass of vehicle collisions, and can simulate the frontal-, the side- and the rear-collision.

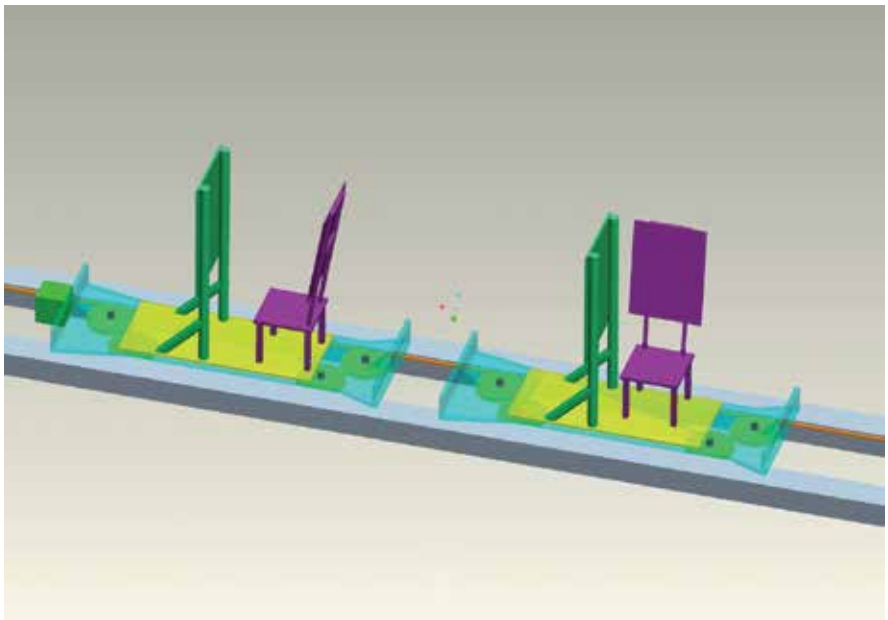


Fig. 3. The animals fixed trolley.

The energy-absorbing device is on the biological trolley or in front of the barrier. In addition to absorbing the energy of the impact moment from the barrier and the trolley, it can generate different damping forces to replicate different deceleration curves to carry out different types of traffic injury experiments for the biological trolley system. There are lots of energy absorbing forms, and usually it takes the hydraulic or combined thin-walled beams.

The structure of the hydraulic buffer is a high-pressure damping chamber. There is an oil pool above the high-pressure chamber. There are several orifices on the chamber wall as the connection of the high-pressure chamber and the oil pool. When the piston rod impacted, its front pushes the oil in to the pool. There is heat from the friction of the oil and the throttle. The majority of car's kinetic energy changses into hydraulic oil heat, and parts of the energy store in the oil pool. By adjusting the orifice area, it can get a specific deceleration waveform to meet the experimental needs.

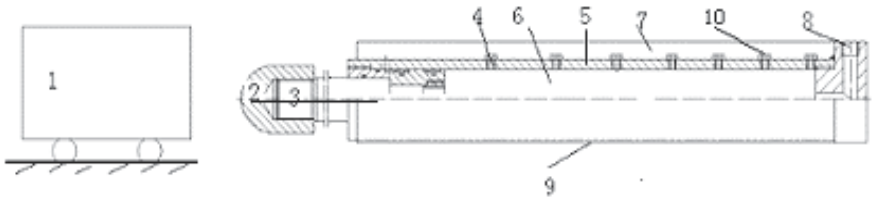


Fig. 4. The schematic diagram of the hydraulic shock absorbers: 1. trolley; 2. buffer block; 3. rod; 4. orifice; 5. Inner liner; 6. high-pressure chamber; 7. oil pool; 8. discharge hole; 9. cylinder; 10. cutting bolts.

The combined thin-walled beams are energy absorption devices taken a standard thin-walled square steel tube with adjusting the length and thickness of thin-walled square steel pipe combinations, to solve problems such as high costs, the single waveform and the poor reproducibility of the existing car-absorbing devices. By controlling the thin-walled square tube length of each group, this combination can control the time to make up peaks and troughs for each other in order to get a smoother deceleration waveform. The combination forms of the thin-walled square tubes are determined by the according to the mass and the deceleration curve of the trolley. The average length of thin-walled square tube is determined by the collision speed. The mounting block has been put on the trolley, so the thin-walled square tube and the trolley can be tight closely. It is applied the pre-strain in front of the thin-walled square tube with the direct rigid collision between the fixed barrier without the buffer block to let the deceleration waveform meet the test requirements. It provides a practical solution for many collision experiments on the safety of the auto parts and for biological experiments.

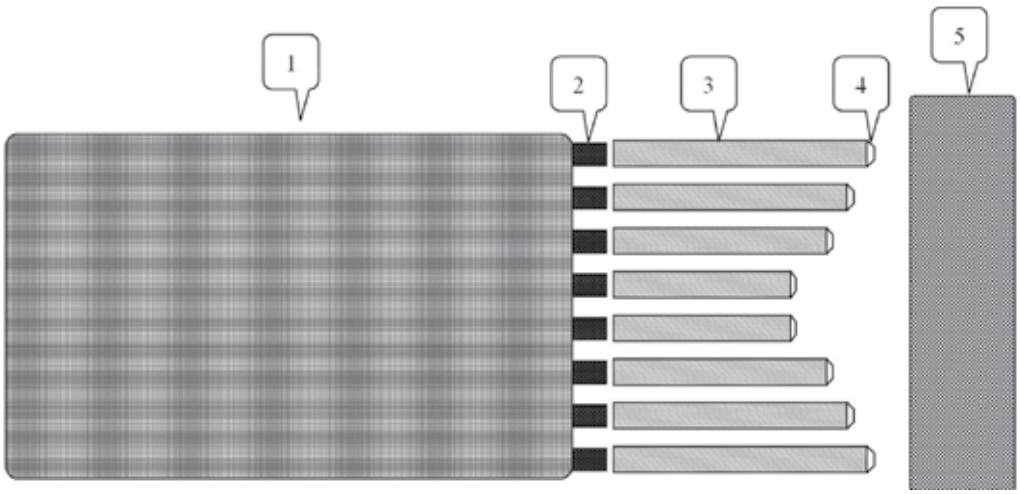


Fig. 5. The combined thin-walled beam of absorption method in trolley crash tests: 1. Trolley; 2. Mounting Block; 3. Thin-walled square steel pipe; 4. Pre-strain; 5. Fixed barrier.

Around the car crash tests, the biological assessment of traffic safety, the vehicle crash and safety protection equipment and the security measures, it can carry out certification tests for vehicle regulations, biological collision experiments, pedestrian crash tests, rollover tests

and motorcycle crash tests, etc. The platform can realistically simulate the impact of various vehicles and traffic injuries. It is an ideal platform for basic research of traffic injury and impact injury. It is also an important technical platform for the development of new vehicles and car parts.

6. Computer simulation tests

Today, computational science has been the third branch of sciences as well as the other two traditional sciences that are the theoretical science and the practical science. When the theoretical model is very complex or has not been established, or the experiment is so expensive that it cannot be done, the computer simulation experiment becomes the the main or only means. In recent years, with the development of the electronic technology, computer science and computational mathematics, it has become a trend to to study the road traffic injury through computer simulation experiments.

The traffic injury study is a wide range including the digital reconstruction of human injury in the accident scene, the biological injury testing in the laboratory and trauma biomechanics research on some individual organ damages. Since there are different purposes and objects for the traffic injury research, there are a variety of computer simulation methods and software systems which can be used to transport medical research.

1) Impulse / momentum method

Large number of real traffic accidents cases is fresh sata for traffic injury research. The traffic accident reconstruction is essential means for real case studies, and the impulse/momentum method is one of the most common means for real digital reconstructions. According to rigid body plane collision theory, the car is simplified as the plane crash model of three freedom degrees with single mass. Assuming a car driving on a flat and regular road before and after the collision, base on linear momentum and angular momentum conservation principle, it considers the energy loss by rebound factor from deformations.

PC-Crash is a typical software based on the impulse/momentum principles. In addition to the digital reconstruction for collisions like motor vehicles - motor vehicles and motor vehicles, it can also do numerical simulation for accidents like the motor vehicle - pedestrian, motor vehicle - cylinder and roll. This software can simulate up to 32 cars multi-hit at the same time and show the three-dimensional animation. A common method in the PC-Crash for accident reconstruction is taking the vehicle brake or the stop location as the optimizing target, and taking the car speed, the initial contact position, the rebound coefficient, the friction coefficient and other parameters as the optimizing variables. When the calculated results of braking traces or the stopping position consists with the real circumstances, parameters corresponding to this state are considered as closest to the real situation that it can analyse the accident cause and the human injury based on the computer simulation.

2) Energy / deformation methods

In car crashes, the deformation of the vehicle depends on the collision energy, while the collision energy depends on the relative speed between two vehicles. It shows that it is an important means of digital reconstruction by the deformation of the vehicle to infer the vehicle collision speed and to estimate the collision point. Its goal is to optimize the vehicle deformation. And the collision speed, the initial contact position, trajectory, friction coefficient and other parameters are optimization variables to do iterative calculation. When the calculation of the vehicle deformation consists with the real circumstances of the

accident, it is considered as the closest to the real situation under the state corresponding to the parameters.

A typical software based on the energy/deformation theory is SMAC (Simulation Model of Automobile Collisions). The software is an open process. In use, the user need to develop in form of card on vehicle size, inertia, deformation and tire characteristics, as well as initial velocity, angle and motion control input, etc. These values can be modified at any time. The program calculated the detailed information such as speed, acceleration, position, angle, deformation and the force of each tire and other values by the collision process. Users modify the initial value by constantly adjusting the input of the initial velocity, impact angle and other variables according to the differences between simulation results and actual results, until the simulation results matched up with physical evidence. In recent years, M-SMAC has been continuously developed. Users need only simply enter the trajectory of the collision, the vehicle deformation and the stop position that the program can optimize computing and eventually get a real situation with the closest results.

3) Multi-body dynamics Method

Currently, human biomechanics research based on numerical simulation techniques receives more and more attention in the analysis of traffic accidents. By the numerical simulation technology, it can reasonablely interpret in mechanics for the collision part of the bodies, the injury degree and causes. The body damage analysis can directly reflect the contact position and force size between people and cars, and people and ground. The accident reconstruction method based on the impulse/momentum principles or energy/deformation theory only depends on the brake marks or deformation of the body contours of the vehicle velocity and other parameters to solve problems. It cannot analyse or describe the human body injuries in the accidents.

Multi-body dynamics analysis is a powerful tool for human injury analysis. It is a mechanical branch combining the rigid body mechanics, analytical mechanics and the computer technology. Multi-body dynamics has versatility in calculating the movement of large displacement etc. with high speed in traffic injury research.

The software of MADYMO (Mathematical Dynamic Model) is based on multi-body dynamics methods. It is now one of simulation softwares for the collision damage simulation with extensive applications. In MADYMO software, it predict the injury of the occupant/pedestrian collision and the dynamic and kinetic characteristics of vehicle structures through the establishment of the appropriate model. MADYMO models are multiple rigid bodies connected by a group of different types of hinges. They usually form an open loop tree by defining the freedom degree of the hinge, binding constraint stiffness and inertia of the rigid body to get the rigid multi-body system dynamics model. The shape of the model is composed of rigid bodies such as the plane, the cylinder, the ellipsoid or other super-ellipsoid shapes, which constitute the contact surface of the multi-body model.

MADYMO software is based on theories of multi-body dynamics to generate motion equations of multi-body system. In addition to describing movement and interaction, the multi-body motion of the system has been affected by the forces from springs, dampers and the restraint system. MADYMO software can not only be used to calculate the acceleration, displacement and contact force, but also provide some body damage index calculation, such as head injury index (HIC), Severity Index, chest resultant acceleration values, chest injury index, viscosity index and femur axial load and so on. Although the original mind of MADYMO design was for the study of vehicle collision mechanics, it has been applied to

analyze other modes of transport such as trains crash, aircraft, motorcycles and bicycles, while the software has also been used to evaluate the applicability of different restraint systems, such as seat belts and airbags.

4) FEM (Finite Element Method)

The finite element method (FEM) is a more innovative and effective numerical method developed for computer use. This method originated from aeronautical engineering in aircraft structure matrix analysis in the middle of 20th century.

For the structure matrix analysis, the overall structure can be seen as a collection interconnected by limited numbers of mechanics units, and each unit can be seen as the building brick that their assemblies can provide the overall mechanical structure properties. In 1960, an aircraft structural engineer named Clough firstly used the term of Finite Element Method in his paper to solve the plane elasticity problem. Since then, not only engineers but also mathematicians and mechanics began to realize the efficacy and the great prospect of the finite element method that they have been in-depth discussed to give it a more solid theoretical foundation. In the joint efforts of engineers and scholars, the finite element method has gone into the real continuum mechanics, becoming one of the most effective ways for solving mechanical problems. In 1969, Fridenbery finited the element theory for the first time, then it has been applied as a new methods and theories to the medical field on human biomechanics research. Currently, FEM has been widely used in the human body biomechanics and collision damage mechanics.

Typical FEM-related softwares include:

1. LS-DYNA

LS-DYNA program is mainly based on Lagrange algorithm, with ALE and Euler algorithms; oriented in the explicit solution, with the implicit solution function; on non-linear dynamic analysis with the static analysis. LS-DYNA program currently has 140 kinds of metal and non-metallic materials, with consideration of material failure, damage, viscosity, creep and strain rate associated with such properties.

2. ABAQUS

ABAQUS provides a wide range of functions, and very easy to use. Input ABAQUS data first, then enter the options block. The beginning of each option is labeled a descriptive name, so it is very convenient for the achieve of ABAQUS data. A lot of complex problems can be simulated easily by different combinations of option blocks. For example, for complex multi-component combination of simulation problems, it is by combination of defining each option bocks of component set sizes with the corresponding material properties. For lots of simulations, even for some higher non-linear problems, users only need to provide some project data, set sizes of the structure, material properties, boundary conditions and load conditions. In some non-linear analysis, ABAQUS can automatically select the appropriate load increments and convergence accuracy. It can not only choose the right parameters, but also to adjust the parameters in the analysis process to ensure effective exact solutions. By accurate definition of parameters, users can control the numerical results effectively.

7. References

[1] Xiaoli Guo, Peifang Zhu, Zhengguo Wang, etc.Animal experimental models of traffic injury caused by car crash to guardrail. Chinese Journal of Clinical Rehabilitation. 2005, 9(30):120-122.

[2] Xiaoli Guo, Zhengguo Wang, Peifang Zhu, etc.Characteristics of traffic injuries caused by lateral impact of cars in pigs.Journal of Traumatic Surgery. 2005, 7(4):251-254.
[3] Xiaoli Guo, Peifang Zhu, Zhengguo Wang, etc. Experimental study of traffic injuries caused by frontal crashes of cars. Chinese Journal of Trauma. 2005, 21(5):378-380.
[4] Xianlong Jin, Xiaoyun Zhang. 2007. Theory and practice of digital traffic accident reconstruction. Beijing: China Communications Press.
[5] Renxian Li.2004. The basis of the finite element method, 2nd edition. Beijing: National Defence Industry Press.
[6] Hui Zhao, Zhiyong Yin, Zhengguo Wang, etc.Study of traffic injury of rear crash in rabbits.Journal of Traumatic Surgery. 2006, 8(3):249-252.
[7] Hui Zhao, Zhiyong Yin, Zhengguo Wang, etc. Study on traffic injury of front crash in rabbits. Acta Academiae Medicinae Militaris Tertiae. 2007, 29(17):1657-1659.
[8] Hui Zhao, Zhiyong Yin, Rong Chen, etc. 2009.Experimental study on deceleration-induced brain injury in rabbits. Journal of Traumatic Surgery. 2009, 11(4): 306-309.
[9] Zhengguo Wang.1997. Traffic Medicine. 1st edition. Tianjin: Tianjin Science and Technology Press.

14

Towards Non-Invasive Bedside Monitoring of Cerebral Blood Flow and Oxygen Metabolism in Brain-Injured Patients with Near-Infrared Spectroscopy

Mamadou Diop, Jonathan T. Elliott, Ting-Yim Lee and Keith St. Lawrence
Imaging Division, Lawson Health Research Institute
London, Ontario
Canada

1. Introduction

Monitoring the injured brain to detect and treat harmful events that can cause secondary injury during the acute recovery period is a central part of neurointensive care. The most basic monitoring tool is the neurological examination, such as the Glasgow Coma Score; however, a large component of this scale involves verbal communication and brain-injured patents are often comatose, mechanically ventilated or sedated. As well, symptoms of neurological deterioration detected by examination often occur at late stages of brain injury. Since the brain is extremely vulnerable to ischemia, a more direct indicator of potential brain injury is detecting impaired cerebral blood flow (CBF). Multiple factors following brain injury can cause ischemia, including systemic hypotension, cerebral hemorrhage, and edema – all of which independently worsen survival (Helmy et al., 2007). This focus has lead to the recognition that continuous monitoring of CBF in patients with, or at risk of, brain injury could improve outcome by providing the ability to detect and prevent cerebral ischemia.

Imaging techniques, such as magnetic resonance imaging (MRI) and computed tomography (CT), are critical to the management of brain-injured patients as they provide detailed structural and functional information of the brain when patients are admitted to an emergency department (Gallagher et al., 2007). CT is the modality of choice because it is widely available and examination times are relatively short. Furthermore, techniques for measuring CBF have been developed on these imaging modalities (Wintermark et al., 2005) and subsequently used to identify CBF abnormalities following brain injury (DeWitt & Prough, 2003; Gowda et al., 2006; Soustiel et al., 2008). Despite these promising advances, conventional imaging modalities suffer from serious disadvantages regarding cerebral monitoring. First, they require transferring patients to imaging facilities which represents a significant risk factor when dealing with critically ill patients. Second, they only provide a single time-point measurement and, therefore, suffer from the possibility of missing flow abnormalities that occur at different times during intensive care. Clearly, effective cerebral monitoring requires bedside techniques.

Since CBF is driven in part by the cerebral perfusion pressure (CPP) which depends on intracranial pressure (ICP), using these pressures as surrogate markers of CBF is becoming an integral part of critical care practice. Intracranial pressure is typically measured using either a ventricular catheter or an intraparenchymal probe, and CPP is determined by the difference between mean arterial pressure (MAP) and ICP. Although there have been no large randomized trials comparing target thresholds, it is generally recommended that the ICP threshold above which treatment should be initiated is between 20-25 mm Hg (Bratton et al., 2007b). Similarly, CPP should be maintained above 60 mm Hg to ensure adequate CBF (Bratton et al., 2007a). However, the thresholds for ICP and CPP remain a source of contention. Increasing CPP (70-90 mm Hg) was found to reduce ischemia (Coles et al., 2004), but comes at the cost of increased incidence of extracranial complications and possibly contributes to vasogenic edema (Robertson et al., 1999). The Lund protocol advises a lower limit of 50 mm Hg to prevent complications while avoiding extremely low CBF (Asgeirsson et al., 1994). It has even been suggested that CPP/ICP therapy only increases therapy intensity without improving outcome (Cremer, 2008). Strictly adhering to targets can therefore give clinicians a false sense of security. A method of measuring CBF at the bedside would remove this ambiguity since the occurrence of ischemia due to inadequate CPP or hyperemia due to excessive CPP therapy would be readily apparent.

The most widely used beside technique for monitoring CBF is transcranial Doppler (TCD). This non-invasive technique derives blood flow velocity from the Doppler shift caused by moving red blood cells in a cerebral artery. Provided the angle of insonation and the diameter of the interrogated vessel remain constant, changes in TCD measurements reflect CBF changes (Valdueza et al., 1997). However, the accuracy of TCD is operator dependent due to problems of probe fixation, the TCD signal is not found in 10-30% of patients, and the technique cannot monitor blood flow in the microvasculature, which can be quite different from flow in the major arteries (Wright, 2007). Invasive techniques for monitoring CBF that require inserting probes directly into brain tissue include laser Doppler (LDF) and thermal diffusion flowmetry (TDF) (Bolognese et al., 1993; Sioutos et al., 1995). These techniques have been used to detect ischemia in brain-injured patients and TDF was shown to be more sensitive than TCD to assessing vasospasm, demonstrating the value of assessing CBF directly (Kirkpatrick et al., 1994; Vajkoczy et al., 2001). Besides being invasive, the major limitation with LDF and TDF is that they can only monitor the vascular territory where the probes are placed. Wider applicability of CBF monitoring requires non-invasive techniques that can assess CBF in multiple brain regions.

A promising alterative to current invasive monitoring techniques is near-infrared spectroscopy (NIRS). Due to the relative transparency of biological tissue to near-infrared (NIR) light, the brain can be interrogated non-invasively by probes placed on the scalp (Jobsis, 1977). Not only is NIRS non-invasive and safe, due to the use of non-ionizing radiation, the major tissue endogenous chromophores – water, oxy- and deoxy-hemoglobin (HbO_2 and Hb), cytochrome c oxidase, and lipids – have unique absorption properties in this wavelength range. The challenge to quantifying tissue chromophores concentrations with NIRS is the need to account for the strong light scattering, which dominates over light absorption. To meet this challenge, techniques that can take into account the effects of scattering have been developed and NIRS is now widely used to continuously monitor HbO_2 and Hb, and derive cerebral blood oxygenation. The most reported NIRS parameter is the ratio of HbO_2 to total haemoglobin (i.e., HbO_2 + Hb), which reflects cerebral blood oxygen saturation and is commonly referred to as the tissue oxygenation index (TOI). The TOI has been proposed for detecting ischemia and

assessing autoregulation by comparison to MAP (Al-Rawi & Kirkpatrick, 2006; Brady et al., 2008). The difficulties with using TOI as a surrogate of CBF are that blood oxygenation is influenced by multiple factors (CBF, cerebral metabolism, arterial saturation and hematocrit) and the "normal range" has yet to be defined. Reported TOI values have ranged from 60 to 85%, and this variation is as great as the change attributed to ischemia (Al-Rawi & Kirkpatrick, 2006; Thavasothy et al., 2002; Yoshitani et al., 2002).

NIRS techniques that can directly measure CBF have been developed. The first method proposed was based on the Fick principle and used a rapid change in arterial oxygen concentration as a blood flow tracer (Edwards et al., 1988). Improved sensitivity was achieved using indocyanine green (ICG) due to its strong light-absorbing properties (Patel et al., 1998). This FDA-approved dye has been in clinical use for over 30 years and has a high safety record; a study involving more than 3000 human subjects, showed that intravenous injection of ICG resulted in significant allergic reaction in only one subject (Hope-Ross et al., 1994). Absolute CBF, rather than a relative measure, was obtained with the ICG approach using a dye densitometer, to measure the arterial blood ICG concentration, a NIRS method capable of quantifying the tissue ICG concentration and a deconvolution method (Brown et al., 2002; Springett et al., 2001). Because of the inherent sensitivity of NIRS to blood oxygenation, $CMRO_2$ can also be measured by combining NIRS measurements of CBF and oxygenation. In addition, $CMRO_2$ has been shown to be a better marker of injury severity in hypoxia-ischemia than CBF alone (Tichauer et al., 2006a). In this chapter we will show that NIRS can be used to measure cerebral blood flow and oxygen metabolism, and to detect general and focal brain injuries.

2. Theory

2.1 Cerebral blood flow model

The hemodynamics of an organ can be quantified by tracking the passage of a contrast agent through the vascular bed, an approach that has been adapted to imaging modalities including MRI and CT, and to NIRS. With this method, the amount of contrast agent in the organ at a given time t ($Q(t)$) is related to the difference between its arterial ($C_a(t)$) and venous concentrations ($C_v(t)$), and to blood flow (F) via the Fick Principle (Meier & Zierler, 1954):

$$\frac{dQ(t)}{dt} = FC_a(t) - FC_v(t). \tag{1}$$

If $Q(t)$, $C_a(t)$ and $C_v(t)$ could be measured non-invasively, then the calculation of flood flow would be a simple mathematical operation but that is not the case since $C_v(t)$ cannot be measured noninvasively. However, the venous concentration of contrast agent can be expressed in term of $C_a(t)$ as (Lee, 2002):

$$C_v(t) = C_a(t) * h(t), \tag{2}$$

where * is the convolution operator and $h(t)$ is the impulse response function of the organ (i.e., the distribution of transit times) and represents the venous concentration of contrast agent if the arterial input was a Dirac-delta function. From Eq. (1) and (2) it can be shown that $Q(t)$ can be expressed as (Lee, 2002):

$$Q(t) = C_a(t) * F \cdot R(t) . \tag{3}$$

Equation (3) shows that CBF can be determined by measuring $Q(t)$ and $C_a(t)$, and applying a deconvolution technique to extract $F \cdot R(t)$. The initial height of the function $F \cdot R(t)$ is CBF, as by definition $R(0) = 1$, and the area under the curve is the cerebral blood volume (CBV) (Brown et al., 2002). It is clear from Eq. (3) that determining CBF requires the quantification of the tracer concentration in the brain. Fig. 1 shows an example of $Q(t)$ and $C_a(t)$ measured on a piglet and the retrieved $F \cdot R(t)$.

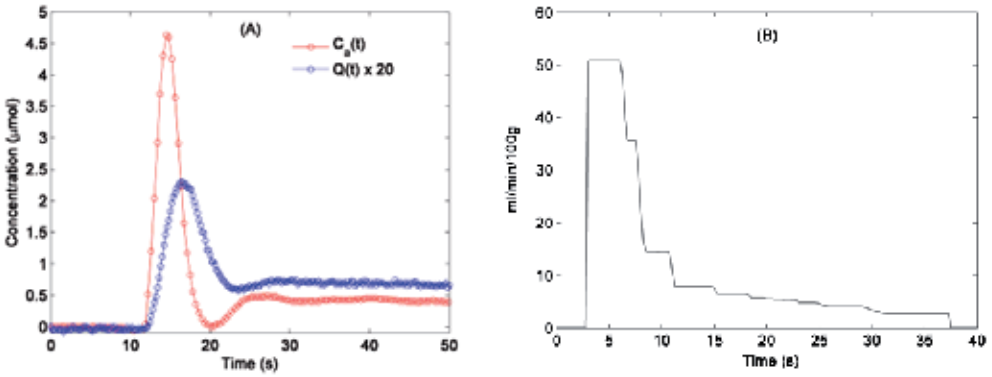


Fig. 1. (A) An example of tissue and arterial ICG concentration curves measured with a NIRS apparatus and a dye densitometer respectively. The curves were measured simultaneously over a period of 50 seconds after injection of an ICG bolus. The peak arterial concentration is approximately 20 times greater than that of tissue. This is because blood volume in tissue is approximately 5 % of the total tissue volume. (B) Cerebral blood flow scaled impulse residue function, $F \cdot R(t)$, retrieved from the deconvolution of the curves in (A). Height of initial plateau yields CBF (50 ml/min/100g) and area under the curve is CBV.

2.2 Measuring the cerebral metabolic rate of oxygen

The NIRS CBF measurements can be used to determine $CMRO_2$ by applying the Fick Principle (Tichauer et al., 2006b):

$$CMRO_2 = F \cdot (AVDO_2), \tag{4}$$

where $AVDO_2$ is the arterial-venous difference of oxygen, or the difference between the oxygen concentration of the arterial system feeding the tissue of interest and the oxygen concentration of the venous system draining the tissue. Assuming that the oxygen content of arterial blood is the same throughout the body (Brown et al., 2003), the arterial O_2 concentration can be obtained from hemoximeter analysis of arterial blood samples from a periphery artery or non-invasively from a pulse oximeter. The venous O_2 concentration is more difficult to determine non-invasively. However, considering that the Hb measured in the brain is due solely to O_2 dissociation from HbO_2, the NIRS measurements of tissue Hb concentration can be used as an indirect measure of the venous O_2 concentration. With this approach, it is necessary to account for the fact that the tissue Hb concentration is a weighted average of the three blood compartments: arterial, capillary and venous. The relative distribution of arterial, capillary, and venous compartments in the total CBV is generally accepted to be approximately 20%, 10%, and 70% respectively (Phelps et al., 1979).

Assuming that the capillary concentration of Hb is the average of arterial and venous concentrations, the venous concentration of Hb can be expressed as:

$$[Hb]_V = \frac{4}{3} \cdot \frac{[Hb]_T}{CBV \cdot \rho} - \frac{1}{3} \cdot [Hb]_a , \tag{5}$$

where $[Hb]_V$, $[Hb]_T$, and $[Hb]_a$ are the venous, tissue, and arterial concentrations of Hb, respectively. The tissue Hb concentration, $[Hb]_T$, is measured by NIRS and $[Hb]_a$ can be measured by either hemoximeter analysis of an arterial blood sample or pulse oximetry. The NIRS measurement of $[Hb]_T$ is normalized by the dilution factor, $CBV \cdot \rho$, to determine the concentration of Hb in just the blood compartment of the tissue region of interest. The density of brain tissue, ρ, is 1.05 g/ml and is used to convert the CBV from volume of blood per mass of tissue to volume of blood per volume of tissue. Finally, with knowledge of the total hemoglobin (tHb = Hb + HbO_2) measured from a blood sample, the venous O_2 concentration can be determined as follows (Brown et al., 2003):

$$\text{Venous}\,O_2\,\text{Concentration} = ([tHb] - [Hb]_v) \cdot 1.39\,\text{ml}\,O_2/\text{gHb} . \tag{6}$$

3. Measurement of *Q(t)* and *Ca(t)*

3.1 Measurement of tissue chromophores concentration

Fig. 2 shows a picture of the cart housing the broadband continuous-wave NIRS instrument. The main components of the apparatus are the light source, the fiber optic cables (optodes), and the spectrometer. The light source is a power-stabilized halogen light bulb and the spectrometer consists of a holographic grating and a cooled CCD camera. During measurements, the two optodes are placed 3.0 cm apart on the skull of the subject. One optode is used to guide the light from the source to the head and the other collects light re-emitted from the head and transmits it to the holographic grating of the spectrometer, where it is dispersed across the cooled CCD chip (cooled to 70°C to reduce electronic dark noise) to generate an attenuation spectrum every 200 ms.

Measured spectra were analyzed on the basis of the principle that when NIR light enters tissue, it is multiply scattered and partially absorbed by endogenous tissue chromophores, such as water, HbO_2, Hb, cytochrome c oxidase, and lipids. Since scattering causes the total pathlength traveled by the NIR light from the emission optode to detection probe to be larger than the physical distance between the two optodes, a simple approach is to take into account the increase in pathlength using the differential pathlength factor (DPF), as first described by Delpy et al. (Delpy et al., 1988). With accurate knowledge of the DPF, a modified version of the Beer-Lambert law can be used to determine absolute changes in the concentrations of the NIR absorbers (Cope, 1991):

$$\Delta A(\lambda) = \sum_i \Delta c_i \cdot DPF \cdot L \cdot \varepsilon_i(\lambda) , \tag{7}$$

where $\Delta A(\lambda)$ is the change in the attenuation spectra, Δc_i is the absolute change in concentration of the i^{th} NIR absorber, L is the physical distance between the emission and detection probes, and $\varepsilon_i(\lambda)$ is the extinction coefficient of the i^{th} NIR absorber as a function of wavelength. In particular, Eq. (7) can be used to quantify tissue ICG concentration, following a bolus injection of the dye, since there is no ICG in the brain prior to the injection.

Furthermore, the second derivative technique described by Matcher et al. (Matcher et al., 1994) can be applied to the broadband data, in conjunction with the assumption of 85% water concentration in the brain in infants and 80% in adults (Matcher & Cooper, 1994), to obtain the DPF and absolute concentrations of endogenous absorbers – as opposed to absolute changes of concentrations – in real time.

Fig. 2. Picture of the potable NIRS apparatus.

3.2 Arterial ICG concentration measurement

Arterial ICG concentration can be measured noninvasively on a peripheral artery (e.g., a foot in the case of a piglet) using a dye densitometer unit (model DDG-2001 A/K, Nihon Kohden, Tokyo, Japan). The probe of the dye densitometer can be held on the skin using a spring-loaded clip, much the same as used for a regular pulse oximeter probe.

4. Validation experiments

4.1 CBF validation

Cerebral blood flow and blood volume were altered in a newborn piglet model, by changing the partial arterial carbon dioxide tension ($PaCO_2$), and concomitantly measured with NIRS and CT perfusion (Brown et al., 2002). Because of the portable nature of the NIRS apparatus, both NIRS and CT measurements were acquired with the piglet in the CT

scanner. Newborn piglets (less than a week old) were chosen to avoid complications due to extracerebral contamination of the NIRS signal that occurs in older subjects, which is discussed section 6. As shown in Fig. 3, there is a strong linear correlation between CT and NIRS hemodynamic measurements over a wide range. In addition, there is no significant statistical difference between CT and NIRS measurements of CBF and CBV, and the estimated bias are small relative to the respective mean values in each case. The precision of NIRS CBF and CBV measurements - determined using analysis of variance of repeated measurements - were 9.71% and 13.05% respectively. These results show that this NIRS technique is capable of accurate and reliable bedside measurement of cerebral hemodynamics in newborn piglets.

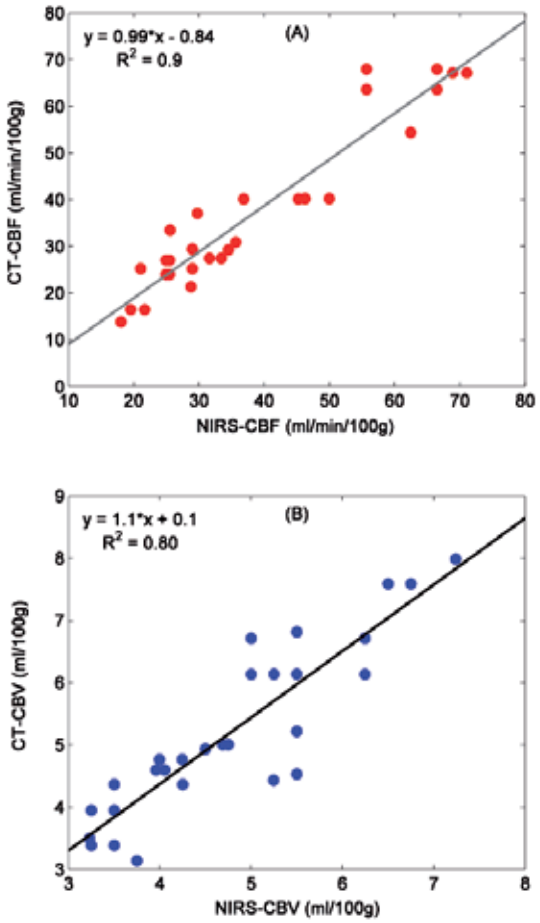


Fig. 3. Comparison of CT and NIRS measurements of cerebral blood flow and blood volume.

There are, however, some challenges facing the clinical application of the technique. Although the piglet head mimics that of a neonate in terms of skull composition and thickness, the two are significantly different in size and dimension. In this validation study an inter-optode distance of 3.0 cm was used and this yields a maximum depth of light

penetration of ~1.73 cm (Brown et al., 2002). This inter-optode separation should be appropriate for neonatal application since it has been shown that in this population, the thickness of the tissue overlying the brain are typically less than 5 mm and do not significantly interfere with NIR monitoring of the brain for inter-optode distance of at least 3 cm (Benaron et al., 1995). A potential inconvenience with this technique is that it requires an ICG injection. Although the use of an exogenous blood flow tracer is somewhat less appealing than using one of the endogenous NIR absorbers (for example HbO_2 or Hb), ICG results in a much higher signal-to-noise ratio.

This study demonstrated the ability of NIRS to accurately measure cerebral hemodynamics at the bedside and showed that this technique can monitor and diagnose ischemic injury in neonates. In ischemic brain injury, CBF is often low for an extended period of time before symptoms of injury are apparent. The ability to detect diminished CBF at an earlier stage would significantly aid earlier intervention and greatly reduce mortality and morbidity. It has also been suggested that loss of $PaCO_2$ reactivity is associated with poor neurodevelopmental outcome and/or hypoxic-ischemic encephalopothy and that the loss of $PaCO_2$ reactivity is a candidate for predicting early severe brain damage in preterm infants (Blankenberg et al., 1997; Muller et al., 1997). Because the NIRS-CBF technique is capable of providing reliable, non-invasive, repeatable measurements it can be used to detect loss of $PaCO_2$ reactivity, thereby greatly improving diagnosis.

4.2 $CMRO_2$ validation

The cerebral metabolic rate of oxygen measured by the NIRS technique were compared to $CMRO_2$ determined from the product of CBF and the cerebral arterial-venous difference in oxygen ($AVDO_2$) measured from blood samples (Tichauer et al., 2006b). The experiments were conducted in newborn piglets subjected to five cerebral metabolic states created by varying the plane of anesthesia. The blood samples were collected from a peripheral artery and the superior sagittal sinus. As such, the two $CMRO_2$ measurements were not collected from exactly the same brain volumes. However, there should be significant overlap between the two sampled volumes since both techniques primarily measure cortical tissue (Scremin et al., 1982). In addition, partial volume errors were avoided by using anesthetics to alter $CMRO_2$ globally (Schultz, 1978). No statistically significant difference was found, within a range of ~ 1.5 to 4.0 $mlO_2/min/100g$, between $CMRO_2$ measurements obtained with the two techniques at any anesthetic level. There was also a strong correlation between concomitant $CMRO_2$ values obtained from the two techniques (Fig. 3 in Tichauer et al., 2006b). Furthermore, repeated measurements obtained while maintaining constant $CMRO_2$ showed that the NIRS technique can measure $CMRO_2$ with a precision of 10.7%. All of this shows that $CMRO_2$ can be determined accurately by combining NIRS measurements of CBF and deoxy-hemoglobin.

It is evident from Eq. 4 that there are two key parameters that must be determined accurately to calculate $CMRO_2$: CBF and $AVDO_2$. Due to the difficulty of measuring these parameters, introduced by the high scattering properties of biological tissue, few studies have attempted to measure *absolute* $CMRO_2$ with NIRS. In this validation, the second derivative technique was applied to broadband data to account for scattering (Matcher et al., 1994) and real-time measurements of the differential pathlength were acquired using the known water concentration in brain (Matcher & Cooper, 1994). A consequence of the second derivative approach is that the $AVDO_2$ must be calculated solely from the Hb

signal as the second derivative of the HbO_2 absorption spectrum is relatively featureless (Cope, 1991). With this approach, an independent measure of the arterial oxygenation must be obtained, which can be determined directly from an arterial blood sample or indirectly from the arterial oxygen saturation. It is also necessary to normalize the tissue by CBV, which was obtained from the ICG measurements, and to assume a relative distribution of in the vasculature.

Quantitative measures of $AVDO_2$ were calculated under the assumption that the relative contribution of venous and arterial blood to the total blood volume in the brain is 3:1 (Phelps et al., 1979). Because of its importance, there have been a number of studies that have attempted to measure the relative vascular distribution (Hueber et al., 2001; Kusaka et al., 2002; Watzman et al., 2000; Wiedeman, 1963). These studies compared NIRS tissue oxygenation measurements to oxygenation measurements from arterial and venous blood samples, and found results similar to the 3:1 ratio which was originally derived from cerebrovascular resistance measurements. In addition, it has been shown that the $AVDO_2$ is relatively insensitive to errors in the assumed relative vascular contribution (Tichauer et al., 2010).

5. Applications

5.1 Detection of hypoxia-ischemia

Recent clinical trials have demonstrated that the incidence of death and disability from hypoxic-ischemic brain injury in newborns can be significantly reduced by initiating treatment strategies – hypothermia for example – after birth and within a 6 h therapeutic window (Chaudhari & McGuire, 2008; Eicher et al., 2005a; Eicher et al., 2005b; Gluckman et al., 2005; Shankaran et al., 2005). Due to the brevity of the therapeutic window, early detection of injury and an early determination of those infants who are likely candidates for treatment are crucial (Gunn & Bennet, 2008). In this regard, traditional early indicators of brain injury – including Apgar scores, umbilical artery acidosis and fetal heart rate monitoring – suffer from poor specificity (Carter et al., 1998; Shankaran, 1998) and more specific indicators of injury, such as magnetic resonance imaging and spectroscopy, are insensitive or difficult to implement within the therapeutic window (Cady, 2001; Martin & Barkovich, 1995). To this point, the most promising and clinically-feasible monitoring technique for early indicator of hypoxia-ischemia (HI) after birth has been amplitude-integrated electroencephalography (aEEG) due to its ease of use, non-invasiveness and high prognostic value as early as 3 h after birth (al Naqeeb et al., 1999; Toet et al., 1999). However, recent studies have questioned the sensitivity of aEEG to detect infants with milder injuries that could still benefit from treatment (Sarkar et al., 2008). Another potentially promising early indicator is reduction in $CMRO_2$ since HI is believed to impair oxidative metabolism.

To test the idea that NIRS-$CMRO_2$ measurements can improve early detection of perinatal HI, measurements were acquired in newborn piglets (Tichauer et al., 2009) before and after 1 h of reperfusion from HI – the duration of which was varied from piglet to piglet with a range of 3-24 min – under fentanyl/nitrous oxide anesthesia to mimic awake-like levels of cerebral metabolism. $CMRO_2$ was found to be significantly depressed following the insult; mean $CMRO_2$ was 2.61 ± 0.11 $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$ prior to the insult and 1.58 ± 0.09 $mlO_2 \cdot min^{-1} \cdot 100g^{-1}$ after 1 h of reperfusion, respectively. The NIRS-$CMRO_2$ measurements were combined with aEEG and both measurements displayed statistically significant correlations

with duration of ischemia ($p < 0.05$; $r = 0.71$ and $r = 0.89$, respectively); however, only $CMRO_2$ was sensitive to milder injuries (< 5 min). These evidences suggest that the NIRS-$CMRO_2$ technique has the potential to delineate different insult severities within 1 h of reperfusion from hypoxia-ischemia in a piglet model of perinatal HI. Since the technique can be applied at the bedside of the sick newborn, these results highlight its potential to improve diagnosis and to monitor treatment of hypoxic-ischemic encephalopathy within the therapeutic window.

5.2 Focal brain injury

Although the previous sections highlight the potential of using NIRS for early detection of brain injury in newborns, CBF and $CMRO_2$ were only measured in a single brain volume as the NIRS instrument had only one detection channel. This limits the clinical applicability of the technique due to selective patterns of damage associated with major causes of neonatal brain injury, such as hypoxia-ischemia and intraventricular hemorrhage, and due to focal injury caused by ischemic stroke. To expand the detection capabilities of the NIRS apparatus to enable the measurements of regional CBF and $CMRO_2$, a relatively simple multiplexing approach based on electronically controlled mechanical shutters has been proposed (Diop et al., 2009). The entire apparatus was housed in a cart (see Fig. 2) and could be moved to the bedside. The ability of this multi-detector NIRS instrument to detect regional CBF and $CMRO_2$ abnormalities was tested in newborn piglets in which the vasoconstrictor Endothelin-1 (ET-1) was injected into the brain to cause a focal ischemic injury. Endothelin-1 is an amino acid peptide that possesses prolonged and profound vasoconstrictive effects on cerebral blood vessels (Diop et al., 2009). For comparison, regional CBF was concurrently measured by CT perfusion. Due to limited space on the piglet head, only four detection channels were used in that study. A general reduction in CBF was observed across all detection channels with the magnitude decreasing farther from the site of ET-1 injection; a trend that was also observed in the CT perfusion images. An excellent agreement was also observed between CBF in the lesion site, as determined from the CT perfusion images, and CBF measured by the NIRS optode nearest to the lesion site (optode 1 in Fig. 4). The NIRS-$CMRO_2$ measurements showed that ET-1 injection did not cause a significant change in cerebral energy metabolism. This was expected as $CMRO_2$ would be maintained, despite the reduction in CBF, by an increase in cerebral oxygen extraction. Although both NIRS and CT measured ~ 50% CBF reduction in the lesion region, this decrease was likely not sufficient to exhaust the compensatory effects of increased oxygen extraction. Typically, reductions in $CMRO_2$ are not observed until CBF falls below 25 ml/100g/min.

A limitation with this multi-channel NIRS apparatus is that it is not immune to partial-volume errors which could result in an overestimation of CBF in focal lesions significantly smaller than the sensitivity volume of a source-detector unit. One approach to improving the spatial resolution would be to combine the multi-channel NIRS instrument with a discrete-wavelength continuous-wave system with multiple emission and detection optodes. Broadband NIRS could be used to determine the DPF and the steady-state Hb concentration needed to determine $CMRO_2$. A discrete-wavelength imaging system could then be used to rapidly acquire dynamic ICG concentration data. The relative ICG data would be converted into absolute units using the DPF measurements. With such an approach, it may be possible to apply diffuse optical tomographic methods to further improve spatial resolution.

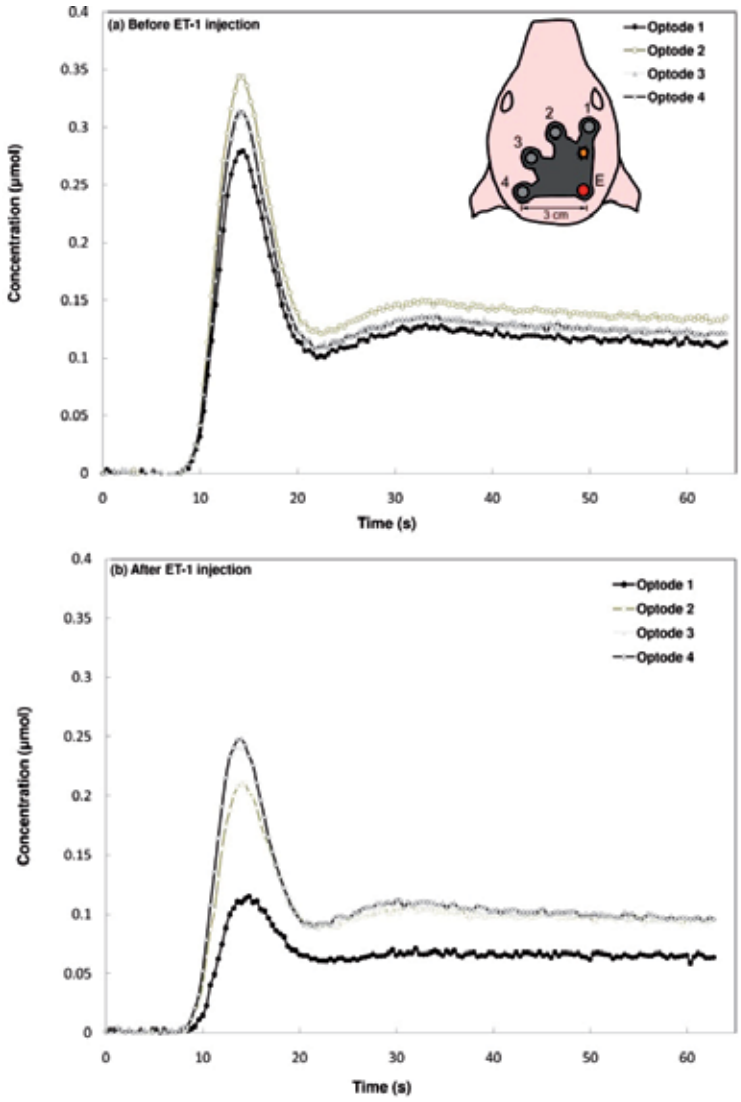


Fig. 4. Brain tissue ICG concentration curves obtained from detection optodes 1-4 before (a) and after (b) ET-1 injection. The reduction in the peak height of the ICG curve for optode 1 after ET-1 injection reflects the reduction in CBF caused by the drug. Shown in the top right hand side of (a) is a cartoon of a piglet head showing the location of the 4 detection optodes (labelled 1-4) relative to the emission optode (labelled E). The emission-detector distance was 3 cm in all cases. The approximate position of the ET-1 injection site is represented by the orange mark between the emission optode and detection optode 1.

6. Extra-cerebral signal contamination

A major obstacle to NIRS clinical applications in adults is the presence of extra-cerebral tissues (scalp, skull and CSF) within the region interrogated by NIRS probes. For CBF

measurements, the measured ICG tissue concentration curve is comprised of both cerebral and non-cerebral components that are highly correlated. Using tracer kinetic models to recover CBF from a contaminated curve would result in an underestimation of CBF (Elliott et al., 2010; Gora et al., 2002; Owen-Reece et al., 1996). Solutions to overcoming this challenge have been proposed, including more advanced instrumentation as well as analytical approaches, with the main goal of increasing sensitivity to cerebral components.

The presence of the extra-cerebral component in the measured signal makes the analytical problem underdetermined when using a single-channel continuous-wave configuration. To differentiate between signal arising from cerebral tissue and extracerebral contamination, more information about the light-tissue interaction is necessary. Several instrumentation approaches have emerged which provide additional information and include multi-distance (also known as spatially resolved), time-resolved and frequency-domain approaches. The additional data provided by these techniques facilitate the removal of extracerebral contamination by further constraining the system of equations which describe the change in light propagation due to the inflow of tracer.

In depth-resolved NIRS, multiple detectors are used to simultaneously measure the optical signal at difference source-detector distances (Hongo et al., 1995; Pucci et al., 2010). This approach is based on the principle that the penetration depth of light increases with source-detector distance. Therefore, the distance that light travels in each tissue type depends on both the geometry of the tissue layers as well as the source-detector distance. Measuring the NIR signal at a variety of source-detector distances for the same tissue structure provides a means of algebraically isolating information from the cerebral tissue. The main limitation of this approach is that the mean partial pathlength of light for a given source-detector distance is unknown and must therefore be determined using mathematical modeling, spectral information, anatomical priors, or a combination of these. Recently, a depth-resolved NIRS method that incorporated CT imaging data into a light propagation model has been validated (Elliott et al., 2010). The determination of mean partial pathlengths using this approach allowed the removal of extracerebral contamination, and the recovered CBF values were in good agreement with independent measurements obtained with CT perfusion. Several other multi-distance techniques have been proposed, using time-resolved or frequency-domain methods to provide additional information (Liebert et al., 2004; Steinbrink et al., 2006).

An effective way to isolate the deeper cerebral component of the signal is to collect information about individual photons, mainly the time required to travel from the source fibre, through the tissue, to the detector. This "time-of-flight" is a function of the optical properties of the tissue and the distribution of times-of-flight for a sample of photons can be considered as a unique signature of the tissue geometry and type. Similar to the depth-resolved approach, photons with late arrival times are more likely to have propagated into cerebral tissue, but unlike in the case of depth-resolved NIRS, the relationship is not straight-forward. Instead, it is necessary to mathematically model the time-of-flight distribution (Kienle et al., 1998; Wang et al., 1995) to determine the optical properties of each medium. Changes in light attenuation recovered from this modeling can be converted to ICG concentration and deconvolved to yield CBF (Diop et al., 2010).

In a similar way, when a frequency-modulated light signal is introduced into a tissue, the amplitude and phase components of the detected light depend on the tissue geometry and optical properties (Choi et al., 2004). In theory, the same time-of-flight information collected using time-resolved NIRS is contained in frequency-domain measurements. Since most analytical models employed in time-resolved analysis have solutions for the frequency domain as well, the two techniques are theoretically equivalent.

When using the methods discussed above, a certain amount of prior information is necessary to properly analyze the measured data. These priors include information regarding the geometry and type of tissue being interrogated, the optical properties of the tissues, and the placement and efficiency of the optical probes. Because the determination of these priors can often be challenging in a clinical environment, it is common to make a limited number of assumptions and simplifications. However, the number and quality of priors will usually affect the accuracy of the CBF measurement, making it necessary to carefully consider this trade-off.

Perhaps the most important prior from a clinical perspective is anatomical information pertaining to the thickness and shape of the extracerebral layers, and must be incorporated into light propagation models. In the absence of this information, the head is treated either as a homogenous medium or as a simple two-layered structure with a top layer thickness based on the population average. Measurements of CBF determined using either of these two assumptions have been shown to be inaccurate under most clinical situations (Gora et al., 2002; Schytz et al., 2009). The accuracy of CBF measurements can be improved when the total thickness of the extra-cerebral layer, or preferably, the individual thicknesses of the scalp, skull and CSF layers are known (Elliott et al., 2010). Recent studies have also suggested that incorporating an imaging dataset from CT or MRI may further increase the accuracy of the measurements (Dehaes et al., 2011), but this has not yet been demonstrated experimentally.

An alternative approach to the quantitative methods discussed above is to use semi-quantitative comparisons to assess the relative change in blood flow as a function of time, or the relative difference in blood flow between different areas on the head (Liebert et al., 2005). While these techniques cannot quantify CBF, they may be effective in specific clinical applications even if no priors are available. Recently, Steinkeller et al. demonstrated the use of a semi-quantitative time-resolved NIRS technique to monitor cerebral perfusion in acute ischemic stroke patients (Steinkellner et al., 2010). In this study, NIRS measurements were acquired over both hemispheres following the injection of tracer, and the time-to-peak, or time between first appearance of tracer and the peak concentration of tracer, was determined. In all patients, a larger time-to-peak was observed in the affected hemisphere, when compared to the normal hemisphere.

7. Conclusion

Near-infrared spectroscopy is already an accepted and widely used monitoring tool for the clinical management of patients since the ubiquitous pulse oximeter is in fact a NIRS instrument. Although NIRS instruments are in general relatively simple, analyzing the measured signal is not always straightforward. For this reason, interpreting NIRS data remains one of the major obstacles preventing its wide spread and routine clinical use. To overcome this challenge, better analytical tools need to be developed as well as stronger collaboration between researchers and clinicians. Another major problem with NIRS is the lack of widely accepted standards. Efforts are being made to solve this issue with multiple research groups working together to develop calibrated tissue mimicking phantoms.

8. Acknowledgment

The authors would like to thank the financial support of the Canadian Institutes of Health Research (CIHR), the Natural Sciences and Engineering Research Council of Canada (NSERC), the Canada Foundation for Innovation, Heart and Stroke Foundation Ontario and the Ontario Neurotrauma Foundation.

9. References

Al-Rawi, P. G. & Kirkpatrick, P. J. (2006): Tissue oxygen index: thresholds for cerebral ischemia using near-infrared spectroscopy. *Stroke*,Vol.37.11: 2720

al Naqeeb, N., Edwards, A. D., Cowan, F. M. & Azzopardi, D. (1999): Assessment of neonatal encephalopathy by amplitude-integrated electroencephalography. *Pediatrics*,Vol.103.6 Pt 1: 1263

Asgeirsson, B., Grande, P. O. & Nordstrom, C. H. (1994): A new therapy of post-trauma brain oedema based on haemodynamic principles for brain volume regulation. *Intensive Care Med*,Vol.20.4: 260

Benaron, D. A., Kurth, C. D., Steven, J. M., Delivoria-Papadopoulos, M. & Chance, B. (1995): Transcranial optical path length in infants by near-infrared phase-shift spectroscopy. *J Clin Monit*,Vol.11.2: 109

Blankenberg, F. G., Loh, N. N., Norbash, A. M., Craychee, J. A., Spielman, D. M., Person, B. L., Berg, C. A. & Enzmann, D. R. (1997): Impaired cerebrovascular autoregulation after hypoxic-ischemic injury in extremely low-birth-weight neonates: detection with power and pulsed wave Doppler US. *Radiology*,Vol.205.2: 563

Bolognese, P., Miller, J. I., Heger, I. M. & Milhorat, T. H. (1993): Laser-Doppler flowmetry in neurosurgery. *J Neurosurg Anesthesiol*,Vol.5.3: 151

Brady, K. M., Lee, J. K., Kibler, K. K., Easley, R. B., Koehler, R. C. & Shaffner, D. H. (2008): Continuous measurement of autoregulation by spontaneous fluctuations in cerebral perfusion pressure: comparison of 3 methods. *Stroke*,Vol.39.9: 2531

Bratton, S. L., Chestnut, R. M., Ghajar, J., McConnell Hammond, F. F., Harris, O. A., Hartl, R., Manley, G. T., Nemecek, A., Newell, D. W., Rosenthal, G., Schouten, J., Shutter, L., Timmons, S. D., Ullman, J. S., Videtta, W., Wilberger, J. E. & Wright, D. W. (2007a): Guidelines for the management of severe traumatic brain injury. IX. Cerebral perfusion thresholds. *J Neurotrauma*,Vol.24 Suppl 1S59

Bratton, S. L., Chestnut, R. M., Ghajar, J., McConnell Hammond, F. F., Harris, O. A., Hartl, R., Manley, G. T., Nemecek, A., Newell, D. W., Rosenthal, G., Schouten, J., Shutter, L., Timmons, S. D., Ullman, J. S., Videtta, W., Wilberger, J. E. & Wright, D. W. (2007b): Guidelines for the management of severe traumatic brain injury. VIII. Intracranial pressure thresholds. *J Neurotrauma*,Vol.24 Suppl 1S55

Brown, D. W., Hadway, J. & Lee, T. Y. (2003): Near-infrared spectroscopy measurement of oxygen extraction fraction and cerebral metabolic rate of oxygen in newborn piglets. *Pediatr Res*,Vol.54.6: 861

Brown, D. W., Picot, P. A., Naeini, J. G., Springett, R., Delpy, D. T. & Lee, T. Y. (2002): Quantitative near infrared spectroscopy measurement of cerebral hemodynamics in newborn piglets. *Pediatr Res*,Vol.51.5: 564

Cady, E. B. (2001): Magnetic resonance spectroscopy in neonatal hypoxic-ischaemic insults. *Childs Nerv Syst*,Vol.17.3: 145

Carter, B. S., McNabb, F. & Merenstein, G. B. (1998): Prospective validation of a scoring system for predicting neonatal morbidity after acute perinatal asphyxia. *J Pediatr*,Vol.132.4: 619

Chaudhari, T. & McGuire, W. (2008): Allopurinol for preventing mortality and morbidity in newborn infants with suspected hypoxic-ischaemic encephalopathy. *Cochrane Database Syst Rev*.2: CD006817

Choi, J., Wolf, M., Toronov, V., Wolf, U., Polzonetti, C., Hueber, D., Safonova, L. P., Gupta, R., Michalos, A., Mantulin, W. & Gratton, E. (2004): Noninvasive determination of the

optical properties of adult brain: near-infrared spectroscopy approach. *J Biomed Opt*,Vol.9.1: 221

Coles, J. P., Steiner, L. A., Johnston, A. J., Fryer, T. D., Coleman, M. R., Smieleweski, P., Chatfield, D. A., Aigbirhio, F., Williams, G. B., Boniface, S., Rice, K., Clark, J. C., Pickard, J. D. & Menon, D. K. (2004): Does induced hypertension reduce cerebral ischaemia within the traumatized human brain? *Brain*,Vol.127.Pt 11: 2479

Cope, M. (1991): *The application of near infrared spectroscopy to non invasive monitoring of cerebral oxygenation in the newborn infant*. Ph. D., University College London, London, U.K.

Cremer, O. L. (2008): Does ICP monitoring make a difference in neurocritical care? *Eur J Anaesthesiol Suppl*,Vol.4287

Dehaes, M., Grant, P. E., Sliva, D. D., Roche-Labarbe, N., Pienaar, R., Boas, D. A., Franceschini, M. A. & Selb, J. (2011): Assessment of the frequency-domain multi-distance method to evaluate the brain optical properties: Monte Carlo simulations from neonate to adult. *Biomed Opt Express*,Vol.2.3: 552

Delpy, D. T., Cope, M., van der Zee, P., Arridge, S., Wray, S. & Wyatt, J. (1988): Estimation of optical pathlength through tissue from direct time of flight measurement. *Phys Med Biol*,Vol.33.12: 1433

DeWitt, D. S. & Prough, D. S. (2003): Traumatic cerebral vascular injury: the effects of concussive brain injury on the cerebral vasculature. *J Neurotrauma*,Vol.20.9: 795

Diop, M., Elliott, J. T., Tichauer, K. M., Lee, T. Y. & St Lawrence, K. (2009): A broadband continuous-wave multichannel near-infrared system for measuring regional cerebral blood flow and oxygen consumption in newborn piglets. *Rev Sci Instrum*,Vol.80.5: 054302

Diop, M., Tichauer, K. M., Elliott, J. T., Migueis, M., Lee, T. Y. & St Lawrence, K. (2010): Comparison of time-resolved and continuous-wave near-infrared techniques for measuring cerebral blood flow in piglets. *J Biomed Opt*,Vol.15.5: 057004

Edwards, A. D., Wyatt, J. S., Richardson, C., Delpy, D. T., Cope, M. & Reynolds, E. O. (1988): Cotside measurement of cerebral blood flow in ill newborn infants by near infrared spectroscopy. *Lancet*,Vol.2.8614: 770

Eicher, D. J., Wagner, C. L., Katikaneni, L. P., Hulsey, T. C., Bass, W. T., Kaufman, D. A., Horgan, M. J., Languani, S., Bhatia, J. J., Givelichian, L. M., Sankaran, K. & Yager, J. Y. (2005a): Moderate hypothermia in neonatal encephalopathy: efficacy outcomes. *Pediatr Neurol*,Vol.32.1: 11

Eicher, D. J., Wagner, C. L., Katikaneni, L. P., Hulsey, T. C., Bass, W. T., Kaufman, D. A., Horgan, M. J., Languani, S., Bhatia, J. J., Givelichian, L. M., Sankaran, K. & Yager, J. Y. (2005b): Moderate hypothermia in neonatal encephalopathy: safety outcomes. *Pediatr Neurol*,Vol.32.1: 18

Elliott, J. T., Diop, M., Tichauer, K. M., Lee, T. Y. & St Lawrence, K. (2010): Quantitative measurement of cerebral blood flow in a juvenile porcine model by depth-resolved near-infrared spectroscopy. *J Biomed Opt*,Vol.15.3: 037014

Gallagher, C. N., Hutchinson, P. J. & Pickard, J. D. (2007): Neuroimaging in trauma. *Curr Opin Neurol*,Vol.20.4: 403

Gluckman, P. D., Wyatt, J. S., Azzopardi, D., Ballard, R., Edwards, A. D., Ferriero, D. M., Polin, R. A., Robertson, C. M., Thoresen, M., Whitelaw, A. & Gunn, A. J. (2005): Selective head cooling with mild systemic hypothermia after neonatal encephalopathy: multicentre randomised trial. *Lancet*,Vol.365.9460: 663

Gora, F., Shinde, S., Elwell, C. E., Goldstone, J. C., Cope, M., Delpy, D. T. & Smith, M. (2002): Noninvasive measurement of cerebral blood flow in adults using near-infrared spectroscopy and indocyanine green: a pilot study. *J Neurosurg Anesthesiol*,Vol.14.3: 218

Gowda, N. K., Agrawal, D., Bal, C., Chandrashekar, N., Tripati, M., Bandopadhyaya, G. P., Malhotra, A. & Mahapatra, A. K. (2006): Technetium Tc-99m ethyl cysteinate dimer brain single-photon emission CT in mild traumatic brain injury: a prospective study. *AJNR Am J Neuroradiol*,Vol.27.2: 447

Gunn, A. J. & Bennet, L. (2008): Timing of injury in the fetus and neonate. *Curr Opin Obstet Gynecol*,Vol.20.2: 175

Helmy, A., Vizcaychipi, M. & Gupta, A. K. (2007): Traumatic brain injury: intensive care management. *Br J Anaesth*,Vol.99.1: 32

Hongo, K., Kobayashi, S., Okudera, H., Hokama, M. & Nakagawa, F. (1995): Noninvasive cerebral optical spectroscopy: depth-resolved measurements of cerebral haemodynamics using indocyanine green. *Neurol Res*,Vol.17.2: 89

Hope-Ross, M., Yannuzzi, L. A., Gragoudas, E. S., Guyer, D. R., Slakter, J. S., Sorenson, J. A., Krupsky, S., Orlock, D. A. & Puliafito, C. A. (1994): Adverse reactions due to indocyanine green. *Ophthalmology*,Vol.101.3: 529

Hueber, D. M., Franceschini, M. A., Ma, H. Y., Zhang, Q., Ballesteros, J. R., Fantini, S., Wallace, D., Ntziachristos, V. & Chance, B. (2001): Non-invasive and quantitative near-infrared haemoglobin spectrometry in the piglet brain during hypoxic stress, using a frequency-domain multidistance instrument. *Phys Med Biol*,Vol.46.1: 41

Jobsis, F. F. (1977): Noninvasive, infrared monitoring of cerebral and myocardial oxygen sufficiency and circulatory parameters. *Science*,Vol.198.4323: 1264

Kienle, A., Patterson, M. S., Dognitz, N., Bays, R., Wagninures, G. & van den Bergh, H. (1998): Noninvasive Determination of the Optical Properties of Two-Layered Turbid Media. *Appl Opt*,Vol.37.4: 779

Kirkpatrick, P. J., Smielewski, P., Czosnyka, M. & Pickard, J. D. (1994): Continuous monitoring of cortical perfusion by laser Doppler flowmetry in ventilated patients with head injury. *J Neurol Neurosurg Psychiatry*,Vol.57.11: 1382

Kusaka, T., Isobe, K., Nagano, K., Okubo, K., Yasuda, S., Kondo, M., Itoh, S., Hirao, K. & Onishi, S. (2002): Quantification of cerebral oxygenation by full-spectrum near-infrared spectroscopy using a two-point method. *Comp Biochem Physiol A Mol Integr Physiol*,Vol.132.1: 121

Lee, T.-Y. (2002): Functional CT: physiological models. *Trends in Biotechnology*,Vol.20.8: S3

Liebert, A., Wabnitz, H., Steinbrink, J., Moller, M., Macdonald, R., Rinneberg, H., Villringer, A. & Obrig, H. (2005): Bed-side assessment of cerebral perfusion in stroke patients based on optical monitoring of a dye bolus by time-resolved diffuse reflectance. *Neuroimage*,Vol.24.2: 426

Liebert, A., Wabnitz, H., Steinbrink, J., Obrig, H., Moller, M., Macdonald, R., Villringer, A. & Rinneberg, H. (2004): Time-resolved multidistance near-infrared spectroscopy of the adult head: intracerebral and extracerebral absorption changes from moments of distribution of times of flight of photons. *Appl Opt*,Vol.43.15: 3037

Martin, E. & Barkovich, A. J. (1995): Magnetic resonance imaging in perinatal asphyxia. *Arch Dis Child Fetal Neonatal Ed*,Vol.72.1: F62

Matcher, S. J. & Cooper, C. E. (1994): Absolute quantification of deoxyhaemoglobin concentration in tissue near infrared spectroscopy. *Phys Med Biol*,Vol.39.8: 1295

Matcher, S. J., Cope, M. & Delpy, D. T. (1994): Use of the water absorption spectrum to quantify tissue chromophore concentration changes in near-infrared spectroscopy. *Phys Med Biol*,Vol.39.1: 177

Meier, P. & Zierler, K. L. (1954): On the theory of the indicator-dilution method for measurement of blood flow and volume. *J Appl Physiol*,Vol.6.12: 731

Muller, A. M., Morales, C., Briner, J., Baenziger, O., Duc, G. & Bucher, H. U. (1997): Loss of CO2 reactivity of cerebral blood flow is associated with severe brain damage in mechanically ventilated very low birth weight infants. *Eur J Paediatr Neurol*,Vol.1.5-6: 157

Owen-Reece, H., Elwell, C. E., Harkness, W., Goldstone, J., Delpy, D. T., Wyatt, J. S. & Smith, M. (1996): Use of near infrared spectroscopy to estimate cerebral blood flow in conscious and anaesthetized adult subjects. *Br J Anaesth*,Vol.76.1: 43

Patel, J., Marks, K., Roberts, I., Azzopardi, D. & Edwards, A. D. (1998): Measurement of cerebral blood flow in newborn infants using near infrared spectroscopy with indocyanine green. *Pediatr Res*,Vol.43.1: 34

Phelps, M. E., Huang, S. C., Hoffman, E. J. & Kuhl, D. E. (1979): Validation of tomographic measurement of cerebral blood volume with C-11-labeled carboxyhemoglobin. *J Nucl Med*,Vol.20.4: 328

Pucci, O., Toronov, V. & St Lawrence, K. Measurement of the optical properties of a two-layer model of the human head using broadband near-infrared spectroscopy. *Appl Opt*,Vol.49.32: 6324

Robertson, C. S., Valadka, A. B., Hannay, H. J., Contant, C. F., Gopinath, S. P., Cormio, M., Uzura, M. & Grossman, R. G. (1999): Prevention of secondary ischemic insults after severe head injury. *Crit Care Med*,Vol.27.10: 2086

Sarkar, S., Barks, J. D. & Donn, S. M. (2008): Should amplitude-integrated electroencephalography be used to identify infants suitable for hypothermic neuroprotection? *J Perinatol*,Vol.28.2: 117

Schultz, J. E. (1978): Brain Energy Metabolism. Von B. K. Siesjö, John Wiley and Sons, Chichester, New York, Brisbane Toronto, 1978, 607 S., DM 78,65. *Pharmazie in unserer Zeit*,Vol.7.6: 192

Schytz, H. W., Wienecke, T., Jensen, L. T., Selb, J., Boas, D. A. & Ashina, M. (2009): Changes in cerebral blood flow after acetazolamide: an experimental study comparing near-infrared spectroscopy and SPECT. *Eur J Neurol*,Vol.16.4: 461

Scremin, O. U., Sonnenschein, R. R. & Rubinstein, E. H. (1982): Cerebrovascular anatomy and blood flow measurements in the rabbit. *J Cereb Blood Flow Metab*,Vol.2.1: 55

Shankaran, S. (1998): Identification of term infants at risk for neonatal morbidity. *J Pediatr*,Vol.132.4: 571

Shankaran, S., Laptook, A. R., Ehrenkranz, R. A., Tyson, J. E., McDonald, S. A., Donovan, E. F., Fanaroff, A. A., Poole, W. K., Wright, L. L., Higgins, R. D., Finer, N. N., Carlo, W. A., Duara, S., Oh, W., Cotten, C. M., Stevenson, D. K., Stoll, B. J., Lemons, J. A., Guillet, R. & Jobe, A. H. (2005): Whole-body hypothermia for neonates with hypoxic-ischemic encephalopathy. *N Engl J Med*,Vol.353.15: 1574

Sioutos, P. J., Orozco, J. A., Carter, L. P., Weinand, M. E., Hamilton, A. J. & Williams, F. C. (1995): Continuous regional cerebral cortical blood flow monitoring in head-injured patients. *Neurosurgery*,Vol.36.5: 943

Soustiel, J. F., Mahamid, E., Goldsher, D. & Zaaroor, M. (2008): Perfusion-CT for early assessment of traumatic cerebral contusions. *Neuroradiology*,Vol.50.2: 189

Springett, R., Sakata, Y. & Delpy, D. T. (2001): Precise measurement of cerebral blood flow in newborn piglets from the bolus passage of indocyanine green. *Phys Med Biol*,Vol.46.8: 2209

Steinbrink, J., Fischer, T., Kuppe, H., Hetzer, R., Uludag, K., Obrig, H. & Kuebler, W. M. (2006): Relevance of depth resolution for cerebral blood flow monitoring by near-infrared spectroscopic bolus tracking during cardiopulmonary bypass. *J Thorac Cardiovasc Surg*,Vol.132.5: 1172

Steinkellner, O., Gruber, C., Wabnitz, H., Jelzow, A., Steinbrink, J., Fiebach, J. B., Macdonald, R. & Obrig, H. (2010): Optical bedside monitoring of cerebral perfusion: technological and methodological advances applied in a study on acute ischemic stroke. *J Biomed Opt*,Vol.15.6: 061708

Thavasothy, M., Broadhead, M., Elwell, C., Peters, M. & Smith, M. (2002): A comparison of cerebral oxygenation as measured by the NIRO 300 and the INVOS 5100 Near-Infrared Spectrophotometers. *Anaesthesia*,Vol.57.10: 999

Tichauer, K. M., Brown, D. W., Hadway, J., Lee, T. Y. & St Lawrence, K. (2006a): Near-infrared spectroscopy measurements of cerebral blood flow and oxygen consumption following hypoxia-ischemia in newborn piglets. *J Appl Physiol*,Vol.100.3: 850

Tichauer, K. M., Elliott, J. T., Hadway, J. A., Lee, D. S., Lee, T. Y. & St Lawrence, K. (2010): Using near-infrared spectroscopy to measure cerebral metabolic rate of oxygen under multiple levels of arterial oxygenation in piglets. *J Appl Physiol*,Vol.109.3: 878

Tichauer, K. M., Elliott, J. T., Hadway, J. A., Lee, T. Y. & St Lawrence, K. (2009): Cerebral metabolic rate of oxygen and amplitude-integrated electroencephalography during early reperfusion after hypoxia-ischemia in piglets. *J Appl Physiol*,Vol.106.5: 1506

Tichauer, K. M., Hadway, J. A., Lee, T. Y. & St Lawrence, K. (2006b): Measurement of cerebral oxidative metabolism with near-infrared spectroscopy: a validation study. *J Cereb Blood Flow Metab*,Vol.26.5: 722

Toet, M. C., Hellstrom-Westas, L., Groenendaal, F., Eken, P. & de Vries, L. S. (1999): Amplitude integrated EEG 3 and 6 hours after birth in full term neonates with hypoxic-ischaemic encephalopathy. *Arch Dis Child Fetal Neonatal Ed*,Vol.81.1: F19

Vajkoczy, P., Horn, P., Bauhuf, C., Munch, E., Hubner, U., Ing, D., Thome, C., Poeckler-Schoeninger, C., Roth, H. & Schmiedek, P. (2001): Effect of intra-arterial papaverine on regional cerebral blood flow in hemodynamically relevant cerebral vasospasm. *Stroke*,Vol.32.2: 498

Valdueza, J. M., Balzer, J. O., Villringer, A., Vogl, T. J., Kutter, R. & Einhaupl, K. M. (1997): Changes in blood flow velocity and diameter of the middle cerebral artery during hyperventilation: assessment with MR and transcranial Doppler sonography. *AJNR Am J Neuroradiol*,Vol.18.10: 1929

Wang, L., Jacques, S. L. & Zheng, L. (1995): MCML--Monte Carlo modeling of light transport in multi-layered tissues. *Comput Methods Programs Biomed*,Vol.47.2: 131

Watzman, H. M., Kurth, C. D., Montenegro, L. M., Rome, J., Steven, J. M. & Nicolson, S. C. (2000): Arterial and venous contributions to near-infrared cerebral oximetry. *Anesthesiology*,Vol.93.4: 947

Wiedeman, M. P. (1963): Dimensions of blood vessels from distributing artery to collecting vein. *Circ Res*,Vol.12375

Wintermark, M., Sesay, M., Barbier, E., Borbely, K., Dillon, W. P., Eastwood, J. D., Glenn, T. C., Grandin, C. B., Pedraza, S., Soustiel, J. F., Nariai, T., Zaharchuk, G., Caille, J. M., Dousset, V. & Yonas, H. (2005): Comparative overview of brain perfusion imaging techniques. *Stroke*,Vol.36.9: e83

Wright, W. L. (2007): Multimodal monitoring in the ICU: when could it be useful? *J Neurol Sci*,Vol.261.1-2: 10

Yoshitani, K., Kawaguchi, M., Tatsumi, K., Kitaguchi, K. & Furuya, H. (2002): A comparison of the INVOS 4100 and the NIRO 300 near-infrared spectrophotometers. *Anesth Analg*,Vol.94.3: 586

Part 4

Protective Mechanisms and Recovery

15

Mechanisms of Neuroprotection Underlying Physical Exercise in Ischemia – Reperfusion Injury

David Dornbos III and Yuchuan Ding
Wayne State University Department of Neurological Surgery
USA

1. Introduction

Cerebrovascular accidents carry significant morbidity and mortality and are vastly present in current society. One of the most prevalent causes of death and long-term disability within the United States, stroke was found to directly cause 6 million deaths in 2006 and was found to be indirectly attributed to $73.7 billion in health care costs in 2010 (Lloyd-Jones et al., 2010). Current standard of care requires that patients present to a health care facility very early in disease onset so thrombolytic therapy can be initiated; however, this therapy, focusing on the establishment of reperfusion is not ideal as reperfusion often worsens ischemic injury (Yang and Betz, 1994). Clearly, new therapeutic and pharmacologic interventions are needed.

Exercise has long been known to provide protection for ischemic stroke through the amelioration of stroke risk factors. Through its beneficial effects on hypertension, lipid profiles, obesity, and diabetes, exercise training has been associated with decreased stroke incidence and better outcomes after stroke (Evenson et al., 1999; Gillum et al., 1996; Hu et al., 2004). Despite risk factor management, exercise has also been shown to provide endogenous neuroprotection, preserving neuronal viability in the setting of ischemia/ reperfusion injury, resulting in decreased infarct volume and improved neurologic recovery (Chaudhry et al., 2010; Curry et al., 2009; Davis et al., 2007; Ding et al., 2004a, 2004b, 2005, 2006a, 2006b; Guo et al., 2008a; Liebelt et al., 2010; Zwagerman et al., 2010a, 2010b). These beneficial endogenous effects of exercise preconditioning have been seen even after multivariate analysis has controlled for risk factor alterations (Hu et al., 2005).

Multiple studies have shown the endogenous protection of rat myocardium following exercise preconditioning (Powers et al., 2002; Siu et al., 2004) through attenuation of apoptosis and better outcomes following reperfusion. Similarly, endogenous neuroprotection has been shown to take place through multiple mechanisms, including upregulation of neurotrophin expression, strengthening of the blood brain barrier (BBB), enhancing the cerebral capillary and arterial networks, decreasing inflammation and apoptosis, and improving cerebral metabolism. Through these mechanisms, exercise preconditioning provides valuable insight into the science of endogenous neuroprotection and its potential therapeutic and clinical implications.

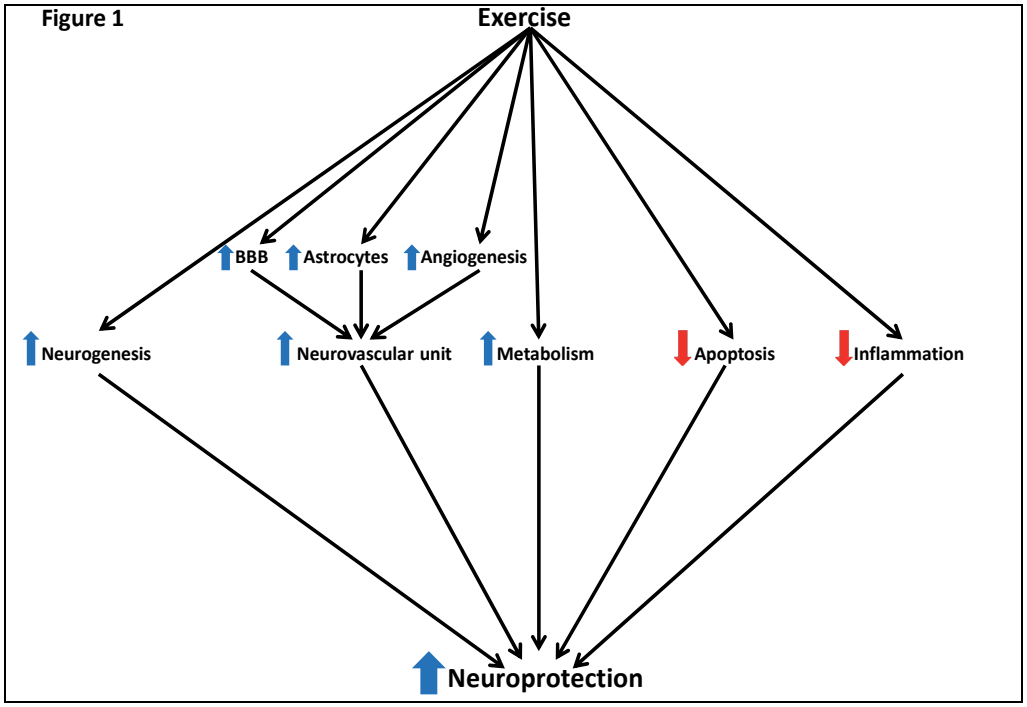


Fig. 1. Exercise generates endogenous neuroprotection through the development of neurogenesis, improved integrity of the neurovascular unit, enhanced metabolism, decreased apoptosis, and amelioration of inflammation.

2. Methods of preconditioning

To date, no specific guidelines have been established through human studies to convey adequate or optimal neuroprotection. Although exercise has been shown to convey endogenous neuroprotection and innate cardioprotection (Hu et al., 2004; Kloner, 2001), the extent, duration, and type of exercise has not been conclusively studied. Despite this, a previous study revealed that moderate intensity and duration of exercise, as opposed to mild or strenuous, correlates with better outcomes and life improvements (Larson et al., 2006). There are specific exercise modalities which convey more potent neuroprotection, and exercise of any duration appears to be neuroprotective. Certainly more studies, especially with human subjects, need to be performed in order to determine exercise regimens resulting in maximal neuroprotection.

2.1 Duration of preconditioning

Despite its known neuroprotective effects, no specific duration of exercise has been shown to provide maximal neuronal survival or lead to better neurologic outcomes. Through multiple studies in rats, it can be seen that exercise preconditioning prior to ischemia/reperfusion injury for a duration of as little as 2 weeks and up to 12 weeks provides similar levels of neuroprotection (Ang et al., 2003; Curry et al., 2009; Davis et al., 2007; Ding et al., 2004a; Stummer et al., 1994; Wang et al., 2001). In addition to the duration of exercise needed, another important factor revolves around the extent of

neuroprotection after exercise has stopped. Another study revealed that the neuroprotective effects of exercise preconditioning appear to be long-lasting, showing that 3 weeks after cessation of exercise, rats still maintained lower levels of neurologic deficit and decreased stroke volume when compared to non-exercise rats (Ding et al., 2004b). Future human studies ought to determine the exact duration needed for maximal endogenous neuroprotection.

2.2 Preconditioning modalities

While various modalities, such as treadmill running, voluntary running, simple and complex exercise, have been shown to provide variable amounts of neuroprotection. When comparing forced exercise on a treadmill to voluntary running on a running wheel, rats forced to exercise on a treadmill tend to have better neurologic outcomes after stroke (Hayes et al., 2008). Previous studies have shown that forced exercise on a treadmill is slower but more constant than voluntary exercise occurs in shorter spurts with faster speed, although the total distance is equal in the two groups (Noble et al., 1999). This neuroprotection in forced exercise subjects led to decreased stroke volume, lessened neurologic deficit, upregulation of heat shock proteins, increased neurogenesis and cerebral metabolism (Hayes et al., 2008; Kinni et al., 2011; Leasure and Jones, 2008). These findings show that moderate exercise over a longer time period conveys more efficient and greater neuroprotection than more vigorous exercise over shorter time periods.

In addition to the studies assessing forced and voluntary exercise, differences have also been established when comparing simple and complex exercise. This study analyzed simple exercise as the repetitive movements of treadmill running, whereas complex exercise constituted enriched activities which required both balance and coordination (Ding et al., 2003). Following ischemia/reperfusion injury, rats preconditioned with complex exercise demonstrated increased synaptogenesis and improved neurologic outcomes when compared to simple treadmill exercise (Ding et al., 2003; Jones et al., 1999). Simple exercise training also alleviates much of the injury following ischemia/reperfusion, but its benefit is less pronounced than what is observed with complex exercise training. Although no human studies have definitively shown the most effective means of exercise for enhanced neuroprotection, these animal studies provide a solid framework for the development of appropriate exercise regimens. Moderate exercise intensity, including components of balance, coordination and stress, taking place over a sustained duration seem to be the most neuroprotective when compared to other exercise modalities and intensities.

3. Mechanisms of neuroprotection

Exercise clearly provides substantial endogenous neuroprotection in addition to its role in risk factor reduction for ischemia/reperfusion injury. These effects have been shown through a wide variety of neuroprotective mechanisms all which increase neuronal survival in the setting of ischemic stroke. These protective effects revolve around the neurovascular unit, composed of neuronal, glial, and vascular cells. This unit is significantly enhanced through exercise training and is injured through ischemia/reperfusion. As such, its integrity is paramount for proper cerebral functioning and is the focal point of the neuroprotective effects of exercise in ameliorating ischemic injury. This neuroprotection is, in part, conveyed through the upregulation of neurotrophin expression, including brain derived neurotrophic factor (BDNF) and nerve growth factor (NGF). These important regulatory proteins increase

neurogenesis, providing a richer neuronal network prior to injury and a more potent neuronal regenerative ability.

In addition to enhanced neurogenesis, exercise neuroprotection also serves to enhance neurovascular unit integrity through strengthening of the blood brain barrier (BBB). Exercise has been shown to enhance the expression of basal lamina proteins and to increase astrocytosis, effectively providing greater stability to the BBB and the neurovascular unit. Matrix metalloproteinases have also been shown to be downregulated following exercise, enzymes which normally degrade the BBB. Furthermore, angiogenic factors, such as vascular endothelial growth factor (VEGF) and angiopoietins (Ang1 and Ang 2), are upregulated following exercise and correlated with increased blood vessel density and increased cerebral blood flow (CBF). These changes provide further stability to the neurovascular unit and have been shown to provide better outcomes following ischemia/reperfusion injury.

Exercise also regulates apoptotic pathways in such a way as to tip the balance in favor of anti-apoptotic to pro-apoptotic gene expression. Carried out through mediators, such as tumor necrosis factor (TNF)-α, extracellular regulated kinase (ERK)-1/2, and heat shock protein (HSP)-70, increasing the anti:pro-apoptotic ratio effectively decrease apoptosis and prolongs neuronal survival, providing obvious benefit following ischemia/reperfusion injury. Furthermore, exercise preconditioning decreases the expression of vascular adhesion molecules (ICAM-1), which decreases leukocyte infiltration and secondary damage following ischemic stroke. These changes ameliorate much of the neuronal damage and death after hypoxic conditions. Finally, neuronal metabolism is upregulated following chronic exercise, correlating with increased ATP production in the acute phase following ischemia/reperfusion injury. This is done through upregulation of glucose transport enzymes, glycolytic enzymes, and the upstream regulator protein hypoxia-inducible factor (HIF)-1α. The pathways underlying these mechanistic changes in response to exercise preconditioning will be defined in detail as each provides valuable clues to understanding the protective ability of exercise following ischemia/reperfusion injury.

3.1 Neurotrophin expression

Neurotrophins are well known to enhance neurogenesis and synaptogenesis while working to promote cerebral integrity at its most basic unit. Multiple human and animal studies have revealed that brain derived neurotrophic factor (BDNF) and nerve growth factor (NGF) facilitate this process (Cohen-Cory et al., 2010; Kim et al., 2004; Kuipers and Bramham, 2006). These trophic factors generate more abundant neuronal and synaptic networks, and they have also been shown to be protective of the neural and cerebrovascular systems. BDNF and NGF mRNA levels have been shown to be upregulated following several weeks of continuous exercise (Ding et al., 2004b), which has been specifically seen in astrocytes and neuronal cells. In addition, providing exercise in an enriched environment, including running wheels, toy balls, ladders, and wooden planks, increased synaptogenesis and neurologic functioning even more than previously seen with basic exercise preconditioning, which was also associated with an increase in BDNF and NGF mRNA levels (Neeper et al., 1996; Ickes et al., 2000). This further implies that complex exercise, requiring balance and coordination, transmits greater neuroprotection that basic repetitive exercise.

Upregulation of these proteins following chronic exercise generates increased neurogenesis, which is protective in advance of ischemia/reperfusion injury. Several studies have shown

that exercise preconditioned rats have elevated levels of BDNF and NGF following ischemia/reperfusion injury, which correlates with decreased neurologic deficit and decreased stroke volume when compared to non-exercised rats (Ang et al., 2003; Ding et al., 2004a). Another study has shown these factors to be upregulated within the reperfusion stage as well (Schabitz et al., 2007), suggesting a possible reparative role post-ischemic insult. The ability to upregulate these neurogenic factors in the reperfusion stage provides the potential for cell survival and regeneration, especially within the ischemic penumbra zone. These results suggest a temporally duel role of BDNF and NGF in exercise preconditioning in which their upregulation via chronic exercise training strengthens the neurovascular unit before and after injury. Altogether, the upregulation of BDNF and NGF seen following exercise preconditioning promotes neuronal survival following ischemia/reperfusion injury.

3.2 Integrity of the neurovascular unit

In addition to neuronal health and viability, integrity of the neurovascular unit as a whole requires maintenance of the blood brain barrier (BBB), structural support provided by astrocytic glial cells, and an intact cerebrovascular network. Composed of capillary endothelial cells, the basal lamina, and astrocytic end-feet, the BBB provides a robust filtration mechanism which serves as the functional barrier for neurovascular unit integrity in the setting of ischemic stroke. Also the first structure injured after ischemia/reperfusion injury, the strength of the BBB is of utmost importance and is a key component to the neuroprotection afforded by exercise training.

While viewing the neurovascular unit as the most basic structure for neuroprotection and stability, astrocytes also play a key role in maintaining the cerebral architecture. Seen following chronic exercise, astrocytosis strengthens the BBB and neurovascular unit, providing a rigid framework to withstand ischemia/reperfusion insults against the neurovascular unit.

Not only does exercise promote neuronal health, BBB integrity, and astrocytic structure, but it also generates an increased blood vessel density. Increased collateral circulation coupled with richer capillary beds allows a more efficient and effective response to ischemia. The angiogenesis and arteriogenesis seen after exercise allow the brain to be rapidly reperfused. In exercised subjects with enhanced BBB integrity, this elevated reperfusion potential occurs much more proficiently and with less damage than is seen in non-exercised subjects. These neuroprotective changes in exercised animals in the setting of ischemia/reperfusion injury underlie the importance of pre-ischemic exercise conditioning and provide potential novel therapeutic interventions.

3.2.1 Blood Brain Barrier integrity

The integrity of the blood brain barrier (BBB) is paramount to maintaining proper filtration of nutrients from the vascular system and in providing the necessary structure to the neurovascular unit. Composed of endothelial cell walls, the basal lamina, and astrocytic end-feet, this filtration barrier is the first structure injured during ischemia/reperfusion injury (del Zoppo and Hallenbeck, 2000; del Zoppo and Mabuchi, 2003), making its integrity a key focal point in the neuroprotective nature of exercise. While an intact endothelial cell wall and astrocytic end-feet are necessary for proper BBB functionality, the basal lamina provides the central structural support and selective permeability necessary to maintain a

healthy cerebral environment. The basal lamina is composed of various proteins of the extracellular matrix, including collagen type IV, laminin, heparan sulphate, proteoglycan, and fibronectin, which are produced by surrounding endothelial cells and astrocytes. The importance for proper integrity and functionality of this structure has been repeatedly established (Lo et al., 2003, Lo et al., 2005). When this barrier is damaged, as is the case following ischemia/reperfusion injury, its ability to selectively discriminate products of the cerebrovascular system is compromised, which is clinically evident as vasogenic edema.

In a hypoxic setting, this loss of BBB integrity and introduction of cerebral edema further disturbs cerebral homeostasis in a step-wise manner. Soon after arterial occlusion, the endothelial permeability barrier is lost and matrix proteins, primarily fibronectin, collagen, and laminin, also begin to lose their integrity (del Zoppo and Mabuchi, 2003). As ischemia continues, a lack of selective permeability and continued vasogenic edema lead to cellular swelling. Swelling of astrocytes and endothelial cells separates them further from the basal lamina, further promoting leakage of vascular contents into the cerebral interstitial spaces (del Zoppo and Mabuchi, 2003). Reperfusion tends to worsen this damage, despite its role as the current mainstay of therapy for ischemic stroke. These alterations in the ischemic phase severely damage the integrity of the BBB, and the sudden increase in cerebrovascular flow in the reperfusion stage leads to excessive leakage of water and generation of oxygen free radicals, further promoting cerebral damage (Ayata and Ropper, 2002; Yang and Betz, 1994).

Studies have shown that exercise training increases basal lamina thickness, adding both strength and stability to the BBB (Davis et al., 2007). These changes were associated with decreases cerebral edema, decreased stroke volume, and improved neuronal recovery following ischemia/reperfusion injury. Collage type IV, a major component of the basal lamina, was found to be upregulated in exercise preconditioned rats, and these subjects maintained a decreased net loss of collagen type IV levels after stroke as well (Davis et al., 2007). Also confirmed by immunocytochemistry, elevated levels of collagen type IV-positive cells were observed in previously exercise rats, and these rats had significantly lower levels of neurologic deficit following stroke (Davis et al., 2007).

In a similar manner to collagen expression, integrin proteins also provide stability to the basal lamina and BBB, providing additional support to the neurovascular unit. Composed of α and β heterodimers, these proteins serve as cell adhesion molecules within the basal lamina and extracellular matrix, anchoring astrocytes and endothelial cells together, ultimately maintaining the integrity of these structures and the neurovascular unit as a whole (Dans and Giancotti, 1999; Hynes, 1992). Integrins, which are anchored to astrocytic and endothelial cytoskeletons, serve as receptors for numerous ligands and proteins within the basal lamina matrix, primarily collagen and laminin (del Zoppo and Mabuchi, 2003; Tawil et al., 1994). These structural proteins also serve as signalling receptors for astrocytes and endothelial cells, allowing for dynamic alterations of the BBB in response to exercise, ischemia, and other noxious stimuli (Hynes, 1992). Following ischemic/reperfusion injury, these proteins rapidly lose affinity for their associated ligands within the basal lamina, decreasing the connective integrity between endothelial and astrocytic cells and the extracellular matrix (Tagaya et al., 2001; Wagner et al., 1997). However, in exercised-trained rats, integrin expression was found to be significantly higher in astroglia and endothelium following ischemic stroke (Ding et al., 2005; Tawil et al., 1994), which correlated with a decrease in neurologic deficit (Ding et al., 2006b). These changes suggest that integrin expression is uniquely important to BBB integrity as it serves to bind all three components

together, and this increased expression following exercise serves as another mechanism of its neuroprotective nature. Through both increased collagen and integrin expression following exercise preconditioning, the enhanced integrity of the BBB decreases vasogenic edema, secondary damage, and neuronal death following ischemia/reperfusion.

While it can clearly be seen that exercise increases the thickness and integrity of the basal lamina and BBB as a whole, pre-ischemic exercise also improves integrity of the neurovascular unit by decreasing BBB breakdown. Matrix metalloproteinase (MMP) is an enzyme produced by endothelial cells, microglia, and astrocytes with a primary function to degrade extracellular matrix and basal lamina proteins (Lo et al., 2003). Expression of MMPs increases exponentially following cerebral damage and, as a result, has been heavily implicated following ischemia/reperfusion injury (Lo et al., 2003). Upregulation of MMP following stroke has been implicated in multiple animal and human studies (Clark et al., 1997; Gasche et al., 1999), leading to increased BBB permeability and vasogenic edema. In addition to these damaging changes, MMP expression has also been correlated with increased inflammation and leukocyte infiltration (Romanic et al., 1998). Pharmacologic inhibition (Romanic et al., 1998) and genetic knockout (Asahi et al., 2001) of MMPs leads to a dramatic decrease in associated edema and neurologic damage. MMP-induced damage following ischemia/reperfusion is mechanistically linked to extracellular matrix breakdown, including degradation of collagen, leading to loss of integrity of the BBB and the neurovascular unit.

Following exercise preconditioning, rats were found to have reduced MMP-9 expression, which correlated with elevated collagen IV levels (Davis et al., 2007), and similar results were seen following pharmacologic inhibition. This neuroprotective effect of exercise is mediated by improved microvascular integrity and basal lamina reinforcement, ultimately decreasing neurologic deficit, infarct volume, and leukocyte infiltration following ischemia/reperfusiom injury (Curry et al., 2009). Interestingly, when these same rats were treated with tumor necrosis factor (TNF)-α antibody or inhibition of extracellular regulated kinase (ERK), MMP-9 levels were not decreased, and the positive effects on neurologic outcome and infarct volume were no longer seen (Curry et al., 2009; Hosomi et al., 2005), suggesting that TNF-α and ERK serve as regulators of MMP expression. Further studies have shown that ERK1/2 mediates a TNF-α induced an increase in MMP-9 expression in the acute setting (Arai et al., 2003), while the gradual increases in TNF-α and ERK1/2 have been shown to decrease MMP-9 expression and lead to decreased neuronal cell death (Chaudhry et al., 2010). These gradual changes are seen following exercise training, in which gradual upregulation of TNF-α and ERK1/2 lead to decreased levels of MMP-9 expression, which also correlates with better outcomes following ischemia/reperfusion injury (Chaudhry et al., 2010). In this study, following ischemic injury, neuronal apoptosis was seen to be reduced in association with decreased levels of MMP-9 expression in exercise preconditioned rats (Chaudhry et al., 2010). Following pharmacologic inhibition of both ERK1/2 and MMP-9, similar effects were also seen, resulting in less neuronal apoptosis and better neurologic outcomes; however, when only ERK1/2 was inhibited, MMP expression returned to the level of non-exercised control rats, suggesting a pathway involving regulation by ERK1/2 and TNF-α of MMP-9 expression (Chaudhry et al., 2010). ERK1/2 has previously been shown to also work through upregulation of tissue inhibitors of metalloproteinases (TIMPs) (Tong et al., 2004), and TIMPs have been previously shown to be upregulated following chronic exercise in association with decreased MMP-9 levels (Guo et al., 2008a). The

upregulation of ERK1/2 and TNF-α following exercise preconditioning, leads to decreased MMP-9 expression, decreased BBB dysfunction, improved neurovascular unit integrity, and better neurologic outcomes. In addition to these important findings regarding the role of ERK1/2 and TNF-α, heat shock proteins (HSP-70) are also thought to play a synergistic role in the downregulation of MMP-9 expression (Liebelt et al., 2010). HSP-70 works in concert with TNF-α to decreased MMP-9 expression and has been shown to be increased following chronic exercise training. Taken together, these various pathways reveal multiple synergistic pathways which function to reduce the breakdown of the BBB, reinforcing the integrity of the neurovascular unit and the cerebral environment.

3.2.2 Astrocytosis

Another vital player in the neurovascular unit is the astrocyte, which also forms the cerebral side of the blood brain barrier (BBB). Astrocytic glial cells are well known to induce strengthening of the BBB (Park et al., 2003; Petty and Lo, 2002). Specifically, these cells have been shown to cover 90% of the cerebrovascular surface and primary function to restrict the permeability across the BBB, providing crucial integrity to the neurovascular unit (Igarashi et al., 1999; Janzer and Raff, 1987; Kondo et al., 1996; Willis et al., 2004). The density and integrity of astrocytes within the brain have also been shown to maintain neurovascular integrity in the setting of acute ischemic stroke (del Zoppo and Mabuchi, 2003; Lo et al., 2005). The damaging effects of ischemia/reperfusion are primarily seen at the BBB, classically affecting endothelial cells and astrocytes and their corresponding adherence to the extracellular matrix. This interaction between endothelial cells, the extracellular matrix, and astrocytes provides the central trigger for neuronal injury and death in the setting acute stroke (Petty and Wettstein, 2001). In addition to its other effects, pre-ischemic exercise conditioning has been shown to upregulate the degree of astrocytosis, and this upregulation has been shown to be correlated with better outcomes following ischemic/reperfusion injury (Li et al., 2005). An increased number of astrocytes within the neurovascular unit, provided by exercise training, cover a greater percentage of the BBB. This increased coverage allows the neurovasculature to be more restricted in the blood products that are allowed to permeate the BBB, providing important integrity in the setting of ischemia/reperfusion injury.

3.2.3 Angiogenesis and arteriogenesis

Exercise training transforms the neurovascular system of the neurovascular unit, developing a vital metabolic response network in response to ischemia/reperfusion injury. Under normal conditions, angiogenesis and endothelial cell proliferation is scant in the adult brain (Ogunshola et al., 2000). Nonetheless, as a major element of the neurovascular unit, it plays a crucial role in maintaining an appropriate and healthy cerebral environment. Previous studies have shown that physical activity on a treadmill increases blood vessel density in the brain (Black et al., 1990; Isaacs et al., 1992; Kleim et al., 2002; Swain et al., 2003), and forced exercise on a treadmill induces cortical and striatal angiogenesis (Ding et al., 2004a, 2004b). In addition to these increases in angiogenesis, exercise preconditioning also increases arteriogenesis, which promotes cerebral blood flow (CBF), increases collateral circulation, and ameliorates neuronal injury and death following ischemia/reperfusion injury (Lloyd et al., 2003, 2005). Closely linked to the metabolic requirements needed by the brain during levels of high activity, the amount of blood supply provides the necessary avenue to

produce the needed glucose and oxygen. Through increasing this metabolic demand, exercise leads to permanent structural alterations, such as angiogenesis and arteriogenesis, and these changes allow the increased delivery of vital nutrients to active neurons (Isaacs et al., 1992; Vissing et al., 1996). These structural changes not only facilitate increased glucose and oxygen delivery, but they have also been shown to reduce brain damage as well (Ding et al., 2004a).

The structural alterations seen with angiogenesis are driven by several regulator proteins, namely vascular endothelial growth factor (VEGF) and angiopoietins (Ang) 1 and 2. VEGF and Ang1/2 are known to be expressed in greater abundance following exercise training, and these changes lead to increased blood vessel density (Ding et al., 2004b). Expression of Ang 1 and 2 mRNA has been shown to be increased as early as 1 week after the onset of exercise training (Ding et al., 2006b), and VEGF mRNA expression has been shown to be mildly increased at 1 week but exponentially higher after 3 weeks of exercise training (Matsumori et al., 2005; Nawashiro et al., 1997; Sawatzky et al., 2006; Yong et al., 2001). Exercise-induced angiogenesis was also seen in aging rats with associated increases in VEGF and Ang1/2 mRNA levels (Ding et al., 2006b), suggesting that angiogenesis can be expected in the adult brain. Although most proliferation of the neurovascular system occurs during cerebral development, these findings reveal that VEGF and Ang1/2 drive this process in aging brains as well, further promoting the endogenous neuroprotection afforded by exercise preconditioning.

These changes occurring after chronic exercise also lead to better outcomes following ischemia/reperfusion injury. In addition to correlating with increased cerebral blood flow and glucose utilization, the angiogenesis and arteriogenesis observed following exercise preconditioning is associated with decreased neuronal cell death following ischemia/reperfusion injury (Li et al., 2005). The process of angiogenesis provides considerably denser cerebrovascular networks, which bathes the brain in a network of vessels more apt to deliver the vital nutrients necessary for proper brain health and functioning. The additional benefit seen from arteriogenesis, following exercise preconditioning underscores the importance of increased collateral circulation, particularly vital in saving vital brain volume in the ischemic penumbra. Especially when coupled with astrocytosis, the angiogenesis seen following exercise preconditioning substantially contributes to the development and integrity of the BBB, further promoting the neurovascular unit and protecting against ischemic/reperfusion injury.

3.2.4 Cerebral blood flow and glucose uptake

As previously discussed, exercise training increases angiogenesis and arteriogenesis, providing more avenues for potential blood delivery to the cerebral system. Previous studies with Laser Doppler flowmtery (LDF) and ^{15}O-H_2O positron emission tomography (PET) have shown that preischemic exercise preserves the cerebral blood flow (CBF) during the reperfusion stage in ischemia/reperfusion injury (Zwagerman et al., 2010b). Although CBF was similar in exercised and non-exercised groups during ischemia, CBF was significantly higher in the exercise preconditioned animals during reperfusion and was associated with a decreased volume of the infarct. This increase in perfusion during the reperfusion stage in preconditioned animals suggests that this training may partial ameliorate the "no reflow" phenomenon often seen following ischemia/reperfusion injury.

In the same study, intracerebral glucose uptake was also assessed using an ^{18}F-fluorodeoxy-D-glucose (FDG) radiotracer (Zwagerman et al., 2010b). Following ischemia/reperfusion injury, cerebral metabolism was significantly reduced as evidenced by a decrease in glucose uptake. However, in rats preconditioned with physical exercise, brain glucose uptake and metabolism was substantially preserved following reperfusion and was associated with a decrease in infarct volume and functional neurologic deficit. CBF and metabolism also increase during the act of physical exercise, signifying the increased metabolic demand on neuronal cells during training (Hellstrom et al., 1996; Ide and Secher, 2000; Vissing et al., 1996; Williamson et al., 1997) and the likely underlying mechanism through which neuroprotection is obtained. The angiogenic changes that occur following exercise preconditioning provide the brain with an enriched vascular bed with an enhanced ability for proper cerebral blood flow and glucose delivery to neurons, yielding more tolerance to reperfusion injury in the setting of ischemia/reperfusion.

3.3 Inflammatory response

The "no reflow" phenomenon refers to the tendency towards hypoperfusion during the reperfusion stage following ischemia. This hypoperfusion following transient ischemia is thought to stem from multiple mechanisms, including microvascular damage and cerebrovascular occlusion from cellular elements (Aspey et al., 1989; Dietrich et al., 1987; Mori et al., 1992; Nishigaya et al., 1991). In addition to these observed changes, hemoconcentration, red blood cell sludging, hyperviscosity, and platelet plugging tend to occur as well in the reperfusion phase, further exacerbating the damage seen in ischemia/reperfusion injury (Choudhri et al., 1998). While these effects of reperfusion injury causes significant damage, the accumulation of polymorphonuclear leukocytes are the primary contributors to the perfusion abnormalities observed following transient ischemia. The secondary inflammation after ischemia/reperfusion injury plays a major role in secondary brain damage through increased leukocyte infiltration, microvascular damage, and free radical accumulation during reperfusion.

3.3.1 Adhesion molecules and toll-like receptors

In the setting of ischemia/reperfusion injury, cytokines tend to stimulate the expression of cellular adhesion molecules, attracting leukocytes to the cerebrovascular system and promoting their diapedesis into the interstitial space. Two central cytokines in this process, interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α), are known to be upregulated following periods of hypoxia and promote the expression of intercellular adhesion molecule 1 (ICAM-1), P-selectin, and E-selectin on leukocytes and endothelial cells. These changes lead to the leukocyte accumulation classically seen in ischemia/reperfusion, leading to adhesion of leukocytes to the damaged vascular endothelium, clogging of the neurovascular vessels, and infiltration into the brain parenchyma. Previous studies have shown that pre-ischemic exercise training reduces the expression of ICAM-1, leading to decreased leukocyte infiltration and accumulation, in the reperfusion stage (Ding et al., 2005). This downregulation leads to decreased inflammation following ischemia/reperfusion injury and serves as another neuroprotective mechanism of exercise preconditioning.

Another mechanism underlying the inflammatory damage in the reperfusion stage pertains to the expression of toll-like receptors. These cell surface receptors are found within the brain and throughout the body and are actively involved in the immune response by

binding endogenous and foreign materials and triggering a cytokine cascade (Gleeson et al., 2006). Previous research studies have also revealed that exercise preconditioning reduces the expression of Toll-like receptor-4 (McFarlin et al., 2006), and reduction of these receptors in Toll-like receptor-4 deficient mice has been shown to decrease tissue damage and neurologic deficits following ischemia/reperfusion injury (Cao et al., 2007). Another study looking at both of these factors has indicated that exercise preconditioning simultaneously reduces expression of Toll-like receptor-4, which leads to a reduction in brain injury following ischemic stroke (Zwagerman et al., 2010a).

3.3.2 Leukocyte invasion

Through the downregulation of adhesion molecules and toll-like receptors, exercise preconditioning is able to significantly reduce the amount of damage seen following ischemia/reperfusion injury. The underlying mechanism through which this process works focuses on the decreased leukocyte migration and diapedesis often seen in the reperfusion stage following stroke. Decreased leukocyte infiltration decreases many of the secondary changes such as free radical formation and subsequent edema formation. Ultimately, the decreased leukocyte invasion seen in exercise preconditioned subjects leads to better neurologic outcome following ischemia/reperfusion injury.

3.4 Neuronal death and survival signalling pathways

Neuronal survival depends on both external and internal stimuli and environments. Many of the changes following exercise preconditioning, involving inflammation, strengthening of the blood brain barrier, and increased integrity of the neurovascular unit, improve the cerebral environment in the setting of acute ischemia/reperfusion injury. In addition, internal cellular stimuli and pathways have a profound effect on neuronal survival. Following exercise preconditioning, apoptosis is attenuated and heat shock proteins are upregulated through a variety of biomolecular mechanisms following ischemia/reperfusion injury. Likewise, external stimuli through tumor necrosis factor (TNF)-α protect the brain during hypoxia. These changes seen in rats preconditioned with physical exercise are neuroprotective in the setting of ischemia/reperfusion injury, decreasing neuronal death and improving neurologic outcomes.

3.4.1 Anti:Pro apoptotic ratio

Neuronal apoptosis following ischemia/reperfusion injury is regulated by cascades of pro- and anti-apoptotic proteins. Notable among these include pro-apoptotic Bax, Bad, and Bak and anti-apoptotic Bcl-2 and Bcl-xL (Lazou et al., 2006; Mayer and Oberbauer, 2003). Upregulation of anti-apoptotic proteins (Bcl-2, Bcl-xL) and corresponding downregulation of pro-apoptotic proteins (Bax, Bad, and Bak) are seen following exercise preconditioning and are protective in the event of cerebral ischemia (Rybnikova et al., 2006; Wu et al., 2003). In addition to these key regulatory proteins, both caspase-dependent and caspase independent pathways appear to be involved in cerebral ischemia and neuronal death (Cao et al., 2003; Joza et al., 2001; Zhu et al., 2003), and apoptosis induced factor (AIF) also plays a key role in pro-apoptosis (Daugas et al., 2000; Susin et al., 1999).

Exercise preconditioning has been shown to not only enhance the expression of anti-apoptotic Bcl-xL, but to also decrease the expression of pro-apoptotic AIF and Bax, ultimately leading to decreased apoptosis and prolonged neuronal survival (Chaudhry et al., 2010). Following

ischemia/reperfusion injury, this increased anti:pro apoptotic ratio generates decreased neuronal death and smaller infarct size. While these neuroprotective mechanisms of exercise are newly evolving, they provide a possible point of therapeutic intervention. While neuroprotective agents that target cell death pathways have been tried in the past, the potential for increasing cell survival reveals a potential novel strategy for improving outcomes following ischemia/reperfusion (Chan, 2004). This could provide a neuroprotective therapy that would simultaneously promote cell survival and decrease neuronal death, thus ameliorating much of the functional loss following acute ischemic stroke.

3.4.2 TNF-α and TNF-α receptor

Tumor necrosis factor (TNF)-α is a major deleterious pro-inflammatory cytokine found throughout the systemic circulation and upregulated following stroke and traumatic brain injury (Botchkina et al., 1997; Sairanen et al., 2001). Despite its inflammatory and injurious effects, evidence also points to TNF-α as a beneficial factor in tissue repair and neuroprotection (Bruce et al., 1996; Feuerstein and Wang, 2001; Wang et al., 2000). Furthermore, this cytokine may serve to induce endogenous neuroprotection following chronic exercise preconditioning. It is believed that exercise training produces a chronic low grade increase in TNF-α concentration, ultimately generating neuronal tolerance and protection in the setting of ischemia/reperfusion injury (Ginis et al., 1999; Liu et al., 2000; Wang et al., 2000). In a similar manner, TNF-α concentration was chronically elevated following exercise, which resulted in reduced myocardial infarction (Yamashita et al., 1999); however, acutely elevated levels of TNF-α following ischemia results in harmful myocardial remodelling (Jobe et al., 2009).

In the brain, exercise preconditioning also chronically increases the level of TNF-α that is exposed to neuronal cells, an effect which prevents the downstream inflammatory reaction which is induced by acutely elevated levels of TNF-α following cerebral ischemia/reperfusion injury (Ding et al., 2005). Furthermore, TNF-α has also been shown to reduce blood brain barrier injury and neuronal damage following ischemic stroke (Guo et al., 2008b). This scenario reveals two sides to the effect of TNF-α on neuronal survival and cerebral integrity. With chronic low levels of the cytokine, it is profoundly beneficial and neuroprotective; however, following ischemia/reperfusion, the acute increase of TNF-α into the cerebrovascular circulation destroys the integrity of the neurovascular unit, increases cell death, and enlarges the infarct volume. Exercise preconditioning enhances the former effect, increasing chronic low grade levels of the cytokine, which ameliorates the latter effect and dampens the injury following acute increases of TNF-α.

Mechanisms underlying this complex picture of TNF-α have not been completely uncovered, but are thought to involve the expression of TNF-α receptors. Previous studies have shown that the chronic exercise-induced levels of TNF-α serve to reduce the expression of TNF-α receptor after ischemia/reperfusion (Reyes, Jr. et al., 2006). Following ischemic injury in a rat model, pre-ischemic exercise was indeed found to decrease the expression of TNF-α receptors I and II, leading to reduced brain damage and enhance neurologic recovery (Reyes, Jr. et al., 2006). These results indicate a classic desensitization of the TNF-α receptor following exercise preconditioning, promoting neuronal tolerance to acutely elevated levels of TNF-α following ischemia/reperfusion injury.

3.4.3 Heat shock proteins

Neuronal survival and neuroprotection appears to be further driven by heat shock protein (HSP)-70, a highly inducible protein of 70 kDa. HSP-70 is well known to respond to various

types of stress, including heat shock, hypoxia, oxidative stress, and exposure to metals and toxins (Kiang and Tsokos, 1998). Constitutively expressed, this molecular chaperone protein assists in the folding both nascent and denatured proteins during times of neuronal stress (Schlesinger, 1990). Overexpression of HSP-70 has previously been shown to be neuroprotective in the setting of ischemia/reperfusion (Giffard and Yenari, 2004). This neuroprotective protein interferes with apoptosis inducing factor (AIF), a pro-apoptotic protein. HSP-70 also increases the levels of anti-apoptotic proteins, Bcl-2 and Bcl-xL, thus serving as another mechanism to increase the anti:pro apoptotic ratio and push neurons toward cellular survival, especially in the setting of ischemia/reperfusion (Liebelt et al., 2010; Ohtsuka and Suzuki, 2000).

Multiple studies have shown that ischemic preconditioning induces HSP-70 expression and promotes neuroprotection (Chen and Simon, 1997; Kirino et al., 1991; Masada et al., 2001). Despite these benefits, the effects of HSP-70 are limited. If the insult is very severe or if expression of HSP-70 is too low, the beneficial effects may not be seen (Giffard and Yenari, 2004; Lee et al., 2001; Matsumori et al., 2005). Nonetheless, HSP-70 mice convey more potent neuroprotection than wild type mice, underlying its importance as a key factor in exercise-induced neuroprotection for ischemia/reperfusion injury (Matsumori et al., 2005). In addition to its neuroprotective effects, HSP-70 has also been seen to be cardioprotective following upregulation after exercise preconditioning (Hamilton et al., 2003; Lennon et al., 2004).

Also, HSP-70 alone does not appear to be neuroprotective, but it requires other proteins for optimal neuroprotection (Lee et al., 2001). Upregulation of TNF-α appears to be critical for HSP-70 to effectively reduce apoptosis and prolong neuronal survival (Liebelt et al., 2010). Furthermore, TNF-α and HSP-70 appear to work in concert through an ERK1/2 signal transduction pathway to increase the expression of anti-apoptotic genes and decrease the expression of pro-apoptotic genes (Goel et al., 2010). These results reveal the neuroprotective nature of HSP-70, but they also reveal its limitations. For HSP-70 to truly be effective in the setting of ischemia/reperfusion injury, it requires a large insult, significant HSP-70 expression, and the upregulation of other proteins as well, including TNF-α. Despite these constraints, HSP-70 does appear to induce neuroprotection in ischemia/reperfusion injury following exercise preconditioning.

3.5 Extracellular signal-regulated kinase

Extracellular signal-regulated kinases (ERK1/2) are involved in mitogen-activated protein kinase pathways and are constitutively expressed in the adult brain (Fiore et al., 1993; Sharony et al., 2005). These ERK1/2-regulated pathways are pivotal in signal transduction and neuroprotection in the setting of ischemia/reperfusion injury. Numerous studies have shown a pro-apoptotic role for ERK1/2 in neurons and other cells as well (Chu et al., 2004; Shackelford and Yeh, 2006; Zhuang and Schnellmann, 2006). Although ERK1/2 has been shown to push cells towards death, a protective and beneficial role has also been established (Rybnikova et al., 2006). Activation of this regulatory kinase has been shown to enable tissue repair in the setting of ischemia/reperfusion injury, thus decreasing cell death (Cavanaugh, 2004; Hetman and Gozdz, 2004; Ostrakhovitch and Cherian, 2005; Sawatzky et al., 2006). Furthermore, exercise preconditioning appears to upregulate ERK1/2, leading to an ischemic neuronal tolerance under hypoxic condition (Gu et al., 2001; Jones and Bergeron, 2004; Shamloo and Wieloch, 1999). While ERK1/2 is upregulated following ischemic

preconditioning (Lecour et al., 2005), ERK1/2 activation also is detrimental following stroke, trauma and degenerative disease (Chu et al., 2004). This dual role of ERK1/2, being both beneficial and detrimental, may be similar in mechanism to the effects of TNF-α in which chronic upregulation following exercise preconditioning generates neuronal tolerance and improved outcome following ischemia/reperfusion injury.

Following inhibition of ERK1/2 in exercise preconditioned animals, neuroprotection was substantially reduced in ischemia/reperfusion, suggesting the pertinent role of ERK1/2 in exercise-induced neuroprotection (Guo et al., 2008b). However, exercise preconditioned animals did have elevated levels of ERK1/2 and TNF-α following exercise training, which correlated with decreased neurologic deficit, smaller brain infarcts, and less inflammation (Curry et al., 2009). A recent study has shown that ERK1/2 activation also helps to regulate apoptosis (Liebelt et al., 2010). Again, pre-ischemic exercise led to minor increases in ERK1/2, which was neuroprotective against the large acute elevations seen following ischemia/reperfusion injury. In the setting of hypoxic injury, ERK1/2 regulates the anti- and pro-apoptotic pathways involving such regulators as Bcl-xL, Bax, and AIF. Following exercise preconditioning, the upregulation of ERK1/2 served to promote anti-apoptosis, leading to decreased neuronal apoptosis and infarct volume.

ERK1/2, while shown to ameliorate ischemia/reperfusion through its apoptotic regulatory effects and inflammatory changes, has also been linked with HSP-70 and TNF-α. Several studies have shown these three important proteins to be fundamentally linked in a relationship where TNF-α and HSP-70 activate the MEK/ERK signalling pathway, leading to neuroprotection (Gortz et al., 2005; Lee et al., 2001,2005). While ischemic preconditioning has been shown to induce ERK1/2-mediated neuroprotection, pharmacologic pre-treatment with TNF-α has not shown similar results (Lecour et al., 2005), suggesting that multiple interacting pathways and proteins are likely necessary to observe the full neuroprotective effect. It seems likely that a cross talk between TNF-α, HSP-70, and ERK1/2 occurs in exercise training, allowing for a greater neuroprotective response following ischemia/reperfusion injury. Nonetheless, these studies have clarified a dual role of ERK1/2 in neuroprotection, in which chronic low levels protect against acute elevations of the protein and ameliorate the damage and neuronal loss following ischemia/reperfusion injury.

3.6 Metabolic enhancement

The association between exercise preconditioning and neuroprotection in ischemia/reperfusion has been well established. In addition to the aforementioned mechanisms, chronic exercise training also affects cerebral metabolism and energy production, allowing neurons to re-establish homeostasis more rapidly following acute ischemic stroke. A recent study revealed that forced exercise resulted in enhanced cerebral glycolysis and cerebral metabolism (Kinni et al., 2011). In addition to increasing glycolysis, exercise preconditioning is also well known to increase cerebral blood flow (CBF) and adenosine triphosphate (ATP) production (Ide and Secher, 2000; McCloskey et al., 2001; Ogoh and Ainslie, 2009). Exercise can be best viewed as a chronic state of metabolic stress, which requires increased glucose delivery, glycolysis, and ATP production in order to match the energy demand. Chronic exercise preconditioning enables the neuronal cells to accumulate the necessary machinery for mass ATP production, which elevates the metabolic and ATP production potential in these cells. In the setting of ischemia/reperfusion injury,

these preconditioned neurons have an increased metabolic capacity and are better able to avoid hypoxic damage.

3.6.1 ATP production

The process of ATP production requires several key steps, involving detection of low ATP stores, glucose transport and glycolysis. 5'-AMP-activated protein kinase (AMPK) serves as an energy sensor and is capable of detecting low ATP stores in cells (Minchenko et al., 2003). The protein is a heterotrimeric serine/threonine kinase, which becomes activated in times of metabolic stress and decreased ATP stores (Emerling et al., 2007; Hardie et al., 2003; Zorzano et al., 2005). When ATP concentrations drop, AMPK activates glycolysis in order to restore the cell's energy balance, allowing it to serve as an excellent marker of metabolic demand (Kahn et al., 2005). Following exercise training, levels of the active phosphorylated form of AMPK are increased in response to the elevated metabolic demand (Aschenbach et al., 2004; Kinni et al., 2011). Following ischemia/reperfusion injury, the active phosphorylated form of AMPK has been shown to be upregulated in preconditioned rats, suggesting an increased metabolic response to hypoxia in these exercised animals (unpublished data). This increased capacity for metabolism upregulation is mirrored in elevated levels of glucose transporters and glycolytic enzymes, which are also seen following exercise training and correlate with better outcomes following ischemia/reperfusion injury.

While increases in cerebral blood flow associated with exercise facilitate greater transport of glucose to the neurovascular system, transport across the BBB and neuronal membrane are controlled by glucose transporters (GLUT). GLUT1 is primarily found in the endothelial cells of the BBB and generates a basal glucose level, while GLUT3 is found exclusively in neurons, and both of these glucose transporters have previously been shown to increase in response to hypoxia and elevated metabolic demand (Maurer et al., 2006). The increased need for energy production following hypoxia requires more glucose to be available within neurons, and these two glucose transporters work together to shuttle glucose from the circulation into neurons. GLUT1 and GLUT3 are known to have increased expression following exercise preconditioning (Kinni et al., 2011). Following ischemia/reperfusion injury, GLUT1 and GLUT3 levels are elevated in the first 4 hours after reperfusion is established in exercise preconditioned rats, but expression is equal to control at 24 hours after injury (unpublished data). This elevation in GLUT expression in the acute phase of reperfusion indicates that these preconditioned animals have the cellular machinery in place immediately when it is needed. Non-exercised rats take longer to transcribe and translate the needed metabolic proteins, leading to increased metabolic dysfunction. Thus, exercise preconditioning provides neuroprotection by increasing the levels of available glucose within neurons, providing the necessary substrate for ATP production.

Once inside neurons, glucose must be metabolized to produce ATP for the cell. The initial steps of glycolysis are primarily regulated by the key rate limiting step of phosphofructokinase (PFK), which catalyzes the phosphorylation of fructose-6-phosphate to fructose-1,6-bisphosphonate. PFK expression is known to be upregulated following increased metabolism and glycolysis and is has been shown to be neuroprotective in hypoglycemic conditions (Minchenko et al., 2003). Another study has revealed PFK is increased following exercise preconditioning (Kinni et al., 2011), and this enzyme is

increased following ischemia/reperfusion injury, leading to increased neuronal metabolism and decreased neurologic deficits (unpublished data). The elevated ability of neurons to process glucose through glycolysis in exercise preconditioned animals underlies another key component of exercise-induced neuroprotection. Not only does exercise training increase glucose delivery to cells through angiogenesis and glucose transport, it also upregulates the machinery needed for glucose breakdown and ATP production.

Ultimately, these processes work to increase ATP production to meet the energy demand of neurons. One test used to assess the ATP production capacity of a neuron is an ADP:ATP ratio, which has been found to be decreased following ischemia/reperfusion injury in exercise preconditioned rats (unpublished data). A lower ADP:ATP ratio indicates a lower level of metabolic dysfunction and increased capacity for ATP production in exercise trained rats. Through the increased expression of various metabolic proteins, including AMPK, GLUT1, GLUT3, and PFK, exercise preconditioning upregulates multiple stages of ATP production, providing cells with the important energy substrate. The increased capacity for ATP production allows neurons in exercise trained rats to decrease their energy deficit following ischemia/reperfusion injury. In turn, this promotes neuronal survival and decreases infarct size, leading to better outcomes following ischemia/reperfusion.

3.6.2 HIF-1α and metabolism

Hypoxic-induced factor-1α (HIF-1α) is a transcription factor, which is normally inhibited by oxygen-dependent hydroxylase enzymes, but in hypoxic conditions, these hydroxylase enzymes lose their ability to function and allow HIF-1α to initiate gene transcription (Bracken et al., 2006). HIF-1α is known to be neuroprotective in rats following ischemia/reperfusion injury (Bernaudin et al., 2002; Schubert, 2005). Not only is HIF-1α increased following hypoxia, but it also is increased in rats that are chronically exposed to hypoxic events (Bernaudin et al., 2002; Bracken et al., 2006). The expression of HIF-1α is also known to be increased following exercise preconditioning (Kinni et al., 2011). In addition to these findings, the transcription factor has also been shown to increase the expression of genes and proteins involved in angiogenesis and glycolysis, revealing its metabolism promoting activity (Bergeron et al., 1999; Bernaudin et al., 2002; Iyer et al., 1998; Jones and Bergeron, 2001; Schubert, 2005; Semenza, 2009). HIF-1α is known to increase the expression of VEGF, glucose transporters and enzymes involved in glycolysis, such as PFK (Bergeron et al., 1999; Jones and Bergeron, 2001; Kim et al., 2006). HIF-1α is not only involved in enhanced expression of metabolic enzymes and pathways, but the expression of HIF-1α is also increased by AMPK, a master regulator of neuronal metabolism (Emerling et al., 2007; Lee et al., 2003; Neurath et al., 2006). In fact, studies in chronically exercised rats have demonstrated that increased levels of AMPK induce HIF-1α to increase the expression of glucose transporters and PFK (Emerling et al., 2007; Hardie et al., 2003; Kahn et al., 2005; McGee and Hargreaves, 2006).

HIF-1α, through its multiple mechanistic pathways, can increase metabolism and angiogenesis in an oxygen-deficient state, allowing it to simultaneously increase the mode of glucose delivery and the mechanism of ATP production. In addition to being increased following exercise preconditioning (Kinni et al., 2011), HIF-1α is also increased in exercise trained animals following ischemia/reperfusion injury, which correlated with better neurologic outcomes and decreased infarct size (unpublished data). These results indicate

that exercise equips neurons with a greater ability to upregulated glucose transport and metabolism, resulting in greater ATP production and decreased metabolic dysfunction, following acute ischemic stroke. This rapid response to an elevated energy demand promotes neuronal recovery and survival and serves as another mechanism of exercise-induced neuroprotection in the setting of ischemia/reperfusion injury.

4. Clinical implications

Exercise-induced preconditioning is useful in prevention and amelioration of neuronal loss and neurologic dysfunction following ischemia/reperfusion injury, but it also has many therapeutic implications as well. The potential for future drug targets at various levels of the neuroprotective mechanisms are abundant as the effects of neurotrophic factors, neurovascular unit integrity, inflammatory markers, and metabolic changes provide potential avenues for future pharmacologic intervention. Particularly evident in patients with a history of ischemic stroke, traumatic brain injury, or transient ischemic attacks, pharmacologic interventions that could increase metabolism, strengthen the neurovascular unit, or decrease inflammation could have profound effects on morbidity and mortality associated with cerebrovascular accidents. Finally, the implications for exercise preconditioning prior to neurosurgical intervention can clearly be seen. Through its ability to strengthen the neurovascular unit and decrease brain inflammation, exercise training should be strongly encouraged in any individuals prior to neurosurgical intervention.

5. Conclusion

Exercise preconditioning clearly is neuroprotective in the setting of ischemia/reperfusion injury, and these protective effects are conveyed through multiple mechanisms. The neuroprotection derived as a result of exercise training is an endogenous effect that occurs independently of the risk factor modification that is also seen following exercise. Innate neuroprotection from exercise is derived from elevated levels of neurotrophin proteins, which increase neuronal abundance and strength. Furthermore, enhanced integrity of the neurovascular unit occurs through strengthening of the blood brain barrier, astrocytosis, angiogenesis, and arteriogenesis. Exercise preconditioning also decreases the inflammatory response and leukocyte invasion following ischemia/reperfusion injury, thus decreasing much of the secondary damage seen following the reperfusion stage. Neuronal apoptosis is reduced as exercise training increases anti-apoptotic factors and simultaneously decreases pro-apoptotic factors, pushing neurons towards a state of survival rather than programmed cell death. Finally, exercise increases the metabolic capacity of neurons through upregulation of cerebral blood flow, glucose transport, glycolysis, and ATP production. Altogether, these changes seen following exercise preconditioning decreased neuronal loss, reduce infarct volume, and improve neurologic outcomes after ischemia/reperfusion injury.

6. References

Ang, E. T., P. T. Wong, S. Moochhala, and Y. K. Ng, 2003, Neuroprotection associated with running: is it a result of increased endogenous neurotrophic factors?: Neuroscience, v. 118, no. 2, p. 335-345.

Arai, K., S. R. Lee, and E. H. Lo, 2003, Essential role for ERK mitogen-activated protein kinase in matrix metalloproteinase-9 regulation in rat cortical astrocytes: Glia, v. 43, no. 3, p. 254-264.

Asahi, M., X. Wang, T. Mori, T. Sumii, J. C. Jung, M. A. Moskowitz, M. E. Fini, and E. H. Lo, 2001, Effects of matrix metalloproteinase-9 gene knock-out on the proteolysis of blood-brain barrier and white matter components after cerebral ischemia: J Neurosci, v. 21, no. 19, p. 7724-7732.

Aschenbach, W. G., K. Sakamoto, and L. J. Goodyear, 2004, 5' adenosine monophosphate-activated protein kinase, metabolism and exercise: Sports Med., v. 34, no. 2, p. 91-103.

Aspey, B. S., C. Jessimer, S. Pereira, and M. J. Harrison, 1989, Do leukocytes have a role in the cerebral no-reflow phenomenon?: J Neurol Neurosurg Psychiatry, v. 52, no. 4, p. 526-528.

Ayata, C., and A. H. Ropper, 2002, Ischaemic brain oedema: J Clin Neurosci, v. 9, no. 2, p. 113-124.

Bergeron, M., A. Y. Yu, K. E. Solway, G. L. Semenza, and F. R. Sharp, 1999, Induction of hypoxia-inducible factor-1 (HIF-1) and its target genes following focal ischaemia in rat brain: Eur.J.Neurosci., v. 11, no. 12, p. 4159-4170.

Bernaudin, M., A. S. Nedelec, D. Divoux, E. T. MacKenzie, E. Petit, and P. Schumann-Bard, 2002, Normobaric hypoxia induces tolerance to focal permanent cerebral ischemia in association with an increased expression of hypoxia-inducible factor-1 and its target genes, erythropoietin and VEGF, in the adult mouse brain: J.Cereb.Blood Flow Metab, v. 22, no. 4, p. 393-403.

Black, J. E., K. R. Isaacs, B. J. Anderson, A. A. Alcantara, and W. T. Greenough, 1990, Learning causes synaptogenesis, whereas motor activity causes angiogenesis, in cerebellar cortex of adult rats: Proceedings of the National Academy of Sciences of the United States of America, v. 87, no. 14, p. 5568-5572.

Botchkina, G. I., M. E. Meistrell, I. L. Botchkina, and K. J. Tracey, 1997, Expression of TNF and TNF receptors (p55 and p75) in the rat brain after focal cerebral ischemia: Mol Med, v. 3, no. 11, p. 765-781.

Bracken, C. P., A. O. Fedele, S. Linke, W. Balrak, K. Lisy, M. L. Whitelaw, and D. J. Peet, 2006, Cell-specific regulation of hypoxia-inducible factor (HIF)-1alpha and HIF-2alpha stabilization and transactivation in a graded oxygen environment: J.Biol.Chem., v. 281, no. 32, p. 22575-22585.

Bruce, A. J., W. Boling, M. S. Kindy, J. Peschon, P. J. Kraemer, M. K. Carpenter, F. W. Holtsberg, and M. P. Mattson, 1996, Altered neuronal and microglial responses to excitotoxic and ischemic brain injury in mice lacking TNF receptors: Nat Med, v. 2, no. 7, p. 788-794.

Cao, C. X., Q. W. Yang, F. L. Lv, J. Cui, H. B. Fu, and J. Z. Wang, 2007, Reduced cerebral ischemia-reperfusion injury in Toll-like receptor 4 deficient mice: Biochem.Biophys.Res.Commun., v. 353, no. 2, p. 509-514.

Cao, G., R. S. Clark, W. Pei, W. Yin, F. Zhang, F. Y. Sun, S. H. Graham, and J. Chen, 2003, Translocation of apoptosis-inducing factor in vulnerable neurons after transient cerebral ischemia and in neuronal cultures after oxygen-glucose deprivation: J Cereb.Blood Flow Metab, v. 23, no. 10, p. 1137-1150.

Cavanaugh, J. E., 2004, Role of extracellular signal regulated kinase 5 in neuronal survival: Eur.J.Biochem., v. 271, no. 11, p. 2056-2059.

Chan, P. H., 2004, Future targets and cascades for neuroprotective strategies: Stroke, v. 35, no. 11 Suppl 1, p. 2748-2750.

Chaudhry, K. et al., 2010, Matrix metalloproteinase-9 (MMP-9) expression and extracellular signal-regulated kinase 1 and 2 (ERK1/2) activation in exercise-reduced neuronal apoptosis after stroke: Neuroscience Letters, v. 474, no. 2, p. 109-114.

Chen, J., and R. Simon, 1997, Ischemic tolerance in the brain: Neurology, v. 48, no. 2, p. 306-311.

Choudhri, T. F., B. L. Hoh, H. G. Zerwes, C. J. Prestigiacomo, S. C. Kim, E. S. Connolly, Jr, G. Kottirsch, and D. J. Pinsky, 1998, Reduced microvascular thrombosis and improved outcome in acute murine stroke by inhibiting GP IIb/IIIa receptor-mediated platelet aggregation: Journal of Clinical Investigation, v. 102, no. 7, p. 1301-1310.

Chu, C. T., D. J. Levinthal, S. M. Kulich, E. M. Chalovich, and D. B. DeFranco, 2004, Oxidative neuronal injury. The dark side of ERK1/21: Eur.J Biochem., v. 271, no. 11, p. 2060-2066.

Clark, A. W., C. A. Krekoski, S. S. Bou, K. R. Chapman, and D. R. Edwards, 1997, Increased gelatinase A (MMP-2) and gelatinase B (MMP-9) activities in human brain after focal ischemia: Neurosci Lett, v. 238, no. 1-2, p. 53-56.

Cohen-Cory, S., A. H. Kidane, N. J. Shirkey, and S. Marshak, 2010, Brain-derived neurotrophic factor and the development of structural neuronal connectivity: Dev.Neurobiol., v. 70, no. 5, p. 271-288.

Curry, A. et al., 2009, Exercise pre-conditioning reduces brain inflammation in stroke via tumor necrosis factor-alpha, extracellular signal-regulated kinase 1/2 and matrix metalloproteinase-9 activity: Neurological Research.

Dans, M. J., and F. G. Giancotti, 1999,Dans, M. J., and F. G. Giancotti Guidebook to the extracellular matrix, anchor, and adhesion proteins: Oxford, Sambrook & Tooze Publication at Osford University Press.

Daugas, E. et al., 2000, Mitochondrio-nuclear translocation of AIF in apoptosis and necrosis: FASEB J, v. 14, no. 5, p. 729-739.

Davis, W., S. Mahale, A. Carranza, B. Cox, K. Hayes, D. Jimenez, and Y. Ding, 2007, Exercise pre-conditioning ameliorates blood-brain barrier dysfunction in stroke by enhancing basal lamina: Neurological Research, v. 29, no. 4, p. 382-387.

del Zoppo, G. J., and J. M. Hallenbeck, 2000, Advances in the vascular pathophysiology of ischemic stroke: Thromb Res, v. 98, no. 3, p. 73-81.

del Zoppo, G. J., and T. Mabuchi, 2003, Cerebral microvessel responses to focal ischemia: J Cereb Blood Flow Metab, v. 23, no. 8, p. 879-894.

Dietrich, W. D., R. Busto, S. Yoshida, and M. D. Ginsberg, 1987, Histopathological and hemodynamic consequences of complete versus incomplete ischemia in the rat: J Cereb Blood Flow Metab, v. 7, no. 3, p. 300-308.

Ding,Y, Y H Ding, J Li, J A Rafols. Exercise induces integrin overexpression and improves neurovascular integrity in ischemic stroke. Stroke 36[2], 470. 2005. Ref Type: Abstract

Ding, Y., J. Li, J. Clark, F. G. Diaz, and J. A. Rafols, 2003, Synaptic plasticity in thalamic nuclei enhanced by motor skill training in rat with transient middle cerebral artery occlusion: Neurological Research, v. 25, p. 189-194.

Ding, Y., J. Li, X. Luan, Y. H. Ding, Q. Lai, J. A. Rafols, J. W. Phillis, J. Clark, and F. G. Diaz, 2004a, Exercise Pre-conditioning Reduces Brain Damage in Ischemic Rats That May be Associated with Regional Angiogenesis and Cellular Overexpression of Neurotrophin: Neuroscience, v. 124, p. 583-591.

Ding, Y. H., Y. Ding, J. Li, D. A. Bessert, and J. A. Rafols, 2006a, Exercise pre-conditioning strengthens brain microvascular integrity in a rat stroke model: Neurol Res, v. 28, no. 2, p. 184-189.

Ding, Y. H., J. Li, W. X. Yao, J. A. Rafols, J. C. Clark, and Y. Ding, 2006b, Exercise preconditioning upregulates cerebral integrins and enhances cerebrovascular integrity in ischemic rats: Acta Neuropathol.(Berl), v. 112, no. 1, p. 74-84.

Ding, Y. H., X. Luan, J. Li, J. A. Rafols, M. Guthikonda, F. G. Diaz, and Y. Ding, 2004b, Exercise-induced overexpression of angiogenic factors and reduction of ischemia/reperfusion injury in stroke: Current Neurovascular Research, v. 1, no. 5, p. 411-420.

Emerling, B. M., B. Viollet, K. V. Tormos, and N. S. Chandel, 2007, Compound C inhibits hypoxic activation of HIF-1 independent of AMPK: FEBS Lett., v. 581, no. 29, p. 5727-5731.

Evenson, K. R., W. D. Rosamond, J. Cai, J. F. Toole, R. G. Hutchinson, E. Shahar, and A. R. Folsom, 1999, Physical activity and ischemic stroke risk. The atherosclerosis risk in communities study: Stroke, v. 30, no. 7, p. 1333-1339.

Feuerstein, G. Z., and X. Wang, 2001, Inflammation and stroke: benefits without harm?: Arch Neurol, v. 58, no. 4, p. 672-674.

Fiore, R. S., V. E. Bayer, S. L. Pelech, J. Posada, J. A. Cooper, and J. M. Baraban, 1993, p42 mitogen-activated protein kinase in brain: prominent localization in neuronal cell bodies and dendrites: Neuroscience, v. 55, no. 2, p. 463-472.

Gasche, Y., M. Fujimura, F. Morita, J. C. Copin, M. Kawase, J. Massengale, and P. H. Chan, 1999, Early appearance of activated matrix metalloproteinase-9 after focal cerebral ischemia in mice: a possible role in blood-brain barrier dysfunction: J Cereb Blood Flow Metab, v. 19, no. 9, p. 1020-1028.

Giffard, R. G., and M. A. Yenari, 2004, Many mechanisms for hsp70 protection from cerebral ischemia: J Neurosurg Anesthesiol., v. 16, no. 1, p. 53-61.

Gillum, R. F., M. E. Mussolino, and D. D. Ingram, 1996, Physical activity and stroke incidence in women and men. The NHANES I Epidemiologic Follow-up Study: Am J Epidemiol, v. 143, no. 9, p. 860-869.

Ginis, I., U. Schweizer, M. Brenner, J. Liu, N. Azzam, M. Spatz, and J. M. Hallenbeck, 1999, TNF-alpha pretreatment prevents subsequent activation of cultured brain cells with TNF-alpha and hypoxia via ceramide: Am J Physiol, v. 276, no. 5 Pt 1, p. C1171-C1183.

Gleeson, M., B. McFarlin, and M. Flynn, 2006, Exercise and Toll-like receptors: Exerc.Immunol.Rev., v. 12, p. 34-53.

Goel, G., M. Guo, J. Ding, D. Dornbos, III, A. Ali, M. Shenaq, M. Guthikonda, and Y. Ding, 2010, Combined effect of tumor necrosis factor (TNF)-alpha and heat shock protein

(HSP)-70 in reducing apoptotic injury in hypoxia: a cell culture study: Neuroscience Letters, v. 483, no. 3, p. 162-166.

Gortz, B., S. Hayer, B. Tuerck, J. Zwerina, J. S. Smolen, and G. Schett, 2005, Tumour necrosis factor activates the mitogen-activated protein kinases p38alpha and ERK in the synovial membrane in vivo: Arthritis Res Ther., v. 7, no. 5, p. R1140-R1147.

Gu, Z., Q. Jiang, and G. Zhang, 2001, Extracellular signal-regulated kinase 1/2 activation in hippocampus after cerebral ischemia may not interfere with postischemic cell death: Brain Research, v. 901, no. 1-2, p. 79-84.

Guo, M., B. Cox, S. Mahale, W. Davis, A. Carranza, K. Hayes, S. Sprague, D. Jimenez, and Y. Ding, 2008a, Pre-ischemic exercise reduces matrix metalloproteinase-9 expression and ameliorates blood-brain barrier dysfunction in stroke: Neuroscience, v. 151, no. 2, p. 340-351.

Guo, M., V. Lin, W. Davis, T. Huang, A. Carranza, S. Sprague, R. Reyes, D. Jimenez, and Y. Ding, 2008b, Preischemic induction of TNF-alpha by physical exercise reduces blood-brain barrier dysfunction in stroke: J.Cereb.Blood Flow Metab, v. 28, no. 8, p. 1422-1430.

Hamilton, K. L., J. L. Staib, T. Phillips, A. Hess, S. L. Lennon, and S. K. Powers, 2003, Exercise, antioxidants, and HSP72: protection against myocardial ischemia/reperfusion: Free Radic.Biol Med, v. 34, no. 7, p. 800-809.

Hardie, D. G., J. W. Scott, D. A. Pan, and E. R. Hudson, 2003, Management of cellular energy by the AMP-activated protein kinase system: FEBS Lett., v. 546, no. 1, p. 113-120.

Hayes, K., S. Sprague, M. Guo, W. Davis, A. Friedman, A. Kumar, D. F. Jimenez, and Y. Ding, 2008, Forced, not voluntary, exercise effectively induces neuroprotection in stroke: Acta Neuropathologica, v. 115, no. 3, p. 289-296.

Hellstrom, G., C. Fischer, N. G. Wahlgren, and T. Jogestrand, 1996, Carotid artery blood flow and middle cerebral artery blood flow velocity during physical exercise: J Appl Physiol, v. 81, no. 1, p. 413-418.

Hetman, M., and A. Gozdz, 2004, Role of extracellular signal regulated kinases 1 and 2 in neuronal survival: Eur.J.Biochem., v. 271, no. 11, p. 2050-2055.

Hosomi, N., C. R. Ban, T. Naya, T. Takahashi, P. Guo, X. Y. Song, and M. Kohno, 2005, Tumor necrosis factor-alpha neutralization reduced cerebral edema through inhibition of matrix metalloproteinase production after transient focal cerebral ischemia
2: J Cereb.Blood Flow Metab, v. 25, no. 8, p. 959-967.

Hu, G., N. C. Barengo, J. Tuomilehto, T. A. Lakka, A. Nissinen, and P. Jousilahti, 2004, Relationship of physical activity and body mass index to the risk of hypertension: a prospective study in Finland: Hypertension, v. 43, no. 1, p. 25-30.

Hu, G., C. Sarti, P. Jousilahti, K. Silventoinen, N. C. Barengo, and J. Tuomilehto, 2005, Leisure time, occupational, and commuting physical activity and the risk of stroke: Stroke, v. 36, no. 9, p. 1994-1999.

Hynes, R. O., 1992, Integrins: versatility, modulation, and signaling in cell adhesion: Cell, v. 69, no. 1, p. 11-25.

Ickes, B. R., T. M. Pham, L. A. Sanders, D. S. Albeck, A. H. Mohammed, and A. C. Granholm, 2000, Long-term environmental enrichment leads to regional increases in neurotrophin levels in rat brain: Exp Neurol, v. 164, no. 1, p. 45-52.

Ide, K., and N. H. Secher, 2000, Cerebral blood flow and metabolism during exercise: Prog Neurobiol, v. 61, no. 4, p. 397-414.

Igarashi, Y. et al., 1999, Glial cell line-derived neurotrophic factor induces barrier function of endothelial cells forming the blood-brain barrier: Biochem Biophys Res Commun, v. 261, no. 1, p. 108-112.

Isaacs, K. R., B. J. Anderson, A. A. Alcantara, J. E. Black, and W. T. Greenough, 1992, Exercise and the brain: angiogenesis in the adult rat cerebellum after vigorous physical activity and motor skill learning: J Cereb Blood Flow Metab, v. 12, no. 1, p. 110-119.

Iyer, N. V. et al., 1998, Cellular and developmental control of O2 homeostasis by hypoxia-inducible factor 1 alpha: Genes Dev., v. 12, no. 2, p. 149-162.

Janzer, R. C., and M. C. Raff, 1987, Astrocytes induce blood-brain barrier properties in endothelial cells: Nature, v. 325, no. 6101, p. 253-257.

Jobe, L. J., G. C. Melendez, S. P. Levick, Y. Du, G. L. Brower, and J. S. Janicki, 2009, TNF-alpha inhibition attenuates adverse myocardial remodeling in a rat model of volume overload: Am.J.Physiol Heart Circ.Physiol, v. 297, no. 4, p. H1462-H1468.

Jones, N. M., and M. Bergeron, 2004, Hypoxia-induced ischemic tolerance in neonatal rat brain involves enhanced ERK1/2 signaling: Journal of Neurochemistry, v. 89, no. 1, p. 157-167.

Jones, N. M., and M. Bergeron, 2001, Hypoxic preconditioning induces changes in HIF-1 target genes in neonatal rat brain: J.Cereb.Blood Flow Metab, v. 21, no. 9, p. 1105-1114.

Jones, T. A., C. J. Chu, L. A. Grande, and A. D. Gregory, 1999, Motor skills training enhances lesion-induced structural plasticity in the motor cortex of adult rats: J Neurosci, v. 19, no. 22, p. 10153-10163.

Joza, N. et al., 2001, Essential role of the mitochondrial apoptosis-inducing factor in programmed cell death: Nature, v. 410, no. 6828, p. 549-554.

Kahn, B. B., T. Alquier, D. Carling, and D. G. Hardie, 2005, AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism: Cell Metab, v. 1, no. 1, p. 15-25.

Kiang, J. G., and G. C. Tsokos, 1998, Heat shock protein 70 kDa: molecular biology, biochemistry, and physiology: Pharmacol.Ther., v. 80, no. 2, p. 183-201.

Kim, H., Q. Li, B. L. Hempstead, and J. A. Madri, 2004, Paracrine and autocrine functions of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) in brain-derived endothelial cells: J.Biol.Chem., v. 279, no. 32, p. 33538-33546.

Kim, H. L., E. J. Yeo, Y. S. Chun, and J. W. Park, 2006, A domain responsible for HIF-1alpha degradation by YC-1, a novel anticancer agent: Int.J.Oncol., v. 29, no. 1, p. 255-260.

Kinni, H., M. Guo, J. Y. Ding, S. Konakondla, D. Dornbos, III, R. Tran, M. Guthikonda, and Y. Ding, 2011, Cerebral metabolism after forced or voluntary physical exercise: Brain Research, v. 1388, p. 48-55.

Kirino, T., Y. Tsujita, and A. Tamura, 1991, Induced tolerance to ischemia in gerbil hippocampal neurons: J Cereb.Blood Flow Metab, v. 11, no. 2, p. 299-307.

Kleim, J. A., N. R. Cooper, and P. M. VandenBerg, 2002, Exercise induces angiogenesis but does not alter movement representations within rat motor cortex: Brain Res, v. 934, no. 1, p. 1-6.

Kloner, R. A., 2001, Preinfarct angina and exercise: yet another reason to stay physically active: J.Am.Coll.Cardiol., v. 38, no. 5, p. 1366-1368.

Kondo, T., H. Kinouchi, M. Kawase, and T. Yoshimoto, 1996, Astroglial cells inhibit the increasing permeability of brain endothelial cell monolayer following hypoxia/reoxygenation: Neurosci Lett, v. 208, no. 2, p. 101-104.

Kuipers, S. D., and C. R. Bramham, 2006, Brain-derived neurotrophic factor mechanisms and function in adult synaptic plasticity: new insights and implications for therapy: Curr.Opin.Drug Discov.Devel., v. 9, no. 5, p. 580-586.

Larson, E. B., L. Wang, J. D. Bowen, W. C. McCormick, L. Teri, P. Crane, and W. Kukull, 2006, Exercise is associated with reduced risk for incident dementia among persons 65 years of age and older: Ann.Intern.Med., v. 144, no. 2, p. 73-81.

Lazou, A., E. K. Iliodromitis, D. Cieslak, K. Voskarides, S. Mousikos, E. Bofilis, and D. T. Kremastinos, 2006, Ischemic but not mechanical preconditioning attenuates ischemia/reperfusion induced myocardial apoptosis in anaesthetized rabbits: the role of Bcl-2 family proteins and ERK1/2: Apoptosis., v. 11, no. 12, p. 2195-2204.

Leasure, J. L., and M. Jones, 2008, Forced and voluntary exercise differentially affect brain and behavior: Neuroscience, v. 156, no. 3, p. 456-465.

Lecour, S., N. Suleman, G. A. Deuchar, S. Somers, L. Lacerda, B. Huisamen, and L. H. Opie, 2005, Pharmacological preconditioning with tumor necrosis factor-alpha activates signal transducer and activator of transcription-3 at reperfusion without involving classic prosurvival kinases (Akt and extracellular signal-regulated kinase): Circulation, v. 112, no. 25, p. 3911-3918.

Lee, J. E., M. A. Yenari, G. H. Sun, L. Xu, M. R. Emond, D. Cheng, G. K. Steinberg, and R. G. Giffard, 2001, Differential neuroprotection from human heat shock protein 70 overexpression in in vitro and in vivo models of ischemia and ischemia-like conditions: Exp.Neurol, v. 170, no. 1, p. 129-139.

Lee, J. S., J. J. Lee, and J. S. Seo, 2005, HSP70 deficiency results in activation of c-Jun N-terminal Kinase, extracellular signal-regulated kinase, and caspase-3 in hyperosmolarity-induced apoptosis: J Biol Chem., v. 280, no. 8, p. 6634-6641.

Lee, M., J. T. Hwang, H. J. Lee, S. N. Jung, I. Kang, S. G. Chi, S. S. Kim, and J. Ha, 2003, AMP-activated protein kinase activity is critical for hypoxia-inducible factor-1 transcriptional activity and its target gene expression under hypoxic conditions in DU145 cells: J.Biol.Chem., v. 278, no. 41, p. 39653-39661.

Lennon, S. L., J. Quindry, K. L. Hamilton, J. French, J. Staib, J. L. Mehta, and S. K. Powers, 2004, Loss of exercise-induced cardioprotection after cessation of exercise: J Appl.Physiol, v. 96, no. 4, p. 1299-1305.

Li, J., Y. H. Ding, J. A. Rafols, Q. Lai, J. P. McAllister, II, and Y. Ding, 2005, Increased astrocyte proliferation in rats after running exercise: Neuroscience Letters, v. 386, p. 160-164.

Liebelt, B. et al., 2010, Exercise preconditioning reduces neuronal apoptosis in stroke by up-regulating heat shock protein-70 (heat shock protein-72) and extracellular-signal-regulated-kinase 1/2: Neuroscience, v. 166, no. 4, p. 1091-1100.

Liu, J., I. Ginis, M. Spatz, and J. M. Hallenbeck, 2000, Hypoxic preconditioning protects cultured neurons against hypoxic stress via TNF-alpha and ceramide: Am J Physiol Cell Physiol, v. 278, no. 1, p. C144-C153.

Lloyd, P. G., B. M. Prior, H. Li, H. T. Yang, and R. L. Terjung, 2005, VEGF receptor antagonism blocks arteriogenesis, but only partially inhibits angiogenesis, in skeletal muscle of exercise-trained rats: Am.J.Physiol Heart Circ.Physiol, v. 288, no. 2, p. H759-H768.

Lloyd, P. G., B. M. Prior, H. T. Yang, and R. L. Terjung, 2003, Angiogenic growth factor expression in rat skeletal muscle in response to exercise training: Am J Physiol Heart Circ.Physiol, v. 284, no. 5, p. H1668-H1678.

Lloyd-Jones, D. et al., 2010, Heart disease and stroke statistics--2010 update: a report from the American Heart Association: Circulation, v. 121, no. 7, p. e46-e215.

Lo, E. H., T. Dalkara, and M. A. Moskowitz, 2003, Mechanisms, challenges and opportunities in stroke: Nat Rev Neurosci, v. 4, no. 5, p. 399-415.

Lo, E. H., M. A. Moskowitz, and T. P. Jacobs, 2005, Exciting, radical, suicidal: how brain cells die after stroke: Stroke, v. 36, no. 2, p. 189-192.

Masada, T., Y. Hua, G. Xi, S. R. Ennis, and R. F. Keep, 2001, Attenuation of ischemic brain edema and cerebrovascular injury after ischemic preconditioning in the rat: J Cereb Blood Flow Metab, v. 21, no. 1, p. 22-33.

Matsumori, Y. et al., 2005, Hsp70 overexpression sequesters AIF and reduces neonatal hypoxic/ischemic brain injury: J Cereb.Blood Flow Metab, v. 25, no. 7, p. 899-910.

Maurer, M. H., H. K. Geomor, H. F. Burgers, D. W. Schelshorn, and W. Kuschinsky, 2006, Adult neural stem cells express glucose transporters GLUT1 and GLUT3 and regulate GLUT3 expression: FEBS Lett., v. 580, no. 18, p. 4430-4434.

Mayer, B., and R. Oberbauer, 2003, Mitochondrial regulation of apoptosis: News Physiol Sci., v. 18, p. 89-94.

McCloskey, D. P., D. S. Adamo, and B. J. Anderson, 2001, Exercise increases metabolic capacity in the motor cortex and striatum, but not in the hippocampus: Brain Res, v. 891, no. 1-2, p. 168-175.

McFarlin, B. K., M. G. Flynn, W. W. Campbell, B. A. Craig, J. P. Robinson, L. K. Stewart, K. L. Timmerman, and P. M. Coen, 2006, Physical activity status, but not age, influences inflammatory biomarkers and toll-like receptor 4: J.Gerontol.A Biol.Sci.Med.Sci., v. 61, no. 4, p. 388-393.

McGee, S. L., and M. Hargreaves, 2006, Exercise and skeletal muscle glucose transporter 4 expression: molecular mechanisms: Clin.Exp.Pharmacol.Physiol, v. 33, no. 4, p. 395-399.

Minchenko, O., I. Opentanova, and J. Caro, 2003, Hypoxic regulation of the 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase gene family (PFKFB-1-4) expression in vivo: FEBS Lett., v. 554, no. 3, p. 264-270.

Mori, E., G. J. del Zoppo, J. D. Chambers, B. R. Copeland, and K. E. Arfors, 1992, Inhibition of polymorphonuclear leukocyte adherence suppresses no-reflow after focal cerebral ischemia in baboons: Stroke, v. 23, no. 5, p. 712-718.

Nawashiro, H., D. Martin, and J. M. Hallenbeck, 1997, Inhibition of tumor necrosis factor and amelioration of brain infarction in mice: J Cereb Blood Flow Metab, v. 17, no. 2, p. 229-232.

Neeper, S. A., P. Gomez, J. Choi, and C. W. Cotman, 1996, Physical activity increases mRNA for brain-derived neurotrophic factor and nerve growth factor in rat brain: Brain Res, v. 726, no. 1-2, p. 49-56.

Neurath, K. M., M. P. Keough, T. Mikkelsen, and K. P. Claffey, 2006, AMP-dependent protein kinase alpha 2 isoform promotes hypoxia-induced VEGF expression in human glioblastoma: Glia, v. 53, no. 7, p. 733-743.

Nishigaya, K., Y. Yoshida, M. Sasuga, H. Nukui, and G. Ooneda, 1991, Effect of recirculation on exacerbation of ischemic vascular lesions in rat brain: Stroke, v. 22, no. 5, p. 635-642.

Noble, E. G., A. Moraska, R. S. Mazzeo, D. A. Roth, M. C. Olsson, R. L. Moore, and M. Fleshner, 1999, Differential expression of stress proteins in rat myocardium after free wheel or treadmill run training: J.Appl.Physiol, v. 86, no. 5, p. 1696-1701.

Ogoh, S., and P. N. Ainslie, 2009, Cerebral blood flow during exercise: mechanisms of regulation: J.Appl.Physiol, v. 107, no. 5, p. 1370-1380.

Ogunshola, O. O., W. B. Stewart, V. Mihalcik, T. Solli, J. A. Madri, and L. R. Ment, 2000, Neuronal VEGF expression correlates with angiogenesis in postnatal developing rat brain: Brain Res Dev Brain Res, v. 119, no. 1, p. 139-153.

Ohtsuka, K., and T. Suzuki, 2000, Roles of molecular chaperones in the nervous system: Brain Res Bull., v. 53, no. 2, p. 141-146.

Ostrakhovitch, E. A., and M. G. Cherian, 2005, Inhibition of extracellular signal regulated kinase (ERK) leads to apoptosis inducing factor (AIF) mediated apoptosis in epithelial breast cancer cells: the lack of effect of ERK in p53 mediated copper induced apoptosis: J Cell Biochem., v. 95, no. 6, p. 1120-1134.

Park, J. A., K. S. Choi, S. Y. Kim, and K. W. Kim, 2003, Coordinated interaction of the vascular and nervous systems: from molecule- to cell-based approaches: Biochem Biophys Res Commun, v. 311, no. 2, p. 247-253.

Petty, M. A., and E. H. Lo, 2002, Junctional complexes of the blood-brain barrier: permeability changes in neuroinflammation: Prog Neurobiol, v. 68, no. 5, p. 311-323.

Petty, M. A., and J. G. Wettstein, 2001, Elements of cerebral microvascular ischaemia: Brain Res Brain Res Rev, v. 36, no. 1, p. 23-34.

Powers, S. K., S. L. Lennon, J. Quindry, and J. L. Mehta, 2002, Exercise and cardioprotection: Curr.Opin.Cardiol., v. 17, no. 5, p. 495-502.

Reyes, R., Jr., Y. Wu, Q. Lai, M. Mrizek, J. Berger, D. F. Jimenez, C. M. Barone, and Y. Ding, 2006, Early inflammatory response in rat brain after peripheral thermal injury: Neuroscience Letters, v. 407, no. 1, p. 11-15.

Romanic, A. M., R. F. White, A. J. Arleth, E. H. Ohlstein, and F. C. Barone, 1998, Matrix metalloproteinase expression increases after cerebral focal ischemia in rats: inhibition of matrix metalloproteinase-9 reduces infarct size: Stroke, v. 29, no. 5, p. 1020-1030.

Rybnikova, E., N. Sitnik, T. Gluschenko, E. Tjulkova, and M. O. Samoilov, 2006, The preconditioning modified neuronal expression of apoptosis-related proteins of Bcl-2 superfamily following severe hypobaric hypoxia in rats: Brain Res, v. 1089, no. 1, p. 195-202.

Sairanen, T. R., P. J. Lindsberg, M. Brenner, O. Carpen, and A. Siren, 2001, Differential cellular expression of tumor necrosis factor-alpha and Type I tumor necrosis factor receptor after transient global forebrain ischemia: J Neurol Sci, v. 186, no. 1-2, p. 87-99.

Sawatzky, D. A., D. A. Willoughby, P. R. Colville-Nash, and A. G. Rossi, 2006, The involvement of the apoptosis-modulating proteins ERK 1/2, Bcl-xL and Bax in the resolution of acute inflammation in vivo: Am J Pathol., v. 168, no. 1, p. 33-41.

Schabitz, W. R., T. Steigleder, C. M. Cooper-Kuhn, S. Schwab, C. Sommer, A. Schneider, and H. G. Kuhn, 2007, Intravenous brain-derived neurotrophic factor enhances poststroke sensorimotor recovery and stimulates neurogenesis: Stroke, v. 38, no. 7, p. 2165-2172.

Schlesinger, M. J., 1990, Heat shock proteins: J Biol Chem., v. 265, no. 21, p. 12111-12114.

Schubert, D., 2005, Glucose metabolism and Alzheimer's disease: Ageing Res.Rev., v. 4, no. 2, p. 240-257.

Semenza, G. L., 2009, Regulation of oxygen homeostasis by hypoxia-inducible factor 1: Physiology.(Bethesda.), v. 24, p. 97-106.

Shackelford, D. A., and R. Y. Yeh, 2006, Modulation of ERK and JNK activity by transient forebrain ischemia in rats: J Neurosci.Res, v. 83, no. 3, p. 476-488.

Shamloo, M., and T. Wieloch, 1999, Changes in protein tyrosine phosphorylation in the rat brain after cerebral ischemia in a model of ischemic tolerance: J.Cereb.Blood Flow Metab, v. 19, no. 2, p. 173-183.

Sharony, R., G. Pintucci, P. C. Saunders, E. A. Grossi, F. G. Baumann, A. C. Galloway, and P. Mignatti, 2005, Matrix Metalloproteinase Expression in Vein Grafts: Role of Inflammatory Mediators and Extracellular Signal-Regulated Kinases-1 and -2 2: Am.J Physiol Heart Circ.Physiol.

Siu, P. M., R. W. Bryner, J. K. Martyn, and S. E. Alway, 2004, Apoptotic adaptations from exercise training in skeletal and cardiac muscles: FASEB J., v. 18, no. 10, p. 1150-1152.

Stummer, W., K. Weber, B. Tranmer, A. Baethmann, and O. Kempski, 1994, Reduced mortality and brain damage after locomotor activity in gerbil forebrain ischemia: Stroke, v. 25, no. 9, p. 1862-1869.

Susin, S. A. et al., 1999, Molecular characterization of mitochondrial apoptosis-inducing factor: Nature, v. 397, no. 6718, p. 441-446.

Swain, R. A. et al., 2003, Prolonged exercise induces angiogenesis and increases cerebral blood volume in primary motor cortex of the rat: Neuroscience, v. 117, no. 4, p. 1037-1046.

Tagaya, M. et al., 2001, Rapid loss of microvascular integrin expression during focal brain ischemia reflects neuron injury: J Cereb Blood Flow Metab, v. 21, no. 7, p. 835-846.

Tawil, N. J., P. Wilson, and S. Carbonetto, 1994, Expression and distribution of functional integrins in rat CNS glia: J.Neurosci.Res., v. 39, no. 4, p. 436-447.

Tong, L., D. Smyth, C. Kerr, J. Catterall, and C. D. Richards, 2004, Mitogen-activated protein kinases Erk1/2 and p38 are required for maximal regulation of TIMP-1 by oncostatin M in murine fibroblasts: Cell Signal., v. 16, no. 10, p. 1123-1132.

Vissing, J., M. Andersen, and N. H. Diemer, 1996, Exercise-induced changes in local cerebral glucose utilization in the rat: J Cereb Blood Flow Metab, v. 16, no. 4, p. 729-736.

Wagner, S., M. Tagaya, J. A. Koziol, V. Quaranta, and G. J. del Zoppo, 1997, Rapid disruption of an astrocyte interaction with the extracellular matrix mediated by integrin alpha 6 beta 4 during focal cerebral ischemia/reperfusion: Stroke, v. 28, no. 4, p. 858-865.

Wang, R. Y., Y. R. Yang, and S. M. Yu, 2001, Protective effects of treadmill training on infarction in rats: Brain Res, v. 922, no. 1, p. 140-143.

Wang, X., X. Li, J. A. Erhardt, F. C. Barone, and G. Z. Feuerstein, 2000, Detection of tumor necrosis factor-alpha mRNA induction in ischemic brain tolerance by means of real-time polymerase chain reaction: J Cereb Blood Flow Metab, v. 20, no. 1, p. 15-20.

Williamson, J. W., A. C. Nobrega, R. McColl, D. Mathews, P. Winchester, L. Friberg, and J. H. Mitchell, 1997, Activation of the insular cortex during dynamic exercise in humans: J Physiol, v. 503 (Pt 2), p. 277-283.

Willis, C. L., C. C. Nolan, S. N. Reith, T. Lister, M. J. Prior, C. J. Guerin, G. Mavroudis, and D. E. Ray, 2004, Focal astrocyte loss is followed by microvascular damage, with subsequent repair of the blood-brain barrier in the apparent absence of direct astrocytic contact: Glia, v. 45, no. 4, p. 325-337.

Wu, C., H. Fujihara, J. Yao, S. Qi, H. Li, K. Shimoji, and H. Baba, 2003, Different expression patterns of Bcl-2, Bcl-xl, and Bax proteins after sublethal forebrain ischemia in C57Black/Crj6 mouse striatum: Stroke, v. 34, no. 7, p. 1803-1808.

Yamashita, N., S. Hoshida, K. Otsu, N. Taniguchi, T. Kuzuya, and M. Hori, 1999, Monophosphoryl lipid A provides biphasic cardioprotection against ischaemia-reperfusion injury in rat hearts: Br J Pharmacol, v. 128, no. 2, p. 412-418.

Yang, G. Y., and A. L. Betz, 1994, Reperfusion-induced injury to the blood-brain barrier after middle cerebral artery occlusion in rats: Stroke, v. 25, no. 8, p. 1658-1664.

Yong, V. W., C. Power, P. Forsyth, and D. R. Edwards, 2001, Metalloproteinases in biology and pathology of the nervous system: Nat Rev Neurosci, v. 2, no. 7, p. 502-511.

Zhu, C., L. Qiu, X. Wang, U. Hallin, C. Cande, G. Kroemer, H. Hagberg, and K. Blomgren, 2003, Involvement of apoptosis-inducing factor in neuronal death after hypoxia-ischemia in the neonatal rat brain: J Neurochem., v. 86, no. 2, p. 306-317.

Zhuang, S., and R. G. Schnellmann, 2006, A death-promoting role for extracellular signal-regulated kinase: J Pharmacol.Exp.Ther., v. 319, no. 3, p. 991-997.

Zorzano, A., M. Palacin, and A. Guma, 2005, Mechanisms regulating GLUT4 glucose transporter expression and glucose transport in skeletal muscle: Acta Physiol Scand., v. 183, no. 1, p. 43-58.

Zwagerman, N., C. Plumlee, M. Guthikonda, and Y. Ding, 2010a, Toll-like receptor-4 and cytokine cascade in stroke after exercise: Neurological Research, v. 32, no. 2, p. 123-126.

Zwagerman, N., S. Sprague, M. D. Davis, B. Daniels, G. Goel, and Y. Ding, 2010b, Pre-ischemic exercise preserves cerebral blood flow during reperfusion in stroke: Neurological Research.

16

Physiological Neuroprotective Mechanisms in Natural Genetic Systems: Therapeutic Clues for Hypoxia-Induced Brain Injuries

Thomas I Nathaniel[1,2], Francis Umesiri[3], Grace Reifler[4], Katelin Haley[4], Leah Dziopa[4], Julia Glukhoy[4] and Rahul Dani[4]
[1]*Center for Natural and Health Sciences, Marywood University*
[2]*Department of Biomedical Sciences, University of South Carolina School of Medicine-Greenville, Greenville*
[3]*Department of Chemistry, University of Toledo, Toledo*
[4]*Center for Natural and Health Sciences, Marywood University*
USA

1. Introduction

Adaptations, conservation or evolution of physiological systems in human indicates that the physiological system for hypoxia tolerance represents an adaptive character that is present in humans, just like in poikilotherms or endotherms(Hochachka 1993; Hochachka et al. 1996). Precisely, brain metabolic organization capability is thought to represent an adaptive physiological system that is present in humans, but composed of so many neurochemical products and physiological mechanisms that make it difficult to account for its plasticity for manipulation to induce protection(Hochachka 1993; Frappell et al. 2002). Evidence that hypoxia tolerating mammals suppressed metabolic demand in response to hypoxia contributed substantially to the understanding of metabolic regulation as a neuroprotective strategy that can be adapted for the protection of tissue hypoxia in humans. In that case, understanding the physiological mechanisms that are involved in such ability in hypoxia tolerating species may provide a clue for specific mechanisms that could be manipulated in the brain of stroke patients to prevent the death of metabolically vulnerable neurons. In this context, knowledge of the specific role of the central neurotransmitter systems and pathways that control metabolic suppression during hypoxia tolerance is important. This is because the neurochemical-mediators that may defend against hypoxic insults during brain injury act through interconnections within the neural systems that are highly protected in hypoxia tolerating species(Mravec et al. 2006). Exposure of non hypoxia tolerating species to severe hypoxia results in the alteration of the connections within the neural systems because of loss control of local microcirculation of oxygen. This leads to ineffectiveness of membrane potentials to modulate neuronal function (Ackland et al. 2007). If this occurs, there is some hope for effective neuropharmacological intervention through neurotransmitter-mediators. The fact that chemical neurotransmitters are part of normal physiology, whether during development or adulthood, means that specific agonists and

antagonists that restore physiological homeostasis can lead to pharmacological repair of hypoxia-induced neuronal damage in humans.

2. Physiological strategies that protect the brain during ischemic stroke

Hypothermia is one strategy that can decrease hypoxic/ischemic injury under regulated conditions (Rincon 2008; Statler 2008). This is possible because a decline in brain temperature can prevent the dead of neurons that are deprived of nutrients. Such a protective strategy has been attributed to the reversible protein phosphorylation that regulates suppression of the rates of multiple ATP-production, ATP-utilization and related cellular processes that allow animals to go through a stable hypometabolic state (Storey and Storey 2007; Storey and Storey 2005). A stable hypometabolic state mechanism is known to protect against pathological effects of cortical neuronal pathology or oxidative stress in stroke patients (Ma et al. 2005). Cumulative evidence from studies of hypometabolism during hibernation in small mammals suggests that hypometabolism is a stable metabolic state that allows timely limited energy regulation during hypoxia (Heldmaier et al. 2004). This is because a stable metabolic state activates defense mechanisms, such as antioxidants, proteins, protease inhibitors that stabilize macromolecules and promote long-term neuronal viability in the stable metabolic state (Zhou et al. 2001; Zhao and Zuo 2005). These adaptations can be manipulated in humans to prevent the death of metabolically vulnerable neurons in stroke patients. Although these adaptations do not fully account for the mechanisms that facilitate the suppression of metabolism during exposure to hypoxia (Nathaniel et al. 2009) or during mammalian hibernation (Drew et al. 2007), it has been proposed that hypoxia itself could facilitate a systematic regulation of metabolism through cooling of core body temperature and stabilization of temperature in the new metabolic rate (Barros et al. 2001). Future studies that explore the mechanisms that switch-off energy demand, when supplies become limited during metabolic suppression could provide clues of how to develop metabolic suppression as novel therapy in the clinical management of stroke.

3. Cellular implications of disruption of brain energy balance when oxygen delivery fails to meet demand

The mammalian brain is a highly oxidative organ that accounts for an inexplicably large percentage of the whole body oxygen consumption that provides the major source of energy during aerobic metabolism (Wang et al. 2002). This implies that structural and functional integrity of brain functions strongly depend on a regular oxygen and glucose supply. Therefore, any disruption of the homeostasis of brain energy demand and supply becomes life threatening. This is because a reduction in oxygen availability due to the abnormality in systemic or local blood circulation cannot be endured for a prolonged period by a non hypoxia tolerant species (Zhou et al. 2001a; Barger et al. 2003). The immediate effect is variation in oxygen partial pressure (PO_2), within the brain that is detected by cellular oxygen sensors. The cellular oxygen sensors, such as hypoxia inducible transcription factors (HIFs), are thought to be available to regulate oxygen homeostasis in the brain. In order for the brain to cope with variations in oxygen partial pressure, these sensors induce adaptive mechanisms to avoid, or at least minimize, brain damage (Nawashiro et al. 1996). Any abnormality in cellular oxygen-sensor response is a reflection of the low partial pressure and

concentrations of oxygen in the brain. The abnormality is primarily because of the inability of a specific oxygen-sensor or target system to tailor adaptive responses according to differences in the cellular oxygen availability in a short or long term. In this context, the arising question is how does low cellular oxygen availability affect neuronal and physiological activities? Major cellular operations in the brain are dependent on the oxygen level in the physiological range (Erecinska et al. 2005). Limitation of oxygen supply to the brain below a critical physiological level blocks oxidative phosphorylation, which drastically decreased cellular ATP leading to a collapse in ion gradients. Consequently, neuronal activity stops. If the process of re-oxygenation is not re-introduced quickly, neurons will die(Nawashiro et al. 1996). In the brain of a stroke patient, early interruption of energy homeostasis when oxygen delivery fails to meet oxygen demand is the first step in a surge of events that leads to cell death of metabolically susceptible neurons. If hypoxia does not set in, availability of glucose will help maintain ATP levels through glycolysis. However, if glucose supply is limited, reduction of high-energy phosphates (e.g. ATP) results in loss of cellular ionic homeostasis (Erecińska and Silver 2001). This is because the disruption of ion homeostasis causes an influx of Na^+ and Cl^- ions, the release of neurotransmitters, opening of voltage-gated Ca^{2+} channels, and anoxic depolarization (Erecinska et al. 2005). This finding suggests that anoxic depolarization is directly associated with hypoxia-induced neuronal loss that leads to irreversible brain damage. An interesting question relevant to this idea is how does anoxic depolarization trigger neuronal death during hypoxia? This question has been investigated in the context of the mechanism of anoxic depolarization. For instance, concurrent changes in $(K^+)_0$t that lead to failure of (Na^+ ,K^+) ATPase occur during hypoxia due to ATP depletion(Balestrino et al. 1989). In support of this idea, electrophysiological evidence reveals that failure of ATPase causes anoxic depolarization through some intermediate event, such as Na^+-induced cell swelling(Rufini et al. 2009). To clarify the direct relationship between anoxic depolarization and ATP levels, synaptic transmission analysis in rat hippocampus revealed that first, Na^+ influx plays a relatively larger role in ATP consumption during hypoxia than Ca^{2+} influx; second, anoxic depolarization imposes a large and rapid drop in ATP levels(Fowler et al. 1999). Taken together, it then implies that depolarization and increased sodium concentration during hypoxia seem to account for a significant portion of the neuronal damage induced by hypoxia that eventually leads to both necrotic and apoptotic processes (Reshef et al. 2000; Gonchar and Mankovskaya 2009).

4. Stimulation of glutamate, activation of NMDA and AMPA receptors regulate calcium influx and promote neuronal damage

Although increased sodium concentration during hypoxia seems to account for a major part of neuronal damage during hypoxia, additional structural damages develop hours or days later because Ca^{2+} influx into neurons is stimulated by glutamate (He et al. 2009), and amplified by activation of NMDA and AMPA receptors (Zamalloa et al. 2009). Glutamate is one of the most widespread excitatory neurotransmitters in the brain. In small amounts, it is indispensable for neuronal function, while in excessive amounts it is a neuronal poison (excitotoxin) that stimulates Ca^{2+} influx into neurons (Liljequist et al. 1995; Bonde et al. 2005). If the excessive extracellular release of glutamate is not regulated, it could lead to brain damage(Robinson 2006). This idea is strengthened by the observation that overstimulation of glutamate receptors promotes neuronal death(Sheldon and Robinson

2007a). Since excessive glutamate is harmful to the brain, how exactly can excess amounts of glutamate be regulated in the brain cells? It is possible that such regulation could be achieved by controlling a specific effector system that modulates the activities of glutamate. In support of this idea, studies by Pellegrini-Giampietro et al.(1999), Sheldon and Robinson (2007a) suggest that the mechanism that regulates glutamate transporter activity within minutes is a major redistributor of proteins in the plasma. This idea is further strengthened by the finding that glutamate transporters are regulated by the mechanism that redistributes proteins to or from the plasma membrane(Savolainen et al. 1995; Noda et al. 2000; Sheldon and Robinson 2007b). Findings from these studies indicate that longer-term activation of protein kinase C (PKC) decreased the activity and cell surface expression of the predominant forebrain glutamate Glu-1 transporter that is being redistributed from the plasma membrane to an intracellular compartment. Taken together, the existing studies reveal that the cellular machinery required for redistribution is via lysosomal degradation and not proteosomal degradation. Since longer-term activation of PKC results in degradation of GLT-1 that can be blocked by inhibitors of lysosomal but not proteosomal degradation, we speculate that regulation of total GLT-1 levels during hypoxia could play a significant role in hypoxia neuroprotection. Since the stimulative effect of glutamate (Noda et al. 2000) and activation of NMDA and AMPA receptors (Sobczyk et al. 2005) led to calcium overload (Mulvey and Renshaw 2009), our interest in this review to determine whether the different roles of glutamate, NMDA and AMPA receptors are the sole means of extracellular accumulation of calcium following hypoxic insults. By analyzing the influence of a specific ion channel inhibitor on the rise of cytosolic free calcium (Ca^{2+}) during hypoxia in a rat's cerebrocortical brain slices, Bickler (1998) found that the cytosolic calcium changes during hypoxia are due to multiple mechanisms. The changes are incompletely inhibited by a combined ion channel blockade. They are associated with the disruption of the cell membrane integrity. There is evidence that other factors may be at work as well. One factor is oxidative stress that is associated with the activation of microglia. Activation of microglia in turn, releases inflammatory cytokines, nitric oxide and extracellular accumulation of calcium, resulting in stress and neuronal death. Another factor is an increase in reactive oxygen species production. This alters cellular components including calcium levels and contributes to cytotoxic events that lead to cell death.

5. Cellular responses in brain neural systems can help in understanding hypoxia-induced hypometabolism and neuroprotection

In the previous section, we described how the stimulation of glutamate, activation of NMDA and AMPA receptors can regulate calcium influx and promote neuronal damage. It is now important to discuss how cellular responses in brain neural systems can help in understanding hypoxia-induced hypometabolism to initiate neuroprotection. Obviously, this proposal needs an explanation on how the cellular responses in any brain neural network would be a major factor for understanding hypoxia-induced hypometabolism and functional integrity. Indeed, a correlative neurophysiological observation tells us that understanding hypometabolism during neuroprotection should be in the context of functional integrity, because hypoxic response of each neuron can be unexpectedly heterogeneous, even within the same brain region(Boutilier 2001). The heterogeneity might be due to the fact that hypoxia itself leads to considerable downregulation of ion channels inside the brain neuronal systems, such that hypoxic reaction of individual neurons vary

(Pena and Ramirez 2005). The variation, in turn, may help in maintaining functional integrity. The idea is supported by the observation that ion channel arrest does not lead to the shutdown of entire networks, nor can networks operate by all cellular components entering a general hypometabolic-neuroprotective state(Ramirez et al. 2007). For instance, a general shutdown of NMDA or Ca^{2+} dependent processes would not be beneficiary to a hypoxia tolerating animal in a hypometabolic state. This is because such a shutdown would severely compromise higher-brain functions. Therefore, hypoxia research should focus on understanding mechanisms of hypometabolism during neuroprotection in the context of functional integrity, and as expressed by hypoxia tolerating species. This disconnect may explain why the protection against tissue hypoxia during stroke is still a main medical problem. There is no doubt that activation of NMDA receptors or intracellular Ca^{2+} may lead to necrosis and apoptosis. However, restraining these mechanisms is not very helpful because they also affect neuroprotection. Taken together, understanding how hypoxia-tolerant neurons sense changes in oxygen dynamics and create signals that have instant and long-term effects on neuronal survival will be significant in developing new strategies for neuroprotection. Studies of how metabolism is regulated at cellular levels during which energy-producing and energy-consuming processes are balanced, facilitated by neurotransmitters-mediated mechanism would be of considerable interest. Since many of the existing studies support the idea that adenosine induces neuroprotection during hypoxia, we will now focus on the different physiological and molecular mechanisms used by adenosine in inducing protection in natural systems of hypoxia tolerance.

6. Adenosine induces neuroprotection by mediating ion signaling during response to chronic hypoxia

Several lines of evidence indicate that reduced ion leakage is an important mechanism for energy conservation during extreme hypoxia, and adenosine has been implicated in ion channel activity. Precisely, it has been shown that A1 receptor stimulation inhibits the brain's electrical activity through K^+ channel activation (direct coupling *via* G-proteins to ion channels) and/or by inhibiting the high-voltage-activated Ca^{2+} channels (Fowler et al. 1999; Reshef et al. 2000). Extracellular adenosine plays a role in the reduction of K^+ influx (channel arrest) that occurs in a brain that was subjected to chronic hypoxia(Reshef et al. 2000). Monitoring of the changes in extracellular K^+ concentration (K+)o in the in-situ brain of the turtle (*Trachemys scripta*), following inhibition of $Na^+/K(^+)$-ATPase with ouabain revealed that the time to reach full depolarization (K^+)o plateau) was 3 times more in the chronic hypoxic brain than in normoxic controls (Pék and Lutz 1997). The initial rate of K^+ leakage was reduced by approximately 70%. Following superfusing the brain, before the onset of chronic hypoxia with the non selective adenosine receptor blocker, theophylline, or the specific adenosine A1 receptor blocker, 8-cyclopentyltheophylline, there was a significant reduction in the time to full depolarization in the ouabain-challenged hypoxic-brain and an increase in the rate of K^+ efflux(Pék and Lutz 1997). The results indicate that A1 receptors are involved in the expression of chronic hypoxia-induced ion channel arrest in the brain. This finding is supported by other studies that indicated that in a chronic hypoxic tolerant specie, the basic strategy for hypoxia survival is the maintenance of ion gradients to avoid anoxic depolarization (Lutz et al. 1996; Perez-Pinzon and Born 1999). To this extent, an important question that re-occurred in many of the most recent theoretical papers is how does hypoxia tolerant species respond to anoxia? In our physiological perspective of

hypoxia neuroprotection, adaption to anoxia (an extreme form of hypoxia) is mainly controlled by hypoxia/ischemic conditioning mechanism(Liu et al. 1991). In addition, the different physiological adaptations for extreme hypoxia have been well described in turtles, one of the species with a unique ability to tolerate extreme hypoxia. In humans, an extreme hypoxia condition causes major histopathological events to the brain. However, in turtles that have the ability to resist extreme form of hypoxia, tolerance is seen when a sublethal ischemic/anoxia insult is induced sometime before a lethal ischemic/anoxia insult is induced(Liu et al. 1991). The mechanisms involved are not well understood. Therefore, a better understanding of the mechanisms that induce extreme tolerance to hypoxia in turtles may provide novel therapeutic interventions that may aide human brain to resist the ravages of extreme hypoxia.

6.1 Adenosine facilitates hypoxia neuroprotection by mediating intracellular signaling pathways

Several lines of evidence indicate that severe hypoxia can cause an imbalance between oxygen supply and consumption. This is usually accompanied by transportation of adenosine down its concentration gradient from the cell via nucleoside transporters, such as ENT1 and the ENT2. These transporters are equilibrative nucleoside transporters that are expressed in the CNS (Chaudary et al. 2004b; Chaudary et al. 2004a), to promote the extracellular accumulation of adenosine. In this context, adenosine seems to act as an autocrine signal that indicates the disproportion between cellular oxygen availability and oxygen usage. Since adenosine is also an essential component of ATP synthesis, adenosine pools need to be vigilantly sustained in metabolically active cells in the brain. This could be done by minimizing extracellular release of adenosine, first by down-regulation of ENT activity. This would aid the restoration of ATP pools once the hypoxic stress has been removed. It is also important to point out that an ENT activity could lead to reuptake of extracellular adenosine to terminate receptor activation, as uniquely done by the neurotransmitter transporters to help restore intracellular pools of adenosine. The precise nature of the relationship between adenosine transporters, adenosine receptors and intracellular signaling pathways is not well known. Hypoxia is known to regulate the adenosine transporter mENT1 (inhibitor-sensitive), and protein kinase c q (PKCq) is involved in regulation of mENT1(Chaudary et al. 2004a), suggesting that chronic hypoxia regulates ENT1, both in terms of protein and overall reactivity through PKC-mediated hypoxia preconditioning.

The PKC-mediated hypoxia protection occurs when extracellular adenosine interacts with adenosine receptors (A_1, A_{2A}, A_{2B}, and/or A_3), and activation of adenosine receptors stimulate signaling pathways that "resist" the stress caused by hypoxia (David et al. 2002). It then implies that the onset of hypoxia leads to a decrease in cellular ATP stores and subsequent generation of adenosine, which is then released extracellularly. An increase in adenosine levels, in addition to bradykinin and opioids, initiate a series of intracellular signaling events via G-protein-coupled receptor signaling leading to activation of PKC activation (Di-Capua et al. 2003). Several studies have shown that injections of adenosine could protect neuronal cells against hypoxic-type injury via a PKC-mediated mechanism (Dave et al. 2009). A series of intracellular signaling that leads to the adenosine-mediated PKC-induced mechanism of hypoxia protection include; i) adenosine stimulates G-protein–coupled receptors, such as the A1/A3 adenosine receptors which activate phospholipases (phospholipase C; PLC) via G-proteins (Gi/o). ii) Other preconditioning stimulus, such as

extracellular glutamate stimulates NMDARs, leading to increased cytosolic calcium and PLC activation. iii) εPKC is activated by PLC, and it increases di-acylglycerol (DAG) production, which in turn activates PKC isozymes including εPKC. Finally, the εPKC activation of extracellular signal-regulated kinase (ERK) promotes survival. Neuronal survival occurs because PKC may induce the opening of *K* (ATP) channels in the mitochondria (*K*ATP) to regulate ATP production, and reduce generation of ROS. Interestingly, mitochondria are ideally located to serve as the cellular oxygen signal and mediator of protective mechanisms, such as ion channel arrest. Thus, regulation of mitochondria based mechanism of ion channel arrest involving ATP-sensitive mitochondrial K(+) channels, cytosolic calcium and ROS concentrations could contribute to neuronal survival.

It is also very important to point out that adenosine-mediated εPKC signaling is mediated in part through ERK, a mitogen-activated protein kinase (MAPK) family member that has been implicated in antiapoptotic signaling(Lange-Asschenfeldt et al. 2004). ERK maintains mitochondrial function by inhibiting deleterious Bcl-2 associated death domain protein BAD activity(Qiu et al. 2001).Taken together, adenosine-mediated εPKC signaling seems to activate ERK. This activation is involved in antiapoptotic signaling and cell survival during the induction of hypoxia preconditioning state, in order to salvage 'at-risk-tissue' with residual energy levels. The role of adenosine-mediated εPKC signaling in altering the expression or activity of calcium/calmodulin-dependent protein kinase II, MAPK family members, c-Jun N-terminal kinase, ERK, protein kinase B and PKC indicates that multiple kinases participate in the response of the tissue to counteract the effect of hypoxia (Obexer et al. 2006). This is important because PKC activity has been implicated in cell injury in the cerebral brain (Bright et al. 2008)indicating that it could be involved in a conserved hypoxia response pathway. εPKC activation delays the collapse of ion homeostasis during ischemia in arctic ground squirrels(Dave et al. 2009). This finding suggests that εPKC mediates 26 collapse of ion homeostasis in Arctic Ground Squirrels. This is possible because εPKC inhibits both 27 Na(+)/K(+)-ATPase and voltage-gated sodium channels, which are primary mediators of 28 the collapse of ion homeostasis during ischemia in Arctic Ground Squirrel (Perez-Pinzon et 29 al. 2005). For this reason, the specific role of εPKC in mediating hypoxia/ischemia-induced brain injury following the onset of stroke will be an interesting area of attention. Even more interesting is that the controversy over whether PKC mediates or is simply activated during hypoxia/ischemia-induced cell injury has been resolved to an extent by studies in ischemic models that suggest that PKC activity occurs via εPKC that mediates adenosine-induced preconditioning via K^{+}_{ATP} function (Chaudary et al. 2004b; Bright et al. 2008).

Several lines of evidence suggest that εPKC can confer cerebral protection partly by maintaining mitochondrial function via ERK activity and by modulating adenosine-induced mK^{+}_{ATP} channel function. It is also possible that εPKC activity inside the mitochondria may facilitate the regulation of mK^{+}_{ATP} channels, which is important for defending mitochondrial membrane potential and robust maintenance of ionic homeostasis. Recently, the role of εPKC as a key mediator of neuroprotection was investigated (Teshima et al. 2003). Finding from this study indicates that epsilonPKC inhibits both Na(+)/K(+)-ATPase and voltage-gated sodium channels, which are primary mediators of the collapse of ion homeostasis during ischemia in Arctic Ground Squirrel. Their results support the hypothesis that εPKC activation is neuroprotective by delaying the collapse of ion homeostasis during ischemia or

hypoxia. This probably involves robust maintenance of ion homeostasis, which leads to the conservation of energy by plummeting calcium influx during metabolic challenges. In summary, studies exploring the specific signaling pathways in which PKC participates, including different downstream effectors in different phases of stroke injury will be significant to develop an adenosine-mediated εPKC signaling therapeutic strategy for the clinical management of stroke patients. It is also important to emphasize that continuous cerebral blood flow, maintenance of cerebral oxygen tension and normal mitochondrial function are vital for the maintenance of brain function and tissue viability in the face of chronic hypoxia. The maintenance of normal brain functions require many parameters to work together and contribute to the homeostasis of brain energy demand and supply. Such a combination of parameters that could, in turn, lead to hypoxia protection include regulation of levels of inducible nitric oxide synthase (Thompson and Dong 2005; Thompson et al. 2009)and expression of HIF-1a(Trollmann and Gassmann 2009). Other parameters include the activation of extracellular-signal-regulated kinase(Osorio-Fuentealba et al. 2009; Wilkerson and Mitchell 2009)and c-Jun N-terminal kinase/stress-activated protein kinases (Comerford et al. 2004). Understanding the central mechanism that regulates the combination of the parameters is necessary when developing new approaches to remedying hypoxia-induced brain injuries. This is because there is the possibility that a single central cellular mechanism could invoke a combination of parameters that could lead to the remarkable tolerance to hypoxia as seen in natural genetic models of hypoxia-tolerance.

7. Conclusion

Physiological mechanisms of hypoxia neuroprotection in natural systems of hypoxia tolerance represent the core of our understanding of how the brain of a stroke patient can be made to resist hypoxic insults. Our understanding of physiological adaptations associated with hypoxia tolerance in natural systems, and the diverse cellular implications of disrupting brain energy balance when oxygen delivery fails to meet demand provide the insights of how the human brain can be made tolerant to hypoxia. In this review, we suggest that the physiological mechanisms used by hypoxia-tolerant species offer clues on strategies to adapt for the clinical management of brain injuries where oxygen demand fails to match the supply.

8. References

Ackland GL, Kasymov V, Gourine AV (2007) Physiological and pathophysiological roles of extracellular ATP in chemosensory control of breathing Biochemical Society Transactions 35:1264-1268

Balestrino M, Aitken PG, Somjen GG (1989) Spreading depression-like hypoxic depolarization in CA1 and fascia dentata of hippocampal slices: Relationship to selective vulnerability. Brain Res 497:102–107

Barger JL, Brand MD, Barnes BM, Boyer BB (2003) Tissue-specific depression of mitochondrial proton leak and substrate oxidation in hibernating arctic ground squirrels. Am. J. Physiol. Reg. Integr. Comp. Physiol. 284:1306 -1313

Barros RC, Zimmer ME, Branco LG, Milsom WK (2001) Hypoxic metabolic response of the golden-mantled ground squirrel. J Appl Physiol, 91:603-612.

Bickler PE (1998) Reduction of NMDA receptor activity in cerebrocortex of turtles (Chrysemys picta) during 6 wk of anoxia. Am. J. Physiol. 275:86-91

Bonde C, Noraberg J, Noer H, Zimmer J (2005) Ionotropic glutamate receptors and glutamate transporters are involved in necrotic neuronal cell death induced by oxygen-glucose deprivation of hippocampal slice cultures. . Neuroscience. 136:779-794.

Boutilier RG (2001) Mechanisms of cell survival in hypoxia and hypothermia. J Exp Biol 204:3171–3181

Bright R, Sun GH, Yenari MA, Steinberg GK, Mochly-Rosen D (2008) epsilonPKC confers acute tolerance to cerebral ischemic reperfusion injury. Neurosci Lett. 441:120-124

Chaudary N, Naydenova Z, Shuralyova I, Coe IR (2004a) Hypoxia regulates the adenosine transporter, mENT1, in the murine cardiomyocyte cell line, HL-1. Cardiovasc Res. 61:780–788

Chaudary N, Naydenova Z, Shuralyova I, Coe IR (2004b) PKC regulates ischemic preconditioning and the adenosine transporter, mENT1, in the mouse cardiac myocyte cell line, HL-1. J Pharmacol Exp Ther 310:1190–1198

Comerford KM, Cummins EP, Taylor CT (2004) c-Jun NH2-terminal kinase activation contributes to hypoxia-inducible factor 1alpha-dependent P-glycoprotein expression in hypoxia. Cancer Res 64:9057-9061.

Dave KR, Anthony R, Defazo R, Rava lAP, Dashkin O, Iceman KE, Perez-Pinzon M (2009) Protein kinase C epsilon activation delays neuronal depolarization during cardiac arrest in the euthermic arctic ground squirrel. J Neurochem. 110(4):1170-9

David B, David G, Marie-Christine G, Pablo A, Kadiombo B, Serge NS (2002) The Adenosine A1 Receptor Agonist Adenosine Amine Congener Exerts a Neuroprotective Effect against the Development of Striatal Lesions and Motor Impairments in the 3-Nitropropionic Acid Model of Neurotoxicity. The Journal of Neuroscience 22:9122-9133

Di-Capua N, Sperling O, Zoref-Shani E (2003) Protein kinase C-epsilon is involved in the adenosine-activated signal transduction pathway conferring protection against ischemia-reperfusion injury in primary rat neuronal cultures. J Neurochem. 84:409–412

Drew KL, Buck CL, Barnes BM, Christian SL, Rasley BT, Harris MB (2007) Central nervous system regulation of mammalian hibernation: implications for metabolic suppression and ischemia tolerance. J Neurochem. 102:1713-1726

Erecinska M, Cherian S, Silver A (2005) Brain Development and Susceptibility to Damage; Ion Levels and Movements Current Topics in Developmental Biology 69:139-186

Erecińska M, Silver IA (2001) Tissue oxygen tension and brain sensitivity to hypoxia. Respir Physiol. 128:263-276.

Fowler JC, Partridge LD, Gervitz L (1999) Hydroxylamine blocks adenosine A1 receptor-mediated inhibition of synaptic transmission in rat hippocampus. Brain Res 815:414–418

Frappell PB, Baudinette RV, MacFarlane PM, Wiggins PR, Shimmin G (2002) Ventilation and metabolism in a large semifossorial marsupial: the effect of graded hypoxia and hypercapnia. Physiol. Biochem. Zool. 75:77-82

Gonchar O, Mankovskaya I (2009) Effect of moderate hypoxia/reoxygenation on mitochondrial adaptation to acute severe hypoxia. Acta Biol Hung 60:185-194.

He Z, Lu Q, Xu X, Huang L, Chen J, Guo L (2009) DDPH ameliorated oxygen and glucose deprivation-induced injury in rat hippocampal neurons via interrupting Ca2+ overload and glutamate release. Eur J Pharmacol 28:50-55

Heldmaier G, Ortmann S, Elver R (2004) Natural hypometabolism during hibernation and daily torpor in mammals. Respiratory Physiology & Neurobiology 141:317-329

Hochachka PW (1993) Hypoxia tolerance in Amazon fishes: status of an underexplored biological ''goldmine''. CRC Press,, Boca Raton

Hochachka WP, Buck LT, Doll CJ, Land SC (1996) Unifying theory of hypoxia tolerance: molecular/metabolic defense and rescue mechanisms for surviving oxygen lack. Biochemistry 93: 9493-9498

Lange-Asschenfeldt C, Raval AP, Dave KR, Mochly-Rosen D, Sick TJ, Perez-Pinzon MA (2004) Epsilon protein kinase C mediated ischemic tolerance requires activation of the extracellular regulated kinase pathway in the organotypic hippocampal slice. J Cereb Blood Flow Metab. 24:636–645

Liljequist S, Cebers G, Kalda A (1995) Effects of decahydroisoquinoline-3-carboxylic acid monohydrate, a novel AMPA receptor antagonist, on glutamate-induced CA2+ responses and neurotoxicity in rat cortical and cerebellar granule neurons. Biochem Pharmacol. 50:1761-1774.

Liu GS, Thornton J, Van Winkle DM, Stanley AW, Olsson RA, Downey JM (1991) Protection against infarction afforded by preconditioning is mediated by A1 adenosine receptors in rabbit heart. Circulation. 84:350–356.

Lutz PL, Nilsson GE, Peréz-Pinzón MA (1996) Anoxia tolerant animals from a neurobiological perspective. Comp Biochem Physiol B Biochem Mol Biol. 113:3-13

Ma YL, Zhu X, Rivera PM (2005) Absence of cellular stress in brain after hypoxia induced by arousal from hibernation in Arctic ground squirrels. Am J Physiol Regul Integr Comp Physiol 289:297–306

Mravec B, Gidron Y, Kukanova B, Bizik J, Kss I, Hulin I (2006) Neural–endocrine–immune complex in the central modulation of tumorigenesis: Facts, assumptions, and hypotheses. Journal of Neuroimmunology. 180:104-116

Mulvey JM, Renshaw GM (2009) GABA is not elevated during neuroprotective neuronal depression in the hypoxic epaulette shark (Hemiscyllium ocellatum). Comp Biochem Physiol A Mol Integr Physiol. 152:273-277

Nathaniel IT, Saras A, Umesiri F, Olajuyigbe F (2009) Tolerance to Oxygen nutrient deprivation in the hippocampous slices of the naked mole rats. Journal of Integrative Neuroscience. 8:123-136

Nawashiro H, Tasaki K, Ruetzler CA, Hallenbeck JM (1996) TNF-alpha pretreatment induces protective effects against focal cerebral ischemia in mice. J Cereb Blood Flow Metab. 17:483-490

Noda M, Nakanishi H, Nabekura J, Akaike N (2000) AMPA-kainate subtypes of glutamate receptor in rat cerebral microglia. J Neurosci. 20:251-258

Obexer P, Geiger K, Ambros PF, Meister B, Ausserlechner MJ (2006) FKHRL1-mediated expression of Noxa and Bim induces apoptosis via the mitochondria in neuroblastoma cells. Cell Death Differ. 14:534-547

Osorio-Fuentealba C, Valdés JA, Riquelme D, Hidalgo J, Hidalgo C, Carrasco MA (2009) Hypoxia stimulates via separate pathways ERK phosphorylation and NF-kappaB activation in skeletal muscle cells in primary culture. J Appl Physiol. 106:1301-1310

Pék M, Lutz PL (1997) Role for adenosine in channel arrest in the anoxic turtle brain. J Exp Biol. 200:1913-1917.

Pellegrini-Giampietro DE, Peruginelli F, E. M, Cozzi A, Albani-Torregrossa S, Pellicciari R, Moroni F (1999) Protection with metabotropic glutamate 1 receptor antagonists in models of ischemic neuronal death: time-course and mechanisms. Neuropharmacology 38:1607-1619.

Pena F, Ramirez JM (2005) Hypoxia-induced changes in neuronal network properties. Mol. Neurobiol. 32:251–283

Perez-Pinzon MA, Born JG (1999) 1999. Rapid preconditioning neuroprotection following anoxia in hippocampal slices: role of the K+ATP channel and protein kinase C. Neuroscience. 89:453-459.

Perez-Pinzon MA, Dave KR, Raval AP (2005) Role of reactive oxygen species and protein kinase C in ischemic tolerance in the brain. . Antioxid Redox Signal 7:1150-1157

Qiu J, Grafe MR, Schmura SM, Glasgow JN, Kent TA, Rassin DK, Perez-Polo JR (2001) Differential NF-kappa B regulation of bcl-x gene expression in hippocampus and basal forebrain in response to hypoxia. J Neurosci Res. 64:223-234.

Ramirez JM, Folkow LP, Blix AS (2007) Hypoxia Tolerance in Mammals and Birds: From the Wilderness to the Clinic

Annu. Rev. Physiol. 69:113-143

Reshef A, Sperling O, Zoref-Shani E (2000) Role of K(ATP) channels in the induction of ischemic tolerance by the "adenosine mechanism" in neuronal cultures. Adv Exp Med Biol. Adv Exp Med Biol 486:217-221

Rincon F (2008) Therapeutic Hypothermia after Cardiac Arrest. , 2008. 148 ANN INTERN MED.:485-486.

Robinson MB (2006) Acute regulation of sodium-dependent glutamate transporters: a focus on constitutive and regulated trafficking. Handb Exp Pharmacol 175:251-275.

Rufini S, Grossi D, Luly P, Tancredi V, Frank C, D'Arcangelo G (2009) Cholesterol depletion inhibits electrophysiological changes induced by anoxia in CA1 region of rat hippocampal slices. Brain Res 1298:178-185.

Savolainen KM, Loikkanen J, Naarala J (1995) Amplification of glutamate-induced oxidative stress. Toxicol Lett. 82:399-405

Sheldon AL, Robinson MB (2007a) The role of glutamate transporters in neurodegenerative diseases and potential opportunities for intervention. Neurochem Int. 51:333-355.

Sheldon AL, Robinson MB (2007b) The role of glutamate transporters in neurodegenerative diseases and potential opportunities for intervention. Neurochem. Int 51:333-355.

Sobczyk A, Scheuss V, Svoboda K (2005) NMDA receptor subunit-dependent [Ca2+] signaling in individual hippocampal dendritic spines. J Neurosci. 25:6037-6046.

Statler KD (2008) Hypothermia to Treat Neonatal Hypoxic Ischemic Encephalopathy. AAP Grand Rounds 19:3-4

Storey JM, Storey KB (2005) Biochemical Adaptation to Extreme Environments Integrative Physiology in the Proteomics and Post-Genomics Age:169-200

Storey KB, Storey JM (2007) putting life on 'pause'--molecular regulation of hypometabolism. J Exp Biol. 10:1700-1714

Teshima Y, Akao M, Li RA, Chong TH, Baumgartner WA, Johnston MV, Marban E (2003) Mitochondrial ATP-sensitive potassium channel activation protects cerebellar

granule neurons from apoptosis induced by oxidative stress. Stroke. 2003 34:1796–1802.

Thompson L, Dong Y, Evans L (2009) Chronic hypoxia increases inducible NOS-derived nitric oxide in fetal guinea pig hearts. Pediatr Res. 65:188-192.

Thompson LP, Dong Y (2005) Chronic hypoxia decreases endothelial nitric oxide synthase protein expression in fetal guinea pig hearts. Soc Gynecol Investig. 12:388-395

Trollmann R, Gassmann M (2009) The role of hypoxia-inducible transcription factors in the hypoxic neonatal brain. Brain Dev.45:503-9.

Wang SQ, Lakatta EG, Cheng H, Zhou ZQ (2002) Adaptive mechanisms of intracellular calcium homeostasis in mammalian hibernators. J. Exp. Biol. 205:2957 -2962

Wilkerson JE, Mitchell GS (2009) Daily intermittent hypoxia augments spinal BDNF levels, ERK phosphorylation and respiratory long-term facilitation. Exp Neurol. 217:116-123

Zamalloa T, Bailey CP, Pineda J (2009) Glutamate-induced post-activation inhibition of locus coeruleus neurons is mediated by AMPA/kainate receptors and sodium-dependent potassium currents. Br J Pharmacol. 156:649-661.

Zhao P, Zuo Z (2005) Prenatal hypoxia-induced adaptation and neuroprotection that is inducible nitric oxide synthase-dependent. Neurobiol Dis. 20:871-880.

Zhou F, Braddock JF, Hu Y, Zhu X, Castellani RJ, Smith MA, Drew KL (2001a) Microbial origin of glutamate, hibernation and tissue trauma: an in vivo microdialysis study. J. Neurosci. Meth. 119:121 -128

Zhou F, Zhu X, Castellani JR, Stimmelmayr R, Perry G, Smith MA, Drew KL (2001) Hibernation, a Model of Neuroprotection. American Journal of Pathology. American Journal of Pathology 158:2146-2150.

Part 5

Management Approaches

17

Competing Priorities in the Brain Injured Patient: Dealing with the Unexpected

Jonathan R. Wisler, Paul R. Beery II,
Steven M. Steinberg and Stanislaw P. A. Stawicki
Department of Surgery, Division of Critical Care
Trauma, and Burn, The Ohio State University
Medical Center, Columbus, Ohio
USA

1. Introduction

Management of the multiply injured trauma patient can be defined by its complex nature and the necessity to reconcile multiple competing clinical priorities. Approach to single anatomic region/organ system traumatic injury tends to be relatively straight forward, although increasing severity of any isolated injury can by itself pose a formidable therapeutic challenge. In fact, any such "isolated" injury can be life threatening if severe enough and/or not managed optimally.

When the effects of simultaneous injuries to different anatomic regions and organ systems are combined, the cumulative complexity of trauma management can increase dramatically.[1] This chapter discusses clinical approaches to patients with traumatic brain injury in the context of multiple simultaneous associated injuries, focusing on addressing competing priorities and triage strategies needed to successfully manage these patients.

2. Team management and leadership

Effective diagnostic and therapeutic approaches to the multiply injured patient require the presence of well-functioning trauma systems and integrated specialty teams.[2] The optimal approach to the multiply injured patient involves the involvement of trauma-trained surgeons, intensivists, orthopedic specialists, urologists, neurosurgeons, and interventional radiologists.[3, 4] Highly skilled team management, leadership, and communication skills are of critical importance.[4] Excellent communication between physicians and teams, including awareness of important clinical pitfalls and constant vigilance on the part of all participating teams (i.e., presence of multiple cross-checks), as well as the need for centralized care planning (including multi-disciplinary patient care conferences) are crucial.[5]

3. Physiologic and outcome considerations

Restoration and maintenance of homeostasis is critical in management of the multiply injured patient. This often formidable task requires the achievement of a delicate balance between satisfying individual organ-system physiologic needs while reconciling the

frequent necessity for micro-management of often competing specific organ- or body system-oriented considerations (i.e., maintenance of relatively lower blood pressure levels in a patient with concomitant aortic and brain injury *versus* maintenance of higher blood pressures to ensure adequate brain perfusion). The critical nature of clinical decision making is exemplified by the finding that morbidity and mortality in head injured patients can as much as triple in the presence of hypotension.[6] Additionally, when matched for injury severity and age, multiply injured patients with brain trauma have a significantly worse long term outcomes compared to multiply injured patients without brain trauma.[1,2]

Early predictors of outcome from traumatic brain injury (TBI) include age, Glasgow Coma Scale, pupillary exam, computed tomographic (CT) characteristics, and the presence of hypotension (systolic blood pressure <90 mmHg).[7] The key consideration is to maintain homeostasis and to triage clinical management in a manner that optimizes recovery of all affected organ systems. This involves the need for intimate knowledge of individual organ system tolerance limits, the knowledge of common clinical management pitfalls, as well as familiarity with temporal evolution of injuries and injury patterns in the context of overlapping priorities and biomedical parameter ranges.

The overarching goal is to prevent the so-called "secondary hits" that have been shown to adversely affect outcomes.[2] These secondary insults, in contrast to the primary trauma, are amenable to prevention and may be reversible if detected and managed promptly.[8] As many as 40% of patients with TBI exhibit some form of significant neurologic deterioration during their hospital stay, most often from secondary insults.[3] Significant proportion of patients with traumatic brain injury are initially lucid after injury but deteriorate quickly. Therefore, practitioners should always have a high index of suspicion and should avoid potentially dangerous clinical assumptions (i.e., assuming acute intoxication) when approaching patients with possible TBI. One must remember that nearly one-third of all head injured patients who die may belong in this category[9], and up to 75% of these have an identifiable and avoidable secondary insult.[10] Hypotension accompanies severe head trauma in approximately 35% of cases and entails a near doubling of mortality (from 27% to 50%).[10] Hypotension in the setting of hemorrhagic shock is also associated with increased mortality. A hematocrit of less than 30% may be associated with a reduction in blood oxygen carrying capacity and potential worsening of cerebral ischemia. Acute anemia associated with head injuries has been cited to carry an associated mortality of 52%.[10] Despite that, universal blood transfusion triggers continue to be controversial, and there is evidence to suggest that "dosing" blood by unit(s) as opposed to absolute hematocrit targets may be more prudent in the context of brain tissue oxygenation.[11-13] The age of the transfused blood may also be an important consideration, with more favorable cerebral oxygenation responses seen following transfusions of blood stored for fewer than 19 days.[11]

Therapy directed at correcting hypovolemic shock includes prompt volume expansion with crystalloid solutions, followed by administration of blood products as per established trauma guidelines. It is hypothesized that, following traumatic brain injury, cerebrovascular dysfunction results in loss of brain compliance, resulting in increased sensitivity to elevated venous pressures. Increased central venous pressure (CVP) occurring with vigorous crystalloid resuscitation may therefore contribute to the loss of brain compliance and the development of intracranial hypertension.[14] Cerebral perfusion pressure (CPP), defined as the difference between mean arterial pressure (MAP) and intracranial pressure (ICP) is an important factor in determining the adequacy of cerebral blood flow. Cerebrovascular autoregulation, present in the uninjured brain, is lost when the CPP falls below 50 mmHg.[15]

Xenon 133 scanning to measure cerebral blood flow in brain injured patients found that cerebrovascular dysfunction and global cerebral ischemia was seen in 13% of patients with a GCS of 8 or less, and of these patients 63% were in a persistent vegetative state or died.[16] In some cases, patients with spinal cord injury may exhibit hypotension secondary to dysfunction of the sympathetic nervous system and loss of peripheral vascular tone, an important consideration due to the association between the relatively frequent co-occurrence of TBI and spinal injury.[17, 18]

4. Overview of injury mechanisms and related considerations

Blunt trauma is associated with some form of brain injury in as many as 40-50% of patients.[19, 20] Among those with TBI, the incidence of associated injuries can exceed 60%.[19] Moreover, the very presence of TBI and an associated injury approximately doubles the mortality (from ~10% to ~20%) when compared to TBI alone.[19] In patients with a GCS of less than 8, mortality is as high as 45%. In terms of the components of the GCS scale, a motor score of 2 or less was associated with the lowest survival.[5] Certain associated injuries, when combined with TBI carry an especially high mortality, including great vessel (50% mortality), liver (39%), bowel (37%), spleen (34%), lung (34%), spine (26-32%), and various skeletal injuries (18-29%).[21] Of note, many of these "high mortality" concurrent injuries tend to be associated with either blood loss and hemorrhage (i.e., femur fracture, splenic/hepatic laceration) or hypoxia (i.e., pulmonary injury).

5. Concurrent spinal injury

Spinal injuries and TBI frequently occur together. Combination of TBI and spinal injury without neurologic deficit carries an approximate mortality of 25%, which increases to about 33% when neurologic deficit is present.[21] In addition to a defined set of priorities associated with the management of TBI, the trauma practitioner must be aware of important considerations unique to the setting of spinal injury. The overarching consideration is the avoidance of secondary injury by preventing both hypotension and hypoxia.[8] This spans an entire spectrum of preventive measures, including adequate spinal immobilization with spinal precautions, avoidance of excessive manipulation during patient transfers and procedures (i.e., endotracheal intubation), and provision of adequate cardio-respiratory support. Patients with spinal cord injuries (SCI) may be at increased risk of respiratory failure due to diaphragmatic and/or intercostal muscle dysfunction, depending on the level of SCI.[22] The most common causes of spinal cord injuries are motor vehicle crashes (48%), falls (21%), assaults (15%) and sports-related accidents (14%).[4] It is imperative that the practitioner be aware of the possibility of spinal cord injury in the multiply injured patient. Of all trauma patients that die within the first 30 minutes after injury, 20-25% have a cervical SCI.[6]

A distinct set of complicating factors can be brought about by hemodynamic derangements associated with spinal cord injury. Included are factors such as hypotension requiring vasopressor administration, bradycardia requiring pharmacologic and/or procedural intervention, patient positioning restrictions, as well as inability to perform a reliable injury assessment below the level of neurologic injury associated with spinal cord disruption. Cervical SCI may cause profound changes in heart rate and rhythm, blood pressure, and cardiac output. Patients may exhibit hypotension secondary to dysfunction of the

sympathetic nervous system and loss of peripheral vascular tone.[17] Patients with high cervical SCI (levels C1-C5) have significantly higher requirements for cardiovascular intervention (i.e., need for of vasoactive agents or assistive device use) than patients with lower injuries (levels C6-C7).[17] Shortly after spinal cord injury (seconds to minutes) there may be a systemic pressor response characterized by widened pulse pressure that results from short-term outflow of sympathetic activity and adrenal hormones.[23] This pressor response is then quickly replaced by neurogenic shock characterized by bradycardia and hypotension.[24] In order to maintain proper systemic tissue perfusion, affected patients commonly require fluid resuscitation, supplemented by administration of vasoactive agents (if patient remains hypotensive despite adequate fluid resuscitation). Because patients with high spinal cord injuries are more susceptible to developing pulmonary edema, it is important to limit the amount of fluid resuscitation while maintaining a systolic blood pressure of 100-110 mmHg.[8]

Ventilatory management strategies for patients with diaphragm dysfunction differ from those used in cases without muscular functional deficits. With injuries involving spinal segments of T1 or higher, the intercostal muscles, important in expanding the anterior-posterior dimension of the thoracic cavity, are flaccid. With higher injuries of the cervical spine, the diaphragm itself may become paralyzed. This results in a paradoxic inward movement of the abdominal wall during inspiration.[25] Pulmonary capacity is further reduced while the patient is supine. Because of the weakened diaphragm, the abdominal contents push cephalad.[26] Initial respiratory management is aimed at providing adequate ventilatory support while reducing ventilator associated complications.

6. Concurrent chest injury

The simultaneous presence of TBI and chest injury involves a distinct set of clinical circumstances and considerations. Life-threatening pulmonary injuries may require aggressive ventilatory approaches, and when considered in the context of TBI, may predispose the patient to both systemic and brain hypoxia. Pericardial tamponade and tension pneumo- and/or hemothorax also represent a life threat by causing hypotension and brain hypoperfusion. In addition, traumatic aortic injury may impose a unique set of hemodynamic restrictions with regards to maintenance of narrow blood pressure and heart rate ranges.[27] For a given mean arterial pressure, any rise in ICP results in a decrease in CPP. In order to maintain adequate cerebral perfusion, CPP should be maintained around 60-70 mmHg.

One special consideration specific to the patient with TBI is the entity of acute lung injury (ALI) associated with isolated brain trauma.[28] This clinico-pathologic entity may not be associated with traumatic pulmonary injury *per se*. Instead, it may be more closely reflective of the global increase in TBI severity (i.e., the presence of a large mass lesion or midline shift on imaging is associated with 5-10 fold increase in risk of ALI).[28]

In addition to ALI, patients with severe brain injury can develop neurogenic pulmonary edema (NPE), which is defined as increased interstitial or alveolar lung water occurring in the absence of cardiac or pulmonary disorders or hypervolemia.[29] The disease process is characterized by alveolar hemorrhage, pulmonary vascular congestion, and the presence of protein rich exudate.[30] Comparatively, the incidence of NPE following severe head injury (20%) is similar to the incidence of NPE in subarachonoid hemorrhage (23%).[9] NPE can present within minutes to hours of the insult and usually resolves by 72 hours. Significant

pulmonary edema past this time point suggests another diagnosis. During the injury there is a sympathetic discharge that causes increases in arterial and venous pressures and subsequent vascular damage. This damage is thought to result in vascular extravasation and the development of NPE. In animal models NPE is most reproducible with insults to the nucleus tractus solitarius or the noradrenergic A1 cell group.[31] Interestingly, the exudate seen in NPE has a much higher protein content than that seen in cardiogenic pulmonary edema, supporting a distinct physiologic process.[32]

7. Concurrent abdominal injury

Traumatic abdominal injuries are among the most lethal overall, with intra-abdominal and pelvic hemorrhage continuing to be associated with significant morbidity and mortality. This section will discuss diagnostic and therapeutic approaches needed to effectively manage concurrent abdominal and traumatic brain injuries. Included in the discussion is the management of the abdominal compartment syndrome and the damage control approach to severe abdominal trauma. At times, increasing ICP may be noted in patients with TBI and significant abdominal injury. In highly select cases, the correct diagnosis and surgical decompression of the abdominal compartment syndrome (ACS) may improve intracranial hypertension that is otherwise unresponsive to traditional medical therapy.[33]

It is not uncommon for the brain injured patient to have simultaneous abdominal injury, especially in the setting of blunt polytrauma. In this case, the presence of intra-abdominal hypertension (IAH) can exacerbate elevations in ICP. Thus, the presence of IAH is an independent risk factor for secondary brain injury.[34] The increase in intraabdominal pressure is directly reflected in intrathoracic pressure and central venous pressure. Elevations in central venous and jugular venous pressures result in increased resistance to cerebral outflow, which causes an increase in ICP and decrease in CPP.[35] Animal experiments have demonstrated that IAH of >20 mmHg causes significant increases in ICP and decreases in CPP. Additionally, elevations in CSF lactate and interleukin-6 were also seen, suggesting the associated presence of cerebral ischemia.[36]

Treatment for refractory ICP elevations in the setting of IAH involves several modalities including neuromuscular blockade, vasopressor use to preserve CPP, and abdominal compartment release.[37] In the setting of new onset end-organ dysfunction (i.e., renal failure, worsening pulmonary dysfunction) many physicians would advocate abdominal fascial release (a.k.a., abdominal damage control).[38] Several authors advocate more liberal use of decompressive laparotomy, extending this paradigm to patients with refractory elevations in ICP without intra-abdominal hypertension. In a group of 17 poly-trauma patients with refractory increases in ICP, decompressive laparotomy resulted in significant ICP reductions from 30.0±4.0 to 17.5±3.2 mmHg.[39]

Occasionally, emergent laparotomy and abdominal damage control may be coupled with damage control neurosurgery (DCNS) in the acute setting. Initial neurosurgical interventions include arrest of intracranial bleeding followed by evacuation of hematoma/mass lesion. Therapeutic craniectomy appears beneficial in children with diffuse brain edema.[40] However, DCNS can not be fully recommended in adults until the ICP becomes uncontrolled despite optimal medical therapy.[41, 42]

Stopping non-cranial hemorrhage is critical in the overall management of the multiply injured patient with brain trauma. Continued emphasis on team work and close

collaboration between clinical teams is crucial.[43] In the multiply injured patient, an ICP monitor (mostly under local anesthesia or during cranitomy) can be inserted in the emergency room or in the intensive care unit while the patient is being stabilized. At times, ICP monitoring is initiated in tandem with emergency laparotomy, thoracotomy or any other life-saving procedures.[43] Occasionally, a craniotomy concurrent to other operative procedures may be required if the patient has a significant intracranial mass lesion and evidence of critical ICP increases on clinical exam. General surgeons may have to occasionally perform neurosurgery in remote locations for patients with TBI as statistics have shown that early simple interventions have resulted in increased rates of survival between 10-50%.[44, 45] Simpson *et al* have recommended evacuation of an extradural hematoma by the general surgeon in a remote location if the trauma center is >1-2 hours away.[44] Rinker et al advise emergency craniotomy if the GCS is <8, there are lateralizing signs such as a dilated pupil, hemiparesis or development of sustained bradycardia and hypertension.[45] Immediate availability of neurosurgeons may not be essential if a properly trained and credentialed trauma surgeon can appropriately monitor patients for neurologic deterioration and facilitate early transfer to a center capable of full-time operative and postoperative neurosurgical care.[46] In certain extreme situations, the performance of an emergency burr hole may be life saving.[47]

8. Concurrent skeletal injuries

Skeletal injuries are associated with a number of unique therapeutic challenges, especially when associated with significant blood loss and need for emergent skeletal fixation. This section discusses best approaches to deal with brain injured patients who also present with fractures, dislocations, and other musculo-skeletal emergencies. Included in the discussion is the topic of extremity compartment syndrome.

The management of skeletal fractures in brain injured patients continues to be a controversial topic. A comparison of early (<24 hours) versus late fracture fixation demonstrated that early fracture stabilization does not result in increased central nervous system complications.[48] Same study showed that patients undergoing delayed fixation experienced significantly higher pulmonary morbidity.[48] Another study showed that lower extremity fracture fixation within 24 hours did not entail greater risk for adverse outcomes in patients with TBI.[49, 50] The authors did emphasize, however, that avoidance of any undue hypoxia and hypotension is critical.[49, 50] Orthopedic "damage control" strategies have evolved in order to assist in early management of skeletal trauma in multiply injured patients with TBI who may be unable to tolerate traditional operative approaches or may not even be stable enough to leave their intensive care bed.[34, 51]

The clinical syndrome of fat embolism can influence the clinical course in the multiply injured patient, especially following long bone extremity fracture fixation.[52] Although early fracture fixation is thought to minimize the risk of this occurrence, some experimental studies show that intramedullary nailing of femoral fractures and subsequent liberation of bone marrow contents may have a negative influence on the central nervous system function.[52]

An important aspect of orthopedic care in the multiply injured patient with brain trauma is the lack of reliable physical examination. Due to this limitation, extremity compartment syndrome may evade timely diagnosis. The reliance on the traditional early clinical signs

and symptoms of compartment syndrome – pain on passive motion that is out of proportion to clinical findings and the presence of paresthesias – has to be substituted with hightened index of clinical suspicion and extremity compartment pressure measurements.[53] Fasciotomies should be performed in a timely fashion when evidence of elevated compartment pressures is present.[54, 55]

9. Concurrent vascular injury

Vascular injuries present a special challenge in the context of simultaneous brain trauma. Specifically, direct managment priority conflicts can be seen with regards to the need for therapeutic anticoagulation and the risk of secondary intracranial hemorrhage. Likewise, the maintenance of cerebral perfusion pressure can pose an increased risk in patients with concurrent traumatic pseudoaneurysms and other injuries that may necessitate strict blood pressure and heart rate control. In addition, high dose vasopressor use to maintain adequate cerebral perfusion may lead to distal extremity ischemic complications up to and including the need for amputation.

With ever improving quality of modern imaging modalities, cerebrovascular injuries are being detected more frequently. Neurologic assessment following blunt cerebrovascular injury can be difficult and distinguishing cerebral ischemia from cerebral infarction is often complex, especially in the setting of altered mental status.[56] The main challenge associated with the diagnosis of blunt carotid or vertebral injury (BCVI) is the relative rarity of BCVI and the need for constant vigilance and high index of suspicion. One of the most important clinical findings associated with BCVI is the presence of an unexplained or new neurologic deficit in the setting of otherwise normal (or unchanged) brain imaging. Trauma practitioners should be familiar with major risk factors for BCVI, both from the injury mechanism standpoint (i.e., cervical seat belt sign, blunt assault to craniofacial area with LeFort III fracture pattern, cervical spine fracture) and from the clinical presentation standpoint (i.e., high-speed motor vehicle crash, flexion-extension neck injury).[57]

10. Missed injuries and diagnostic delays

Important in the context of multiple trauma patient with concomitant brain injury are the concepts of missed injury and delayed diagnosis. Delay in diagnosis occurs when an injury is identified after the usually accepted initial phases of trauma evaluation (i.e., primary, secondary, or tertiary surveys) but before the injury manifests as an overt clinical problem.[58] Missed injury can be defined as a delay in diagnosis that is associated with clinical symptoms and/or is not identified until after discharge from hospital.[58] Although the incidence goal for missed injuries and diagnostic delays should be "zero", this target remains elusive. Major series cite missed injury rates between 0.5% and 65%, with anywhere between 1 and 2.3 missed injuries per patient, depending on population under study, type of study (prospective versus retrospective), and diagnostic definitions (missed injury versus delayed diagnosis).[58] Among missed injuries, over 10% are clinically significant and, of those, 14% to 50% can be associated directly with patient mortality.[1, 58, 59]

The importance of this topic to the brain injured trauma population becomes obvious when one considers the most common contributing factors to missed injury: (a) altered mental

status; (b) presence of distracting injury; (c) administration of analgesia and sedation; and (d) overwhelming or multiple simultaneous injuries.[58] Whenever the patient's sensorium is diminished, it becomes more difficult to identify injuries as the patient loses the ability to effectively express complaints related to pain and discomfort. Alterations in pain processing may occur with traumatic brain injury, spinal cord injury, hypoxia, shock, intoxication/substance abuse, and administration of sedation for various reasons (i.e., combative patient). The pain response can be altered after a major injury and the patient may not be able to process pain from all injuries equally. For example, a non-displaced ankle fracture may not be readily evident with a concurrent presence of an open femur fracture. Often direct palpation over a specific injury site will elicit a pain response. Therefore, comprehensive repetitive physical examinations may be required in order to effectively identify the complete injury list in the presence of distracting pain. However, even the most detailed physical examination may fail to detect traumatic injuries in the multiply injured patient with concomitant moderate to severe brain trauma. Moreover, concurrent administration of analgesia and sedation may additionally affect the practitioner's ability to reliably detect various types of injuries, from minor to life threatening.[58] It is important to note that cranio-facial injuries constitute as many as 5%-30% of missed injuries, depending on study cited.[1, 59, 60]

11. Pitfalls and controversies

This section highlights important pitfalls and controversies associated with management of the multiply injured patients with concurrent brain trauma. We emphasize the need for continuous reassessment of competing priorities and need for centralized team coordination. Because many of the topics mentioned are beyond the scope of this chapter, the reader is referred to other sources as referenced herein. Practitioners should always be aware of potential complications related to massive fluid resuscitation, up to and including the abdominal compartment syndrome.[61] On the opposite end of the hemodynamic spectrum, one should always be cognizant of complications related to use of escalating doses of vasoactive agents, including phenomena such as tachyphylaxis [62] and the possibility of skin/limb ischemia due to high-dose vasopressor use.[63] Although the authors encourage the use of advanced hemodynamic monitoring (both invasive and non-invasive), there are many potential complications associated with both errors in hemodynamic data interpretation and iatrogenic injury related to invasive line placement.[64]

Intra-hospital patient transfers (i.e., transport to operating room or imaging suite) carry its own set of complications, with serious adverse outcomes attributed to such transfers in over 30% of critically ill patients.[65] Use of any therapies or diagnostic tests that could potentially contribute to additional complications should always be considered in the context of risk-benefit ratio.[66] For example, although still controversial, evidence suggests that early use of prophylactic anticoagulation is more beneficial than withholding this therapy in the TBI population.[67, 68]

Additional considerations include the effect of sedative agents on both hemodynamic and metabolic aspects of patient management. For example, the use of propofol for sedation may be associated with complications such as hypotension [69], pancreatitis [70], and propofol infusion syndrome.[71] In addition, adjunctive approaches such as therapeutic hypothermia and chemically induced coma are mentioned and referenced for the reader.[72, 73] These

Provider- and Team-related considerations

- Skilled team management, leadership, and effective communication are of critical importance.
- Coordinated care planning, including multi-disciplinary conferences and open dialogue between various clinical specialties, is important to optimizing patient care.
- Trauma teams should work efficiently, utilizing protocolized care as well as well-functioning clinical management / surveillance systems.
- The overall goal of the trauma team is to reconcile conflicting priorities with the overarching goal of maximizing the outcome from the perspective of the "whole patient".

Injury-related considerations

- Knowledge of injury patterns is useful in determining the likelihood of any potential associated non-TBI injuries, especially in the setting of concurrent neurological impairment.
- The very presence of associated injuries in the setting of concurrent brain trauma is associated with significantly increased mortality. Among such associated injuries, those that carry highest mortality usually involve risks of hypotension, hemorrhage and/or hypoxia.
- Familiarity with acceptable physiologic parameter ranges inherent to the management of each injured anatomic area or organ system is important to patient care optimization and reconciliation of potentially conflicting therapeutic priorities.
- Non-conventional measures, including various "damage control" approaches permit the most critical injuries to be given higher priority while adequately temporizing other, less critical injuries.

Patient care-related considerations

- The overarching goal is to prevent secondary physiologic insults that have been shown to adversely affect outcomes.
- Maintenance of adequate cerebral perfusion pressure while minimizing hypotensive and hypoxic events is crucial. It is important to note that while these priorities do not change over time, the nature of inciting events may differ (i.e., hemorrhage causing early hypotension versus sepsis causing late hypotension).
- Intra-hospital patient transfers (i.e., for procedural interventions or imaging studies) carry a significant risk, with nearly one-third of such transfers associated with some sort of adverse event (i.e., hypotension, hypoxia, etc). Therefore, such transfers should be undertaken only if absolutely indicated.
- Lack of reliable physical examination in multiply injured patients with TBI predisposes this group to missed injuries and diagnostic delays. Although modern technolgical advances enable practitioners to partially "compensate" for the lack of adequate bedside assessment, there are no true substitutes for an experienced practitioner with an adequate level of clinical suspicion.

Miscellaneous considerations

- Use of any therapies or diagnostic tests that carry a defined potential for complications should always be considered in the context of careful risk-benefit determination.

Providers should be familiar with potential complications associated with each and every therapeutic agent and procedure. Early recognition of such complications can be life-saving.

Table 1. Important points in management of multiply injured patients with concurrent traumatic brain injury

therapies are still controversial and further research is needed to better define their safety profiles and risk-benefit characteristics, especially in the setting of multiple trauma and competing clinical priorities.

12. The multiply injured patient with TBI – Putting it all together

Management of the multiply injured patient with TBI involves close collaboration of multiple specialties, including critical care, neurosurgery, orthopedic, and trauma experts. Practitioners must always be aware of all competing priorities, including cross-specialty considerations for specific injury patterns and associated pitfalls and complications. Life-threatening injuries should be approached according to the magnitude of the most immediate mortality risk. At times, simultaneous management of multiple injuries may require the initiation of various "damage control" techniques. Complications related to the primary injuries as well as any secondary insults must be recognized and addressed promptly. Early rehabilitation is crucial in order to optimize long-term outcomes in this population. The achievement of these goals requires that trauma teams work efficiently, utilizing protocolized care and well-functioning clinical surveillance systems.[74, 75] The overall goal of the trauma healthcare team is to reconcile any conflicting priorities with the goal of maximizing the outcome from the perspective of the "whole patient".

13. References

[1] Houshian S, Larsen MS, Holm C. Missed injuries in a level I trauma center. *J Trauma.* Apr 2002;52(4):715-719.

[2] Mirza A, Ellis T. Initial management of pelvic and femoral fractures in the multiply injured patient. *Crit Care Clin.* Jan 2004;20(1):159-170.

[3] Dickinson K. The acute management of pelvic ring injuries. In: Kellman JF, Fisher TJ, Tornetta P, Bosse MJ, Harris MB, eds. *Orthopaedic knowledge update: trauma 2*. Rosemont, IL: American Academy of Orthopaedic Surgeons; 2000:229-237.

[4] Hoff WS, Reilly PM, Rotondo MF, DiGiacomo JC, Schwab CW. The importance of the command-physician in trauma resuscitation. *J Trauma.* Nov 1997;43(5):772-777.

[5] Ruchholtz S, Waydhas C, Lewan U, et al. A multidisciplinary quality management system for the early treatment of severely injured patients: implementation and results in two trauma centers. *Intensive Care Med.* Oct 2002;28(10):1395-1404.

[6] Chesnut RM, Marshall LF, Klauber MR, et al. The role of secondary brain injury in determining outcome from severe head injury. *J Trauma.* Feb 1993;34(2):216-222.

[7] Bullock R, Chesnut RM, Clifton G, et al. Guidelines for the management of severe head injury. Brain Trauma Foundation. *Eur J Emerg Med.* Jun 1996;3(2):109-127.

[8] Chesnut RM. Management of brain and spine injuries. *Crit Care Clin.* Jan 2004;20(1):25-55.

[9] Rose J, Valtonen S, Jennett B. Avoidable factors contributing to death after head injury. *Br Med J.* Sep 3 1977;2(6087):615-618.

[10] Miller JD, Becker DP. Secondary insults to the injured brain. *J R Coll Surg Edinb.* Sep 1982;27(5):292-298.

[11] Leal-Noval SR, Munoz-Gomez M, Arellano-Orden V, et al. Impact of age of transfused blood on cerebral oxygenation in male patients with severe traumatic brain injury. *Crit Care Med.* Apr 2008;36(4):1290-1296.

[12] Leal-Noval SR, Rincon-Ferrari MD, Marin-Niebla A, et al. Transfusion of erythrocyte concentrates produces a variable increment on cerebral oxygenation in patients with severe traumatic brain injury: a preliminary study. *Intensive Care Med.* Nov 2006;32(11):1733-1740.

[13] Smith MJ, Stiefel MF, Magge S, et al. Packed red blood cell transfusion increases local cerebral oxygenation. *Crit Care Med.* May 2005;33(5):1104-1108.

[14] Hariri RJ, Firlick AD, Shepard SR, et al. Traumatic brain injury, hemorrhagic shock, and fluid resuscitation: effects on intracranial pressure and brain compliance. *J Neurosurg.* Sep 1993;79(3):421-427.

[15] Rosner MJ, Daughton S. Cerebral perfusion pressure management in head injury. *J Trauma.* Aug 1990;30(8):933-940; discussion 940-931.

[16] Muizelaar JP. Cerebral ischemia-reperfusion injury after severe head injury and its possible treatment with polyethyleneglycol-superoxide dismutase. *Ann Emerg Med.* Jun 1993;22(6):1014-1021.

[17] Bilello JF, Davis JW, Cunningham MA, Groom TF, Lemaster D, Sue LP. Cervical spinal cord injury and the need for cardiovascular intervention. *Arch Surg.* Oct 2003;138(10):1127-1129.

[18] Stawicki SP, Holmes JH, Kallan MJ, Nance ML. Fatal child cervical spine injuries in motor vehicle collisions: Analysis using unique linked national datasets. *Injury.* Aug 2009;40(8):864-867.

[19] Siegel JH. The effect of associated injuries, blood loss, and oxygen debt on death and disability in blunt traumatic brain injury: the need for early physiologic predictors of severity. *J Neurotrauma.* Aug 1995;12(4):579-590.

[20] Siegel JH, Mason-Gonzalez S, Dischinger P, et al. Safety belt restraints and compartment intrusions in frontal and lateral motor vehicle crashes: mechanisms of injuries, complications, and acute care costs. *J Trauma.* May 1993;34(5):736-758; discussion 758-739.

[21] Siegel JH, Gens DR, Mamantov T, Geisler FH, Goodarzi S, MacKenzie EJ. Effect of associated injuries and blood volume replacement on death, rehabilitation needs, and disability in blunt traumatic brain injury. *Crit Care Med.* Oct 1991;19(10):1252-1265.

[22] Brown R, DiMarco AF, Hoit JD, Garshick E. Respiratory dysfunction and management in spinal cord injury. *Respir Care.* Aug 2006;51(8):853-868;discussion 869-870.

[23] Piepmeier JM, Lehmann KB, Lane JG. Cardiovascular instability following acute cervical spinal cord trauma. *Cent Nerv Syst Trauma.* Fall 1985;2(3):153-160.

[24] Lehmann KG, Lane JG, Piepmeier JM, Batsford WP. Cardiovascular abnormalities accompanying acute spinal cord injury in humans: incidence, time course and severity. *J Am Coll Cardiol.* Jul 1987;10(1):46-52.

[25] Luce JM, Culver BH. Respiratory muscle function in health and disease. *Chest.* Jan 1982;81(1):82-90.

[26] Bergofsky EH. Mechanism for Respiratory Insufficiency after Cervical Cord Injury; a Source of Alveolar Hypoventilation. *Ann Intern Med.* Sep 1964;61:435-447.

[27] Stawicki SP. Trends in nonoperative management of traumatic injuries: a synopsis. *OPUS 12 Scientist.* 2007;1(1):19-35.

[28] Bratton SL, Davis RL. Acute lung injury in isolated traumatic brain injury. *Neurosurgery.* Apr 1997;40(4):707-712; discussion 712.

[29] Ledingham IM, Watt I. Influence of sedation on mortality in critically ill multiple trauma patients. *Lancet.* Jun 4 1983;1(8336):1270.

[30] Miller SM. Management of central nervous system injuries. In: Capan LM, ed. *Trauma Anesthesia and Intensive Care*. Philadelphia: JB Lippincott Co; 1991:321.

[31] Simon RP, Gean-Marton AD, Sander JE. Medullary lesion inducing pulmonary edema: a magnetic resonance imaging study. *Ann Neurol.* Nov 1991;30(5):727-730.

[32] Theodore J, Robin ED. Pathogenesis of neurogenic pulmonary oedema. *Lancet.* Oct 18 1975;2(7938):749-751.

[33] Bloomfield GL, Dalton JM, Sugerman HJ, Ridings PC, DeMaria EJ, Bullock R. Treatment of increasing intracranial pressure secondary to the acute abdominal compartment syndrome in a patient with combined abdominal and head trauma. *J Trauma.* Dec 1995;39(6):1168-1170.

[34] Scalea TM, Boswell SA, Scott JD, Mitchell KA, Kramer ME, Pollak AN. External fixation as a bridge to intramedullary nailing for patients with multiple injuries and with femur fractures: damage control orthopedics. *J Trauma.* Apr 2000;48(4):613-621; discussion 621-613.

[35] Citerio G, Vascotto E, Villa F, Celotti S, Pesenti A. Induced abdominal compartment syndrome increases intracranial pressure in neurotrauma patients: a prospective study. *Crit Care Med.* Jul 2001;29(7):1466-1471.

[36] Marinis A, Argyra E, Lykoudis P, et al. Ischemia as a possible effect of increased intra-abdominal pressure on central nervous system cytokines, lactate and perfusion pressures. *Crit Care.* 2010;14(2):R31.

[37] Cheatham ML. Nonoperative management of intraabdominal hypertension and abdominal compartment syndrome. *World J Surg.* Jun 2009;33(6):1116-1122.

[38] Smith BP, Adams RC, Doraiswamy VA, et al. Review of abdominal damage control and open abdomens: focus on gastrointestinal complications. *J Gastrointestin Liver Dis.* Dec 2010;19(4):425-435.

[39] Joseph DK, Dutton RP, Aarabi B, Scalea TM. Decompressive laparotomy to treat intractable intracranial hypertension after traumatic brain injury. *J Trauma.* Oct 2004;57(4):687-693; discussion 693-685.

[40] Taylor A, Butt W, Rosenfeld J, et al. A randomized trial of very early decompressive craniectomy in children with traumatic brain injury and sustained intracranial hypertension. *Childs Nerv Syst.* Feb 2001;17(3):154-162.

[41] Honeybul S, Ho KM, Lind CR, Gillett GR. Decompressive craniectomy for diffuse cerebral swelling after trauma: long-term outcome and ethical considerations. *J Trauma.* Jul 2011;71(1):128-132.

[42] Soukiasian HJ, Hui T, Avital I, et al. Decompressive craniectomy in trauma patients with severe brain injury. *Am Surg.* Dec 2002;68(12):1066-1071.

[43] Hansen KS, Uggen PE, Brattebo G, Wisborg T. Team-oriented training for damage control surgery in rural trauma: a new paradigm. *J Trauma.* Apr 2008;64(4):949-953; discussion 953-944.
[44] Simpson DA, Heyworth JS, McLean AJ, Gilligan JE, North JB. Extradural haemorrhage: strategies for management in remote places. *Injury.* Sep 1988;19(5):307-312.
[45] Rinker CF, McMurry FG, Groeneweg VR, Bahnson FF, Banks KL, Gannon DM. Emergency craniotomy in a rural Level III trauma center. *J Trauma.* Jun 1998;44(6):984-989; discussion 989-990.
[46] Esposito TJ, Reed RL, 2nd, Gamelli RL, Luchette FA. Neurosurgical coverage: essential, desired, or irrelevant for good patient care and trauma center status. *Ann Surg.* Sep 2005;242(3):364-370; discussion 370-364.
[47] Rosenfeld JV. Damage control neurosurgery. *Injury.* Jul 2004;35(7):655-660.
[48] Starr AJ, Hunt JL, Chason DP, Reinert CM, Walker J. Treatment of femur fracture with associated head injury. *J Orthop Trauma.* Jan 1998;12(1):38-45.
[49] Poole GV, Miller JD, Agnew SG, Griswold JA. Lower extremity fracture fixation in head-injured patients. *J Trauma.* May 1992;32(5):654-659.
[50] Schmeling GJ, Schwab JP. Polytrauma care. The effect of head injuries and timing of skeletal fixation. *Clin Orthop Relat Res.* Sep 1995(318):106-116.
[51] Pape HC, Hildebrand F, Pertschy S, et al. Changes in the management of femoral shaft fractures in polytrauma patients: from early total care to damage control orthopedic surgery. *J Trauma.* Sep 2002;53(3):452-461; discussion 461-452.
[52] Nau T, Aldrian S, Koenig F, Vecsei V. Fixation of femoral fractures in multiple-injury patients with combined chest and head injuries. *ANZ J Surg.* Dec 2003;73(12):1018-1021.
[53] Kosir R, Moore FA, Selby JH, et al. Acute lower extremity compartment syndrome (ALECS) screening protocol in critically ill trauma patients. *J Trauma.* Aug 2007;63(2):268-275.
[54] Branco BC, Inaba K, Barmparas G, et al. Incidence and predictors for the need for fasciotomy after extremity trauma: A 10-year review in a mature level I trauma centre. *Injury.* Jul 31 2010.
[55] Farber A, Tan TW, Hamburg NM, et al. Early fasciotomy in patients with extremity vascular injury is associated with decreased risk of adverse limb outcomes: A review of the National Trauma Data Bank. *Injury.* Jun 28 2011.
[56] Phillips CV, Jacobsen DC, Brayton DF, Bloch JH. Central vessel trauma. *Am Surg.* Aug 1979;45(8):517-530.
[57] Fusco MR, Harrigan MR. Cerebrovascular dissections: a review. Part II: blunt cerebrovascular injury. *Neurosurgery.* Feb 2011;68(2):517-530; discussion 530.
[58] Stawicki SP, Lindsey DE. Missed traumatic injuries: a synopsis. *OPUS 12 Scientist.* 2009;3(2):35-43.
[59] Buduhan G, McRitchie DI. Missed injuries in patients with multiple trauma. *J Trauma.* Oct 2000;49(4):600-605.
[60] Enderson B, Maull KI. Missed injuries: the trauma surgeon's nemesis. *Surg Clin North Am.* 1991;71:399-418.
[61] Balogh Z, McKinley BA, Cocanour CS, et al. Secondary abdominal compartment syndrome is an elusive early complication of traumatic shock resuscitation. *Am J Surg.* Dec 2002;184(6):538-543; discussion 543-534.

[62] Lehmann G, Randall LO. Pharmacological properties of sympathomimetic diamines. *J Pharmacol Exp Ther.* May 1948;93(1):114-125.

[63] Dunser MW, Mayr AJ, Tur A, et al. Ischemic skin lesions as a complication of continuous vasopressin infusion in catecholamine-resistant vasodilatory shock: incidence and risk factors. *Crit Care Med.* May 2003;31(5):1394-1398.

[64] Evans DC, Doraiswamy VA, Prosciak MP, et al. Complications associated with pulmonary artery catheters: a comprehensive clinical review. *Scand J Surg.* 2009;98(4):199-208.

[65] Beckmann U, Gillies DM, Berenholtz SM, Wu AW, Pronovost P. Incidents relating to the intra-hospital transfer of critically ill patients. An analysis of the reports submitted to the Australian Incident Monitoring Study in Intensive Care. *Intensive Care Med.* Aug 2004;30(8):1579-1585.

[66] Stawicki SP, Grossman MD, Cipolla J, et al. Deep venous thrombosis and pulmonary embolism in trauma patients: an overstatement of the problem? *Am Surg.* May 2005;71(5):387-391.

[67] Kim J, Gearhart MM, Zurick A, Zuccarello M, James L, Luchette FA. Preliminary report on the safety of heparin for deep venous thrombosis prophylaxis after severe head injury. *J Trauma.* Jul 2002;53(1):38-42; discussion 43.

[68] Norwood SH, Berne JD, Rowe SA, Villarreal DH, Ledlie JT. Early venous thromboembolism prophylaxis with enoxaparin in patients with blunt traumatic brain injury. *J Trauma.* Nov 2008;65(5):1021-1026; discussion 1026-1027.

[69] Muzi M, Berens RA, Kampine JP, Ebert TJ. Venodilation contributes to propofol-mediated hypotension in humans. *Anesth Analg.* Jun 1992;74(6):877-883.

[70] Leisure GS, O'Flaherty J, Green L, Jones DR. Propofol and postoperative pancreatitis. *Anesthesiology.* Jan 1996;84(1):224-227.

[71] Diedrich DA, Brown DR. Analytic reviews: propofol infusion syndrome in the ICU. *J Intensive Care Med.* Mar-Apr 2011;26(2):59-72.

[72] Clifton GL, Miller ER, Choi SC, et al. Lack of effect of induction of hypothermia after acute brain injury. *N Engl J Med.* Feb 22 2001;344(8):556-563.

[73] Lee MW, Deppe SA, Sipperly ME, Barrette RR, Thompson DR. The efficacy of barbiturate coma in the management of uncontrolled intracranial hypertension following neurosurgical trauma. *J Neurotrauma.* Jun 1994;11(3):325-331.

[74] Gracias VH, Sicoutris CP, Stawicki SP, et al. Critical care nurse practitioners improve compliance with clinical practice guidelines in "semiclosed" surgical intensive care unit. *J Nurs Care Qual.* Oct-Dec 2008;23(4):338-344.

[75] Stawicki SP, Gracias VH, Lorenzo M. Surgical critical care: from old boundaries to new frontiers. *Scand J Surg.* 2007;96(1):17-25.

18

Traumatic Brain Injury – Acute Care

Angela N. Hays and Abhay K. Varma
Medical University of South Carolina
USA

1. Introduction

Traumatic brain injury (TBI) is a spectrum of pathological changes in the brain that result from application of external mechanical force(s) leading to temporary or permanent impairment of neurological function. TBI is a global health problem. In 1990, 9.5 million individuals worldwide sustained a TBI severe enough to warrant medical attention or result in death (Corrigan, Selassie, & Orman 2010). In the United States, the annual incidence of TBI is 506.4 per 100,000 population (Langlois JA 2006), in Europe it is 235 per 100,000 population (Tagliaferri et al. 2006), and in Asia it is reported to range from 160 (Gururaj G 2004) to 344 (Chiu et al. 1997) per 100,000 population (incidence rate from United States is inclusive of emergency department visits, hospital discharges and deaths, incidence rates from Europe and Asia are inclusive of hospital discharges and deaths only). Severe TBI has long term consequences for the individual and the society. In the United States, it is estimated that annually 43.1% (124,000) of the individuals discharged after acute hospitalization for TBI (Selassie et al. 2008) develop long term disability. The prevalence of individuals in the United States with disability secondary to TBI is estimated to be 3.2 million (Zaloshnja et al. 2008). The total life time cost of treating TBI cases in the United States, including loss of productivity, is estimated to be $ 60.4 billion for the year 2000 (Corrigan, Selassie, & Orman 2010).The enormous economic and social burden imposed by TBI demands aggressive measures to prevent and treat TBI.

Technological developments and better understanding of brain physiology have resulted in a dramatic improvement in mortality related to TBI. Mortality rates declined precipitously between 1970 and 1990, likely related to the routine use of computed tomography (CT) scans and intracranial pressure (ICP) monitoring, as well as improvement in trauma resuscitation generally. However, despite more recent advances in neuromonitoring and neuroimaging, mortality has remained steady at approximately 35% since the 1990s (Stein et al. 2010). The reasons for this lack of progress are multifactorial; no doubt the increase in severe TBI among the elderly is a contributing factor (Colantonio et al. 2008). Nevertheless, the field of neurotrauma has suffered from the lack of well organized multicenter, randomized clinical trials designed to assess the efficacy of the newer techniques and interventions for the management of TBI. Efforts to design and conduct clinical trials in TBI have been challenging for many reasons including the heterogeneity of the disease, barriers to recruitment, and lack of standardized management protocols across centers (Narayan et al. 2002). This chapter presents an overview of the pathophysiology and basic principals of management of TBI, and the challenges facing the scientific community in dealing with this debilitating disorder.

2. Mechanism

TBI results from the application of external force to cranium and its contents. The severity of injury is determined by the nature, magnitude and duration of load applied to cranium and the force vector (rotational, translational or angular) (Gennarelli 1993). Force applied to cranium can be static (delivered over more than 200 milliseconds) or dynamic (delivered over less than 200 milliseconds). Static loading is a less common cause of TBI in clinical situations, and usually leads to focal brain damage. Dynamic loading is a more common cause of TBI, and produces more complex and widespread brain damage. Dynamic loading itself results from direct blow to the cranium (contact load), or rapid acceleration or deceleration of the cranium (inertial load) that causes differential motion of the brain relative to the skull. Inertial loading can cause translational movement (linear motion of brain in the skull), rotational movement (shearing motion of different layers of brain in relation to one another), and angular movement (a combination of translational and rotational movements). In most clinical situations, severe TBI is initiated by impact to the head of a solid object at a high velocity, leading to brain damage by a combination of contact and inertial loading (Graham, Adams, & Gennarelli 1988).

Traumatic brain injury can be focal or diffuse. Focal injury manifests as fractures, contusion or hematoma with mass effect. Contact loading, wherein a significant force is applied to cranium, can cause fracture at the site of impact with underlying contusion or epidural/subdural hematoma. If contact loading also produces translational acceleration or deceleration of the brain within the cranium, it can also cause focal damage by compression of brain under the site of impact (coup contusion) or remote from it (counter coup contusion). Diffuse brain injury results from rotational or angular acceleration or deceleration of the brain; the presentation can vary from transient loss of consciousness (concussion) to diffuse axonal injury (DAI) with prolonged coma. With increasing magnitude of inertial force progressively deeper structures in the brain suffer DAI (Adams et al. 1989). In clinical situations focal lesions and DAI frequently coexist (Skandsen et al. 2010).

3. Pathophysiology

TBI is not a static event, but a constantly evolving dynamic process. The initial insult leads to instant brain damage known as primary brain injury. Primary brain injury triggers a cascade of events that cause brain edema, intracranial hypertension, and consequent ischemia, categorized as secondary brain damage (Enriquez & Bullock 2004). In diffuse injury the primary insult is at the cellular level and initiates events that lead to release of excitatory neurotransmitters, loss of ionic homeostasis, disruption of ATP production, toxicity from free radical production, loss of auto regulation and breakdown of blood brain barrier (BBB). These pathological changes lead to secondary injury. Similar secondary changes also occur in the region surrounding an area of contusion or traumatic hematoma, with a zone of ischemia and swelling developing around the focal injury. Tissue in this "ischemic penumbra" is at risk of dying but also salvageable (Schroder et al. 1995; Leker & Shohami 2002). Treatment of acute TBI is directed at minimizing secondary brain damage that follows focal as well as diffuse TBI.

A good understanding of cellular and biochemical changes that follow brain trauma is required for the optimal management of brain injury, and for directing future research aimed at design of new therapies and protocols. A detailed discussion of these changes is

beyond the scope of this chapter; however, a brief summary is presented. Following trauma, the excitatory amino acid glutamate is released from the presynaptic neurons and activates both post synaptic neurons and glial cells. Under physiological conditions both glial cells and postsynaptic neurons actively remove glutamate from the synaptic space. Following trauma the high levels of glutamate activate receptors in the cell membranes of postsynaptic neurons and glial cells, resulting in Ca^{2+} influx into the cell with consequent free radical formation and increased oxidative stress. Calcium ions also accumulate in the mitochondria leading to mitochondrial membrane damage and disruption of energy cycle. These events eventually trigger necrosis and apoptosis in the cells (Enriquez & Bullock 2004; Shohami et al. 1997).

TBI disrupts the BBB that allows fluid to move from intravascular compartment to extravascular compartment in the brain with resultant brain swelling and intracranial hypertension, which in turn leads to fall in cerebral perfusion thus further exacerbating brain ischemia. Aquaporins, matrix metalloproteinases and vasoactive inflammatory agents are potential mediators of BBB breakdown following trauma (Donkin & Vink 2010). A porous BBB allows infiltration of inflammatory cells into damaged brain. These cells (neutrophils and macrophages) release free radicals and cytokines into the brain tissue accentuating the damage (Soares et al. 1995).

4. Initial management at the scene of injury

Pre-hospital management is a critical step in the overall care of acute brain injury. Early institution of optimal care will minimize secondary injury while the patient is transported to a tertiary care facility. About half the deaths following TBI occur within first 2 hours of the injury (Badjatia, Carney et al. 2008) thereby signifying the importance of optimal pre-hospital care.

Pillars of pre-hospital care are restoring and maintaining airway, breathing and circulation (ABC) (Dewall 2010). The cervical spine must be immobilized as the patient is resuscitated, and spine precautions should be maintained till the patient is transferred to a trauma center. Intubation is indicated to secure the airway if the Glasgow Coma Scale (GCS) is less than 9, if the patient is hypoxic (SpO_2 < 90%) despite supplemental oxygen, or if the patient is unable to maintain a patent airway (Gabriel et al. 2002). The SpO_2 should be kept above 95%, while maintaining eucapnea with an end tidal carbon dioxide ($EtCO_2$) of 35 to 40 mm Hg (Badjatia, Carney et al. 2008). Hypotension accelerates secondary injury, and must be promptly recognized and treated. Systolic blood pressure below 90 mm Hg has been shown to increase mortality by two fold in TBI (Chesnut et al. 1993). Intravenous access should be promptly established, and normal saline is a reasonable choice for pre hospital fluid resuscitation (Dewall 2010). Intravenous dextrose should be administered only if blood glucose is less than 70 mg/dl (Badjatia, Carney et al. 2008). Persistent hypotension should prompt search for extracranial injury or spinal cord injury. Neurogenic hypotension presents with bradycardia and hypotension as opposed to hypovolemic shock that is characterized by tachycardia and hypotension.

Glasgow coma scale (GCS) and pupillary response should be documented after the ABCs of resuscitation are complete, and if more than one provider is available, this can be done while the ABCs are being secured. Assessment of the GCS and pupillary response in pre-hospital setting allows the emergency medical personnel to communicate to the receiving hospital the neurological status of the patient, screen for cerebral herniation and trend the

neurological status (Badjatia, Carney et al. 2008). A quick secondary survey for extracranial injuries should be performed prior to transport (Dewall 2010).
Timely transport of these patients to tertiary care center is critical to a favorable outcome. Patients with severe TBI (GCS < 9) should be transported directly to a facility equipped with an immediately available CT scanner, prompt neurosurgical care and the ability to monitor intracranial pressure and treat intracranial hypertension (Badjatia, Carney et al. 2008), even if it is not the closest hospital (Hartl et al. 2006). Mortality can increase by 50%, if a severe TBI patient is not directly transported to such a facility (Hartl et al. 2006). Mode of transport (by road or air) should be chosen to minimize the transit time (Dewall 2010), as mortality following acute subdural hematoma has been shown to be directly linked to time to surgical intervention (Seelig et al. 1981).

5. Medical management

5.1 Principles of intensive care

Management of the patient with traumatic brain injury is directed predominantly at minimizing and preventing secondary brain injury, which can result from intracranial or systemic causes. Although the mechanisms of secondary brain injury are incompletely understood, inadequate cerebral perfusion and oxygenation are integral factors. Systemic hypoxia and hypotension are significantly associated with increased morbidity and mortality. This effect was demonstrated in a data set from the Traumatic Coma Data Bank, in which a single episode of systemic hypotension was associated with double the risk of death (Chesnut et al. 1993; Bratton et al. 2007). This observation serves to demonstrate that good basic critical care is paramount. Current guidelines recommend a systolic blood pressure greater than 90 mmHg as a resuscitation end-point (Bratton et al. 2007). Adequate fluid resuscitation is important, but vasopressors should be used if fluids are insufficient to maintain adequate systemic blood pressures. Intubation and mechanical ventilation are necessary for patients presenting with severe brain injuries in order to ensure adequate oxygenation and ventilation, and to minimize the risk of aspiration (Valadka & Robertson 2007). Admission to a dedicated neuroscience critical care unit, when available, may also result in improved outcomes (Patel et al. 2005).
Ventilator management in patients with severe TBI presents some unique challenges because of the effect of $PaCO_2$ on cerebrovascular autoregulation. Hyperventilation has long been recognized as an effective means for treating acutely elevated intracranial pressure. Hyperventilation results in a systemic respiratory alkalosis, which in turn lowers the pH of the cerebrospinal fluid. In patients with intact cerebral autoregulation, this change in pH results in constriction of cerebral arterioles, thereby decreasing the cerebral blood volume and lowering the intracranial pressure. As a result of this phenomenon, routine hyperventilation of TBI patients has been advocated in the past. However, experimental evidence has demonstrated that the vasoconstrictive effect is relatively short-lived, (Muizelaar et al. 1988) and there is concern that continued hyperventilation could potentiate cerebral ischemia (Bratton et al. 2007). Furthermore, subsequent efforts to correct an iatrogenic respiratory alkalosis sometimes precipitate rebound increases in intracranial pressure (Valadka & Robertson 2007). For these reasons, hyperventilation is usually employed only as a temporizing measure in patients suffering an acute neurological decline.
The relationship between $PaCO_2$ and cerebral blood flow becomes particularly problematic in TBI patients with co-morbid lung injury. Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are not uncommon in the neurological critical care unit. Since the

publication of the ARDSnet trial in 2000, the mainstay of therapy for patients with ALI and ARDS has been lung-protective ventilation, which minimizes barotrauma to the lungs by employing lower tidal volumes (The Acute Respiratory Distress Syndrome Network 2000). This strategy results in a moderate degree of hypercapnea and hypoxemia. While lung-protective ventilation significantly improved mortality in the study population, the relative hypercarbia and hypoxemia may have deleterious effects in a patient who is at risk for cerebral edema, ischemia, or vasospasm. Randomized trials in TBI patients with ARDS are lacking. Monitoring of cerebral perfusion and oxygenation, which is discussed in subsequent sections, is particularly crucial in this scenario (Young et al. 2010).

Because of the brain's role in regulating metabolism, patients with severe traumatic brain injury demonstrate a number of metabolic derangements which ultimately result in systemic catabolism (Cook, Peppard, & Magnuson 2008). Adequate nutritional support is essential to forestall adverse consequences, including protein loss and immunocompromise (Sacks et al. 1995). Early institution of nutritional support has a significant impact on mortality and decreases the rate of hospital-acquired infections (Hartl et al. 2008; Taylor et al. 1999). Patients with severe TBI will require replacement of approximately 140% of their resting energy expenditure, though this requirement will be decreased somewhat in the setting of pharmacological paralysis or deep sedation. Protein requirements are estimated at about 1.5-2 grams/kg of protein (Cook, Peppard, & Magnuson 2008). Close monitoring is essential to ensure adequate supplementation and avoid complications such as hyperglycemia. Early hyperglycemia has been associated with poor outcome in TBI patients (Liu-DeRyke et al. 2009), but intensive glycemic control may exacerbate metabolic stress in some patients (Vespa et al. 2006). Although the optimal glucose range for TBI patients has yet to be determined, our institution has chosen 140-180 mg/dL as a reasonable target range.

Because of their increased sympathetic tone, severity of illness, and degree of immobility, TBI patients are at high risk of complications of critical illness including infection and thromboembolism. Long-term use of prophylactic antibiotics is discouraged, though there is some evidence that brief treatment at the time of endotracheal intubation reduces the incidence of ventilator-associated pneumonia (Sirvent et al. 1997). Fevers are common in patients with traumatic brain injury, and may represent an underlying infection, hypothalamic dysfunction, or other non-infectious cause. Hyperpyrexia must be investigated exhaustively in order to rule out the possibility of an infectious etiology; however, any fever in a patient with neurological injury must be treated aggressively. Increased temperature results in increased cerebral blood flow, which can exacerbate intracranial hypertension and reduce intracranial compliance (Segatore 1992). Furthermore, animal studies have indicated that hyperthermia can increase infarct size and potentiate cellular damage (Dietrich et al. 1996; Dietrich & Bramlett 2007). For these reasons, aggressive treatment of fever in brain-injured patients is warranted. First-line treatments generally include antipyretics such as acetaminophen; cooling blankets, fans, and chilled fluids have also been employed (Johnston et al. 2006). Control of shivering is also important, as shivering can increase metabolic demand and impede efforts to maintain normothermia (Badjatia, Strongilis et al. 2008).

Prevention of thromboembolic events is a major consideration in patients with severe TBI, especially in poly-trauma patients. Mechanical prophylaxis with graduated compression stockings and/or pneumatic compression devices is recommended, though these interventions may be contraindicated in the presence of significant injury to the lower extremities (Bratton et al. 2007). Pharmacological prophylaxis with unfractionated (UH) or

low-molecular weight heparin (LMWH) has been the subject of some debate. There is concern that use of pharmacological interventions could result in exacerbation of cerebral contusions, intracranial hemorrhage, and systemic bleeding. Evidence in a neurosurgical population indicates that pharmacological prophylaxis used in conjunction with mechanical devices is more effective than mechanical measures alone (Nurmohamed et al. 1996; Agnelli et al. 1998). Recent observational evidence suggests that initiation of prophylactic treatment with LMWH within 48 hours of injury or neurosurgical intervention is effective and associated with an acceptably low risk of complications (Norwood et al. 2008; Dudley et al. 2010). Unfortunately, the most effective agent and dosing strategy is yet to be determined.

5.2 Cerebral edema, intracranial hypertension and cerebral perfusion pressure

The components of the intracranial compartment under normal circumstances consist of brain tissue, cerebrospinal fluid, and blood. Because the intracranial contents are contained within the rigid confines of the skull, any increase in volume—in the form of cerebral edema or a pathological mass lesion—must be met with a compensatory decrease in one of the other components, or the ICP will rise. Elevated ICP can result in cerebral ischemia, an important cause of secondary brain injury, by reducing cerebral perfusion pressure (CPP):

$$CPP = MAP - ICP$$

Where MAP is the mean arterial pressure.

There is a considerable amount of debate regarding the relative importance of CPP and ICP thresholds. On one hand, observational data indicates that ICP > 20 mmHg is an independent predictor of morbidity and mortality (Helmy, Vizcaychipi, & Gupta 2007; Valadka & Robertson 2007; Robertson et al. 1999; Hiler et al. 2006; Czosnyka et al. 2005); however, good outcomes are possible even in the setting of exceptionally high ICPs, provided that cerebral perfusion is maintained. As a consequence, some centers advocate a CPP guided strategy, where as other clinical protocols focus predominantly on ICP and minimization of cerebral edema (Eker et al. 1998). Guidelines recommend ICP monitoring in patients with a Glasgow Coma Score (GCS) ≤ 8 who have abnormalities visible on computed tomography (CT). In the absence of CT abnormalities, ICP monitoring is indicated in comatose patients meeting 2 of the following criteria: age > 40 years, presence of motor posturing on exam, and/or SBP < 90 mmHg(Bratton et al. 2007). ICP values in excess of 20 mmHg are considered pathological, though it is important to note that herniation can occur at lower ICPs, especially in the presence of intracranial mass lesions (Bratton et al. 2007).

The situation is complicated by the fact that the relationship between ICP and CPP is affected by the integrity of cerebrovascular autoregulatory mechanisms. When cerebral autoregulation is intact, a fall in CPP is met with reflex cerebral vasodilation. This can precipitate an increase in ICP, thereby further diminishing cerebral perfusion (Robertson 2001). In this setting, efforts to augment CPP artificially may abort a destructive cycle and arrest ongoing secondary brain injury. However, if CPP falls outside of the range of normal autoregulation, or if cerebral autoregulation is impaired, this relationship is reversed: ICP will rise in a linear fashion with increasing CPP, and artificial augmentation of cerebral blood flow may result in worsening cerebral edema and hyperemia. Initially, observational data using historical controls lead to the conclusion that CPP should be maintained at > 70 mmHg. However, in a retrospective analysis of the Selfotel trial, Juul *et al.* found that there was no outcome benefit obtained by maintaining CPP > 60; interestingly, ICP ≥ 20 was the

most powerful predictor of outcome (Juul et al. 2000). The only randomized trial to date addressing the question of CPP vs. ICP was published by Robertson, *et al.* in 1999. In this trial, the CPP in one group was maintained at > 70 mmHg; in the other group, the ICP was maintained at ≤ 20 mmHg and CPPs as low as 50 mmHg were permitted. The investigators found no significant difference in outcome, but the CPP-targeted strategy carried five times the risk of ARDS (Robertson et al. 1999). An increasing appreciation of the role of cerebrovascular autoregulation in secondary brain injury has led some investigators to incorporate assessment of autoregulatory function into clinical protocols. The most readily available measure is the pressure reactivity index (PRx), which is a moving correlation coefficient calculated from measurements of ICP and MAP (Czosnyka et al. 1998). Centers that employ this measurement have reported that patients with intact autoregulation (PRx is low) have improved outcomes when managed according to a CPP guided paradigm, whereas those with impaired autoregulation (PRx is elevated) benefit from an ICP targeted strategy (Howells et al. 2005). Clearly, this observation warrants additional study.

Interventions to control intracranial hypertension generally act to decrease the volume of one component of the intracranial compartment: blood, brain, or CSF. First line interventions include patient positioning, adequate sedation and analgesia, and maintenance of normothermia. The head of the bed should be elevated and any obstructions to jugular venous outflow, such as cervical collars, should be minimized. Adequate sedation and analgesia serve to minimize ventilator dyssynchrony, lower intrathoracic and intraabdominal pressure, and decrease agitation (Helmy, Vizcaychipi, & Gupta 2007). Treating systemic fever can mitigate the associated CNS hyperemia prevent shivering and rigors, which often result in increased ICP. If an external ventricular drain is present, diversion of cerebrospinal fluid can be helpful as well.

Use of hyperosmolar substances is the mainstay of therapy for cerebral edema, with or without associated intracranial hypertension (Bhardwaj 2007). Infusion of a hyperosmolar substance serves to generate an osmotic gradient across the blood-brain-barrier, which reduces cerebral edema by drawing water out of the brain tissue. Mannitol and hypertonic saline (HTS) are the most commonly used agents in clinical practice. Mannitol is generally given as a bolus in doses ranging from 0.25-1.5 grams/kg. Hypertonic saline can be administered as a bolus or as a continuous infusion. For continuous use, concentrations ranging from 2% to 7.5% have been reported; boluses of 23.4% NaCl can be used in the setting of acute neurological decline. Use of either agent can result in rapid improvement in ICP; therefore, selection of the appropriate treatment will depend upon the clinical scenario. Mannitol can easily be infused through a peripheral intravenous line, whereas concentrations of HTS > 2% require central access. Mannitol acts as an osmotic diuretic, and can result in intravascular volume depletion, hypokalemia, and hypotension. There is concern that repeated dosing can result in renal impairment, especially if the drug is not adequately cleared by the kidneys; this can be monitored by calculating the osmolal gap. Hypertonic saline, on the other hand, acts as a volume expander and has been shown to be an effective means of reducing ICP even in patients who did not respond to mannitol (Ogden, Mayer, & E. Sander Connolly 2005; Vialet et al. 2003; Schwarz et al. 2002). Potential adverse events include congestive heart failure and hyperoncotic hemolysis; rapid administration of concentrated solutions may also result in transient hypotension. Either agent has the potential to accumulate in brain tissue in the presence of disruption of the blood-brain-barrier, Also, continued exposure to a hypertonic environment induces the generation of idiogenic osmoles within brain tissue, possibly setting the stage for rebound

intracranial hypertension when the serum osmolarity begins to fall (Diringer & Zazulia 2004). Consequently, close monitoring of the patients' ICP, CPP, serum sodium, and serum osmolarity is required. Despite the lack of convincing evidence, general consensus it that driving the serum sodium above 160 mEq/L is rarely beneficial, and most clinicians try to maintain the serum osmolarity below 320 mOsm/L (Hays et al. 2011).

When intracranial hypertension persists despite maximal osmotherapy, ICP may be controlled by interventions designed to decrease the cerebral blood volume. This can be accomplished by suppressing brain metabolism, either by inducing a pharmacological coma or with therapeutic hypothermia. Induction of a pharmacological coma is usually accomplished using barbiturates, such as pentobarbital. Barbiturates have been shown experimentally to mitigate the development of brain edema (Mishina & Yabuki 1994), decrease cerebral metabolism, and lower cerebral blood flow (Kassell, Hitchon et al. 1980). Early experimental data suggested a neuroprotective effect in models of focal cerebral ischemia (Smith et al. 1974); however, subsequent small-scale clinical trials were disappointing (Ward et al. 1985; Kassell, Peerless et al. 1980). Studies in brain injured patients indicate that barbiturates may be less effective than mannitol as an initial intervention, but nevertheless are useful in patients who have failed maximal osmotherapy (Eisenberg et al. 1988; Schwartz et al. 1984; Marshall et al. 2010). Pentobarbital has a number of undesireable side-effects including systemic hypotension, decreased gastric motility, and bone marrow suppression (Meyer et al. 2010). In addition, continuous infusion of pentobarbital results in delayed drug clearance, which can delay detection of neurological improvement or decline. Continuous electroencephalography allows the clinician to titrate to burst-suppression, thereby ensuring that the minimum effective dose is employed. Several other agents, including propofol and midazolam, can be used to suppress CNS metabolism and induce burst-suppression, though they do not appear to be as effective as barbiturates in the treatment of intracranial hypertension (Meyer et al. 2010; Stewart et al. 1994). Unfortunately, no comparative trials are available.

Induced hypothermia has been investigated as a neuroprotective strategy in many disease states, and is currently the standard of care for comatose patients following cardiac arrest (Bernard & Buist 2003; Mild therapeutic hypothermia to improve the neurologic outcome after cardiac arrest 2002; Bernard et al. 2002). Moderate hypothermia has been shown to decrease the cerebral metabolic rate considerably (Rosomoff & Holaday 1954). In models of ischemia, it serves to decrease the concentrations of lactate and excitotoxic neurotransmitters (Illievich et al. 1994). Although clinical trials have failed to demonstrate an outcome benefit for early hypothermia in traumatic brain injury (Clifton et al. 2001), there is ample evidence that moderate hypothermia is an effective intervention for intracranial hypertension (Meyer et al. 2010). Most investigators used a target temperature between 33-35^{o}C, which can be achieved using a variety of cooling methods (Marion et al. 1993; Marion et al. 1997; Liu et al. 2006; Qiu et al. 2007; Jiang, Yu, & Zhu 2000; Shiozaki et al. 1993). Complications of moderate hypothermia include an increased susceptibility to infection, cardiac arrhythmias, coagulopathy, and electrolyte disturbances. Iatrogenic hypothermia can also mask a fever, and potentially delay the diagnosis of an underlying infection; for that reason, the authors advocate following the white blood cell count and obtaining periodic cultures in all hypothermic patients. Shivering is often encountered during the induction and rewarming phases, and this can counteract the beneficial effects of hypothermia on ICP and cerebral metabolic rate. Pharmacological paralysis is often used to prevent shivering, but other interventions including magnesium, dexmedetomidine, and meperidine have also been

used successfully (Weant et al. 2010). Many of these complications of systemic hypothermia may be mitigated by the use of selective brain cooling devices, which are employed at some institutions (Qiu et al. 2006; Liu et al. 2006).

The duration of hypothermia therapy can vary widely depending upon the clinical needs of the individual patient; reports in the literature range from 24 hours to 14 days (McIntyre et al. 2003). The rewarming phase of therapy is critical due to the risk of mitochondrial injury, vascular dysregulation, and rebound increases in ICP (Jiang, Yu, & Zhu 2000; Jiang & Yang 2007; Povlishock & Wei 2009). Although the optimum rewarming rate has yet to be determined, most authors advocate rates of approximately 0.5-1^{o}/hour (Bernard & Buist 2003; Bernard et al. 2002; Alzaga, Cerdan, & Varon 2006). At our institution, cooling is generally performed for a minimum of 48 hours. If the patient's ICP remains well-controlled during that period, rewarming is initiated at a rate of approximately 0.5^{0}/hr. In the event of recurrent intracranial hypertension, rewarming is halted for an additional 12-24 hours before repeating the attempt.

5.3 Advanced neuromonitoring

In an effort to elucidate the mechanisms of secondary brain injury in severe TBI and improve outcomes from this highly morbid condition, a number of technologies have been developed to provide information regarding brain function, oxygenation, and perfusion. Neurophysiologic techniques such as electroencephalography have been used intermittently in brain injured patients for decades; however, recent evidence suggests that continuous monitoring may provide considerable benefit. Similarly, recent technological advances have enabled the bedside assessment of cerebral perfusion and oxygenation at the bedside. These techniques will be discussed briefly in the following sections.

5.3.1 Continuous electroencephalography

Electroencephalography (EEG) is used for a variety of indications in brain injured patients. Continuous electroencephalography (cEEG) can be employed for the diagnosis and treatment of seizures, in order to titrate barbiturate therapy, as an indicator of cerebral ischemia, and to provide information regarding prognosis. Seizures are a well known complication of TBI, and can contribute to ongoing secondary brain injury (Claassen et al. 2004; Vespa et al. 1999). The incidence of electrographic seizures in severely head injured patients has been reported at 22-33%, with more than half of these events being clinically silent (Vespa et al. 1999; Ronne-Engstrom & Winkler 2006). Moreover, investigators utilizing intracortical electroencephalography report recording several seizures which were occult on simultaneous scalp EEG (Waziri et al. 2009). Seizures are both more likely and more difficult to detect in comatose patients, suggesting that these patients are most likely to benefit from continuous EEG (cEEG) monitoring (Claassen et al. 2004). Early post-traumatic seizures have been associated with adverse physiological events, including elevation of intracranial pressure and an increase in the lactate to pyruvate ratio (Vespa et al. 2007). There is no clear association between isolated post-traumatic seizures and increased mortality; however, case series suggest that post-traumatic status epilepticus carries a high risk of death (Vespa et al. 1999; Bratton et al. 2007). Although most investigators advocate treatment of early post-traumatic seizures as a means for reducing secondary brain injury, it remains to be determined if this intervention improves patient outcome.

5.3.2 Cerebral blood flow and brain tissue oxygenation

The recognition of cerebral ischemia as a major contributing factor to secondary brain injury has generated considerable interest in measurement of cerebral perfusion in head injured patients. A number of imaging techniques, including transcranial Doppler ultrasound, xenon-enhanced CT, CT perfusion imaging, perfusion weighted MRI, and positron emitted tomography are available to evaluate cerebral perfusion. Using these techniques, investigators have demonstrated that cerebral blood flow following head trauma follows three distinct hemodynamic phases: initial hypoperfusion, accompanied by reduced cerebral metabolic rate of oxygen ($CMRO_2$); subsequent hyperemia, without an associated increase in $CMRO_2$, which may be associated with intracranial hypertension; followed by a period in which cerebral blood flow (CBF) may be reduced secondary to vasospasm (Martin et al. 1997). However, the physiological and pathophysiological significance of these phases has been debated, and significant regional variation of cerebral perfusion is present (Wintermark et al. 2004). As discussed previously, cerebral perfusion can be significantly affected by systemic factors that impact the MAP and the CPP; the magnitude of this effect is dependent upon the individual's autoregulatory status (Czosnyka et al. 1998; Howells et al. 2005). Furthermore, the reports of the incidence and significance of cerebral vasospasm after trauma are variable, and likely are affected by the mechanism of injury (Martin et al. 1997; Zubkov et al. 2000). MR, CT, and PET based imaging techniques provide information regarding cerebral perfusion over large regions of the brain parenchyma, but for the most part the data obtained is useful only for a single time point. Transcranial Doppler can be performed for extended periods of time, but it is limited to the evaluation of the large vessels surrounding the Circle of Willis, and image quality is highly operator-dependent. More recently, parenchymal monitors have been used to provide a continuous measurement of cerebral blood flow in a specific, small region of tissue using thermal diffusion. This device is placed in the perilesional white matter via a burr hole in the skull. Use of CBF monitoring is currently employed in some centers and may provide a method for continuous assessment of cerebrovascular autoregulation in the future (Rosenthal et al. 2011).

In addition to CBF, brain oxygenation is often used as an indicator of cerebral ischemia. For decades, brain oxygenation could be assessed only indirectly, by intermittent sampling of the jugular venous blood. However, in the 1990s, a fiber-optic catheter which permitted continuous assessment of jugular venous oxygen saturation ($SjvO_2$) became available (Ritter et al. 1996). $SjvO_2$ provides a global measure of cerebral oxygenation and can permit early detection of cerebral ischemia. Studies have shown that jugular venous desaturations (< 50%) are correlated with poor outcome, especially if prolonged and/or recurrent (Gopinath et al. 1994). However, cerebral metabolism in TBI demonstrates regional heterogeneity, and a global measure such as $SjvO_2$ may not permit detection of focal regions of ischemia (Valadka et al. 2000). More recently, intraparenchymal monitors have become available for the purpose of monitoring local brain tissue oxygen tension ($PbrO_2$). These monitors are typically inserted in normal-appearing brain tissue adjacent to a focal lesion, or in the right frontal region in the setting of diffuse axonal injury. As with $SjvO_2$ monitoring, episodes of desaturation detected by $PbrO_2$ monitoring have been shown to correlate with increased mortality (Stiefel et al. 2005). The critical value appears to be approximately 15 mmHg, though some clinicians prefer a treatment threshold of 20 mmHg (Bratton et al. 2007). Various investigators have studied the relationships between $PbrO_2$ and more conventional clinical parameters. In particular, positive correlations have been described between $PbrO_2$ and various determinants of oxygen supply to the CNS, including FiO_2, PaO_2, CPP, and

hemoglobin concentration (Stiefel et al. 2005). However, studies using PET imaging and monitoring of the arteriovenous oxygen tension difference have provided evidence that barriers to local oxygen diffusion, rather than determinants of oxygen supply, are significant determinants of $PbrO_2$ (Rosenthal et al. 2008; Bratton et al. 2007).

A drop in cerebral oxygenation (as measured by $PbrO_2$ or $SjvO_2$) should prompt an investigation for an underlying cause, such subclinical seizures; an expanding mass lesion; suboptimal CPP; or systemic derangements such as hypotension or worsening lung function. The two monitoring methods are often complementary. For instance, $PbrO_2$ is more sensitive to changes in the arterial oxygen content, and would be more likely to signal the expansion of a nearby contusion (Valadka et al. 2000). Cerebral oxygen desaturation can be successfully treated using measures designed to augment oxygen delivery, such as optimizing the CPP, increasing the fraction of inspired oxygen (FiO_2), and transfusion of red blood cells depending upon the patient's specific clinical characteristics; however, these interventions carry some risk. Normobaric hyperoxia has been shown to improve $PbrO_2$ as well as some other markers of brain metabolism, but prolonged use exposes the patient to the risk of oxygen toxicity (Tisdall et al. 2008). Similarly, the optimal transfusion threshold for brain injured patients has yet to be determined, and injudicious use of blood products carries the risk of transfusion related lung injury, as well as other potential complications. A recent trial of 70 patients reported that a group of patients with severe TBI who received $PbrO_2$ guided therapy (goal > 20 mmHg) demonstrated improved mortality and better functional outcomes than those managed using traditional ICP (< 20 mmHg) and CPP goals (> 60 mmHg)(Spiotta et al. 2010). However, this trial relied on historical controls, and the authors did not report on the incidence of ARDS or other medical complications in either cohort. A multicenter randomized controlled trial addressing the use of $PbrO_2$ monitoring in severe TBI is currently underway.

6. Surgical management

Surgical intervention is indicated in TBI for control of intracranial hypertension or restoring integrity of structures covering the brain as in compound injuries. High ICP, following TBI, can result from a focal intracranial hematoma or diffuse brain swelling. "Moderate" sized contusion hematomas can present the surgeon with a dilemma about whether to operate or observe the patient. Presence of alcohol or drugs on board can further compound the situation. Close clinical observation, ICP monitoring, and a follow-up head CT scan can help to guide the therapy, but ultimately surgeon's experience and clinical judgment plays a crucial role in decision making.

Evidence based guidelines are available to help with decision-making (Bullock et al. 2006, 2006; Bullock et al. 2006, 2006, 2006). Any mass lesion with progressive neurological decline referable to the lesion should be operated upon. A supratentorial lesion with a midline shift of 5 mm or more and effacement of the basal cisterns, and a posterior fossa lesion with compression or distortion of fourth ventricle or effacement of basal cisterns or obstructive hydrocephalus in patients with GCS of 8 or less should be evacuated. Epidural hematomas (EDH) larger than 30 cm^3 and subdural hematomas greater than 10mm in thickness or causing more than 5 mm of midline shift should be evacuated, independent of the GCS. An EDH less than 30 cm^3, *and* less than 15mm in maximal thickness, *and* with less than 5 mm midline shift, in a patient with a GCS of 9 or more *without* a focal deficit, can be managed non-surgically with close monitoring and repeat CT scans. Frontal or temporal lobe

contusions greater than 20 cm^3 associated with midline shift of 5 mm or more, in patients with GCS of 6 to 8 or any parenchymal lesion greater than 50 cm^3 should be operated upon. An open (compound) cranial fracture depressed greater than the thickness of cranium should be operated upon to prevent infection. An open (compound) depressed fracture *may* be treated non surgically if it is not depressed more than 1 cm, there is no dural penetration, no intracranial mass lesion, no frontal sinus involvement, no pneumocephalus, no cosmetic deformity, and no wound contamination or infection.

Decompressive craniectomy (DC) is being increasingly used for patients with severe diffuse TBI and medically refractory intracranial hypertension (Ahmad & Bullock 2011). DC is effective in reducing the ICP and increasing the survival, but has not been shown to improve long term neurological outcome(Li, Timofeev et al. 2010). In fact, there is strong evidence to suggest that DC in patients with severe TBI (GCS 3-8) with medically refractory intracranial hypertension is associated with a worse neurological outcome at 6 months after injury compared to similar patients who were treated with controlled ventilation, mannitol, hypertonic saline, external ventricular drainage, mild hypothermia and barbiturates(Cooper et al. 2011). DC for treatment of medically refractory intracranial hypertension, in severe diffuse TBI, is discouraged till parameters other than ICP control are available, to gauge the success of therapy (Marion 2011).This procedure can still be an effective therapy in very select group of patients such as those undergoing craniotomy for intracranial hematoma (particularly SDH) with significant brain swelling. Unwarranted use of DC may prove counterproductive in terms of neurological recovery in patients with severe TBI.

7. Future directions

7.1 Microdialysis

Microdialysis is an invasive monitoring technique which allows sampling of the extracellular fluid within the brain parenchyma in order to test for a variety of substances. Although a wide variety of substances can be analyzed successfully, glucose, lactate, pyruvate, glycerol, and glutamate are the most commonly employed. Glycerol is monitored primarily as a marker of membrane breakdown, whereas glucose, lactate, pyruvate, and the lactate/pyruvate ratio are used as markers of cerebral energy metabolism. The lactate/pyruvate ratio, in particular, is often employed as an indicator of the brain redox state and an early sign of focal ischemia. Microdialysis probes are usually placed in the frontal white matter in patients with diffuse brain injury, or in the pericontusional tissue in patients with focal radiographic abnormalities (Bellander et al. 2004). Although cerebral microdialysis has been widely used in animal studies for more than 30 years, its use in clinical practice is limited primarily to large academic centers where it is generally used in conjunction with other monitoring devices to provide an early indicator of evolving secondary brain injury. For instance, in cases of cerebral ischemia, the lacate/pyruvate ratio increases markedly, whereas the glucose level declines to near zero. These findings have been shown to correlate with $SjVO_2$ and $PbrO_2$ measurements, as well as the oxygen extraction fraction as measured by PET (Robertson et al. 1995; Valadka et al. 1998; Hutchinson et al. 2002). When used in conjunction with CPP and ICP monitoring, microdialysis may have a role in early detection of focal (as opposed to global) pathological processes, such as vasospasm or expanding contusions (Valadka & Robertson 2007; Hillered, Vespa, & Hovda 2005).

The data acquired by centers that utilize microdialysis routinely provides considerable insight into the pathophysiology of secondary brain injury in this population. For instance, Vespa et al. have reported persistently low glucose levels in cerebral microdialysate in the first 50 hours following brain injury in patients with poor outcome, which was unrelated to cerebral ischemia (Vespa et al. 2003). This may reflect a disorder of energy metabolism related to ongoing brain injury in patients with severe TBI. Work from the same group has demonstrated that intensive insulin therapy, as practiced in many surgical ICUs, often results in evidence of metabolic stress in patients with TBI (Vespa et al. 2006). Investigators studying the relationship between CPP on cerebral glucose metabolism have noted significant increases in the lactate/pyruvate ratio at CPPs less than 50mmHg which were confined to perilesional tissue. This work would seem to support the contention that susceptibility to ischemic insult is greater in a region of "tissue at risk" adjacent to contused brain (Nordstrom et al. 2003). Data on hyperventilated patients have been conflicting, with some studies reporting no effect (Letarte et al. 1999). In contrast, another study demonstrated that increased glutamate and lactate/pyruvate ratio could be demonstrated in TBI patients hyperventilated within 36 hours of injury, though these changes were seen much less commonly at later time points (Marion et al. 2002). This observation reinforces the hypothesis that sustained hyperventilation is particularly detrimental in the early stages following TBI.

Microdialysis data has also been used to investigate the physiological effects of various therapeutic interventions. Hyperbaric oxygen therapy has been shown to result in a reproducible decrease in lactate and lactate/pyruvate ratio, which was accompanied by improvements in cerebral blood flow, $CMRO_2$, and ICP (Rockswold et al. 2010). Similarly, microdialysis has been used to investigate the effect of barbiturates and propofol on cerebral energy metabolism. Thiopental coma was shown to reduce lactate in a small number of TBI patients, suggesting reduced anaerobic metabolism (Goodman et al. 1996). In contrast, propofol was not shown to have any significant effect on lactate, pyruvate, or glucose concentrations even when titrated to burst suppression (Johnston et al. 2003). Although both studies involved small numbers of patients, this would seem to suggest that barbiturates are more effective for the prevention of secondary brain injury.

7.2 Biomarkers

One of the major goals of ongoing research in TBI is the identification of reliable biomarkers. The ideal biomarker would enable early diagnosis of underlying brain injury, provide an early indicator of secondary brain injury, and enable clinicians to monitor the patient's response to therapy. Conceivably, use of a variety of biomarkers specific to different tissue types or mechanisms of injury may help to guide therapy and provide insight into ongoing pathophysiology. Over the past 40 years, a variety of candidate molecules have been evaluated in both clinical settings and in animal models. Perhaps the most widely studied is S100B, a calcium-binding protein that is found in astrocytes. S100B has a half-life of < 60 min in serum; consequently, elevated levels are unlikely to persist for more than 24 hours in the absence of severe TBI (Berger 2006). This protein can be quantified in the serum and in the CSF; the peak serum levels appear to occur roughly 48 hours after the peak CSF concentration is reached (Petzold et al. 2003). Elevated levels of S100B have been shown to correlate with poor outcome, contusion volume, and (inversely) with certain quality of life measures (Raabe et al. 1998; Woertgen, Rothoerl, & Brawanski 2002). Unfortunately, the

time course of S100B release, the dynamics of transport across the blood-brain-barrier, and the patho- physiological implications of elevated S100B levels remain to be determined (Kleindienst et al. 2010). Other candidate biomarkers associated with outcome in TBI patients include neuron specific enlace, a cytoplasm protein found in neuronal tissue, and glial fibrillary acidic protein (GFAP), an intermediate filament protein found in astrocytes (Vos et al. 2004) GFAP, in particular, has been shown to correlate with CT findings and with outcome in trauma patients (Lumpkins et al. 2008; Nylen et al. 2006). Although no large scale studies are available, some investigators suggest that GFAP may prove to be more sensitive than neuron-specific enolase (NSE) or S100B, due to the lack of extracerebral sources (Honda et al. 2010).

Whereas NSE, S100B, and GFAP are markers of cellular breakdown, certain other candidate biomarkers serve as indicators of ongoing pathophysiological processes. For instance, diffuse axonal injury is characterized pathologically by axonal degradation, which is associated with the breakdown of axonal microtubules. This results in the release of microtubule-associated protein tau, which is cleaved into fragments which are known as cleaved-tau (C-tau) (Li, Li et al. 2010). The presence of C-tau in the CSF has been found to be a sensitive indicator of diffuse axonal injury, and levels correlate inversely with clinical improvement (Zemlan et al. 1999). Other putative biomarkers have the potential to allow investigators to differentiate between cell death related to apoptosis as opposed to necrosis. The alpha-II spectrin protein, for example, is a component of the axonal cytoskeleton which is cleaved by enzymes involved in both cellular necrosis (caspase-3) and apoptosis (calpain 1 and 2). Western blot analysis of the alpha-II spectrin breakdown products present in a given sample can therefore provide insight into the predominant mechanism of cellular loss (Li, Li et al. 2010). Preliminary studies using alpha-II spectrin breakdown products in humans have demonstrated that necrosis, mediated by calpain, is the predominant mechanism involved in acute DAI, suggesting that calpain inhibitors may be a potential therapeutic target (Brophy et al. 2009).

7.3 Pharmacotherapy

A major factor contributing to intractable intracranial hypertension following severe TBI is cerebral edema. Both cytotoxic and vasogenic edema contribute to post traumatic cerebral edema (Barzo et al. 1997). Therapeutic interventions directed at mediators of cerebral edema offer potential treatment options for TBI. Aquaporins (AQPs) are integral membrane proteins that form pores in membranes of mammalian cells, and play an important role in development and resolution of cerebral edema (Pasantes-Morales & Cruz-Rangel 2010). AQPs modulation offers a potential therapeutic intervention to prevent or treat brain swelling (Manley et al. 2000; Papadopoulos & Verkman 2008; Taya et al. 2008). Matrix metalloproteinases (MMPs) are zinc dependent endopeptidases involved in the process of tissue remodeling following brain injury from stroke or trauma. MMPs are upregulated following TBI, causing disruption of BBB and cerebral edema in the early phase, followed by neurogenesis and neurovascular remodeling in the later stages of recovery. Thus selective therapeutic blocking of the detrimental effects in the early phase offers the possibility of preventing cerebral edema without disrupting the later reparative phase (Donkin & Vink 2010). A group of neuropeptides released from sensory neurons have been implicated in neurogenic inflammation. Neurogenic inflammation that includes vasodilation, plasma extravasation and neuronal hypersensitivity, has been shown to play a role in the

development of post traumatic cerebral edema. Therapeutic inhibition of neurogenic inflammation offers another potential target to prevent cerebral edema after TBI (Donkin & Vink 2010). Progesterone is another agent with potential therapeutic role in the management of TBI. Progesterone receptors are distributed throughout the central nervous system, and the steroid has neuroprotective properties. Progesterone decreases brain edema, attenuates free radicals and reduces neuronal loss in TBI animal model. There is class II evidence to suggest it may improve neurologic outcome in patients with TBI (Junpeng, Huang, & Qin 2011).

8. Conclusion

TBI is a serious disorder with significant morbidity, mortality and economic implications. It is a dynamic process, and timely intervention can prevent progression of neurological decline. Foundation of acute care is built upon prompt resuscitation and transport from the site of accident to a tertiary care facility, appropriate imaging, and intensive monitoring focused on minimization and prevention of secondary damage. Considerable progress has been made in understanding the physiology of ICP and treatment of intracranial hypertension, but control of ICP does not always translate into good neurological outcome. Better understanding of pathophysiology, identification of newer parameters of brain function, and development of innovative therapeutic modalities is required to improve the outcome from TBI.

9. References

Adams, J. H., Doyle, D., Ford, I., Gennarelli, T. A., Graham, D. I., & McLellan, D. R. (1989). Diffuse axonal injury in head injury: definition, diagnosis and grading, *Histopathology*, Vol.15, No. 1, (Jul 1989), pp. 49-59, ISSN 0309-0167

Agnelli, G., Piovella, F., Buoncristiani, P., Severi, P., Pini, M., D'Angelo, A., Beltrametti, C., Damiani, M., Andrioli, G. C., Pugliese, R., Iorio, A., & Brambilla, G. (1998). Enoxaparin plus compression stockings compared with compression stockings alone in the prevention of venous thromboembolism after elective neurosurgery, *N Engl J Med*, Vol.339, No. 2, (Jul 9 1998), pp. 80-85, ISSN 0028-4793 (Print)

Ahmad, F. U., & Bullock, R. (2011). Decompressive craniectomy for severe head injury, *World Neurosurg*, Vol.75, No. 3-4, (Mar-Apr 2011), pp. 451-453, ISSN 1878-8750

Alzaga, A. G., Cerdan, M., & Varon, J. (2006). Therapeutic hypothermia, *Resuscitation*, Vol.70, No. 3, (Sep 2006), pp. 369-380, ISSN 0300-9572

Badjatia, N., Carney, N., Crocco, T. J., Fallat, M. E., Hennes, H. M., Jagoda, A. S., Jernigan, S., Letarte, P. B., Lerner, E. B., Moriarty, T. M., Pons, P. T., Sasser, S., Scalea, T., Schleien, C. L., & Wright, D. W. (2008). Guidelines for prehospital management of traumatic brain injury 2nd edition, *Prehosp Emerg Care*, Vol.12 Suppl 1, No., 2008), pp. S1-52, ISSN 1545-0066

Badjatia, N., Strongilis, E., Gordon, E., Prescutti, M., Fernandez, L., Fernandez, A., Buitrago, M., Schmidt, J. M., Ostapkovich, N. D., & Mayer, S. A. (2008). Metabolic impact of shivering during therapeutic temperature modulation: the Bedside Shivering Assessment Scale, *Stroke*, Vol.39, No. 12, (Dec 2008), pp. 3242-3247, ISSN 1524-4628

Barzo, P., Marmarou, A., Fatouros, P., Hayasaki, K., & Corwin, F. (1997). Contribution of vasogenic and cellular edema to traumatic brain swelling measured by diffusion-

weighted imaging, *J Neurosurg*, Vol.87, No. 6, (Dec 1997), pp. 900-907, ISSN 0022-3085

Bellander, B. M., Cantais, E., Enblad, P., Hutchinson, P., Nordstrom, C. H., Robertson, C., Sahuquillo, J., Smith, M., Stocchetti, N., Ungerstedt, U., Unterberg, A., & Olsen, N. V. (2004). Consensus meeting on microdialysis in neurointensive care, *Intensive Care Med*, Vol.30, No. 12, (Dec 2004), pp. 2166-2169, ISSN 0342-4642

Berger, R. P. (2006). The use of serum biomarkers to predict outcome after traumatic brain injury in adults and children, *J Head Trauma Rehabil*, Vol.21, No. 4, (Jul-Aug 2006), pp. 315-333, ISSN 0885-9701

Bernard, S. A., & Buist, M. (2003). Induced hypothermia in critical care medicine: a review, *Crit Care Med*, Vol.31, No. 7, (Jul 2003), pp. 2041-2051, ISSN 0090-3493 (Print)

Bernard, S. A., Gray, T. W., Buist, M. D., Jones, B. M., Silvester, W., Gutteridge, G., & Smith, K. (2002). Treatment of comatose survivors of out-of-hospital cardiac arrest with induced hypothermia, *N Engl J Med*, Vol.346, No. 8, (Feb 21 2002), pp. 557-563, ISSN 1533-4406

Bhardwaj, Anish. (2007). Osmotherapy in neurocritical care, *Current Neurology and Neuroscience Reports*, Vol.7, No., 2007), pp. 513-521

Bratton, S. L., Chestnut, R. M., Ghajar, J., McConnell Hammond, F. F., Harris, O. A., Hartl, R., Manley, G. T., Nemecek, A., Newell, D. W., Rosenthal, G., Schouten, J., Shutter, L., Timmons, S. D., Ullman, J. S., Videtta, W., Wilberger, J. E., & Wright, D. W. (2007). Guidelines for the management of severe traumatic brain injury. I. Blood pressure and oxygenation, *J Neurotrauma*, Vol.24 Suppl 1, No., 2007), pp. S7-13, ISSN 0897-7151

Bratton, S. L., Chestnut, R. M., Ghajar, J., McConnell Hammond, F. F., Harris, O. A., Hartl, R., Manley, G. T., Nemecek, A., Newell, D. W., Rosenthal, G., Schouten, J., Shutter, L., Timmons, S. D., Ullman, J. S., Videtta, W., Wilberger, J. E., & Wright, D. W. (2007). Guidelines for the management of severe traumatic brain injury. V. Deep vein thrombosis prophylaxis, *J Neurotrauma*, Vol.24 Suppl 1, No., 2007), pp. S32-36, ISSN 0897-7151

Bratton, S. L., Chestnut, R. M., Ghajar, J., McConnell Hammond, F. F., Harris, O. A., Hartl, R., Manley, G. T., Nemecek, A., Newell, D. W., Rosenthal, G., Schouten, J., Shutter, L., Timmons, S. D., Ullman, J. S., Videtta, W., Wilberger, J. E., & Wright, D. W. (2007). Guidelines for the management of severe traumatic brain injury. VI. Indications for intracranial pressure monitoring, *J Neurotrauma*, Vol.24 Suppl 1, No., 2007), pp. S37-44, ISSN 0897-7151

Bratton, S. L., Chestnut, R. M., Ghajar, J., McConnell Hammond, F. F., Harris, O. A., Hartl, R., Manley, G. T., Nemecek, A., Newell, D. W., Rosenthal, G., Schouten, J., Shutter, L., Timmons, S. D., Ullman, J. S., Videtta, W., Wilberger, J. E., & Wright, D. W. (2007). Guidelines for the management of severe traumatic brain injury. VIII. Intracranial pressure thresholds, *J Neurotrauma*, Vol.24 Suppl 1, No., 2007), pp. S55-58, ISSN 0897-7151

Bratton, S. L., Chestnut, R. M., Ghajar, J., McConnell Hammond, F. F., Harris, O. A., Hartl, R., Manley, G. T., Nemecek, A., Newell, D. W., Rosenthal, G., Schouten, J., Shutter, L., Timmons, S. D., Ullman, J. S., Videtta, W., Wilberger, J. E., & Wright, D. W. (2007). Guidelines for the management of severe traumatic brain injury. X. Brain

oxygen monitoring and thresholds, *J Neurotrauma*, Vol.24 Suppl 1, No., 2007), pp. S65-70, ISSN 0897-7151

Bratton, S. L., Chestnut, R. M., Ghajar, J., McConnell Hammond, F. F., Harris, O. A., Hartl, R., Manley, G. T., Nemecek, A., Newell, D. W., Rosenthal, G., Schouten, J., Shutter, L., Timmons, S. D., Ullman, J. S., Videtta, W., Wilberger, J. E., & Wright, D. W. (2007). Guidelines for the management of severe traumatic brain injury. XIII. Antiseizure prophylaxis, *J Neurotrauma*, Vol.24 Suppl 1, No., 2007), pp. S83-86, ISSN 0897-7151

Bratton, S. L., Chestnut, R. M., Ghajar, J., McConnell Hammond, F. F., Harris, O. A., Hartl, R., Manley, G. T., Nemecek, A., Newell, D. W., Rosenthal, G., Schouten, J., Shutter, L., Timmons, S. D., Ullman, J. S., Videtta, W., Wilberger, J. E., & Wright, D. W. (2007). Guidelines for the management of severe traumatic brain injury. XIV. Hyperventilation, *J Neurotrauma*, Vol.24 Suppl 1, No., 2007), pp. S87-90, ISSN 0897-7151

Bratton, S. L., Chestnut, R. M., Ghajar, J., McConnell Hammond, F. F., Harris, O. A., Hartl, R., Manley, G. T., Nemecek, A., Newell, D. W., Rosenthal, G., Schouten, J., Shutter, L., Timmons, S. D., Ullman, J. S., Videtta, W., Wilberger, J. E., & Wright, D. W. (2007). Guidelines for the management of severe traumatic brain injury. XV. Steroids, *J Neurotrauma*, Vol.24 Suppl 1, No., 2007), pp. S91-95, ISSN 0897-7151

Brophy, G. M., Pineda, J. A., Papa, L., Lewis, S. B., Valadka, A. B., Hannay, H. J., Heaton, S. C., Demery, J. A., Liu, M. C., Tepas, J. J., 3rd, Gabrielli, A., Robicsek, S., Wang, K. K., Robertson, C. S., & Hayes, R. L. (2009). alphaII-Spectrin breakdown product cerebrospinal fluid exposure metrics suggest differences in cellular injury mechanisms after severe traumatic brain injury, *J Neurotrauma*, Vol.26, No. 4, (Apr 2009), pp. 471-479, ISSN 1557-9042

Bullock, M. R., Chesnut, R., Ghajar, J., Gordon, D., Hartl, R., Newell, D. W., Servadei, F., Walters, B. C., & Wilberger, J. (2006). Surgical management of depressed cranial fractures, *Neurosurgery*, Vol.58, No. 3 Suppl, (Mar 2006), pp. S56-60; discussion Si-iv, ISSN 1524-4040

Bullock, M. R., Chesnut, R., Ghajar, J., Gordon, D., Hartl, R., Newell, D. W., Servadei, F., Walters, B. C., & Wilberger, J. (2006). Surgical management of posterior fossa mass lesions, *Neurosurgery*, Vol.58, No. 3 Suppl, (Mar 2006), pp. S47-55; discussion Si-iv, ISSN 1524-4040

Bullock, M. R., Chesnut, R., Ghajar, J., Gordon, D., Hartl, R., Newell, D. W., Servadei, F., Walters, B. C., & Wilberger, J. (2006). Surgical management of traumatic parenchymal lesions, *Neurosurgery*, Vol.58, No. 3 Suppl, (Mar 2006), pp. S25-46; discussion Si-iv, ISSN 1524-4040

Bullock, M. R., Chesnut, R., Ghajar, J., Gordon, D., Hartl, R., Newell, D. W., Servadei, F., Walters, B. C., & Wilberger, J. E. (2006). Surgical management of acute epidural hematomas, *Neurosurgery*, Vol.58, No. 3 Suppl, (Mar 2006), pp. S7-15; discussion Si-iv, ISSN 1524-4040

Bullock, M. R., Chesnut, R., Ghajar, J., Gordon, D., Hartl, R., Newell, D. W., Servadei, F., Walters, B. C., & Wilberger, J. E. (2006). Surgical management of acute subdural hematomas, *Neurosurgery*, Vol.58, No. 3 Suppl, (Mar 2006), pp. S16-24; discussion Si-iv, ISSN 1524-4040

Chesnut, R. M., Marshall, L. F., Klauber, M. R., Blunt, B. A., Baldwin, N., Eisenberg, H. M., Jane, J. A., Marmarou, A., & Foulkes, M. A. (1993). The role of secondary brain injury in determining outcome from severe head injury, *J Trauma*, Vol.34, No. 2, (Feb 1993), pp. 216-222, ISSN 0022-5282

Chiu, W. T., Yeh, K. H., Li, Y. C., Gan, Y. H., Chen, H. Y., & Hung, C. C. (1997). Traumatic brain injury registry in Taiwan, *Neurol Res*, Vol.19, No. 3, (Jun 1997), pp. 261-264, ISSN 0161-6412

Claassen, J., Mayer, S. A., Kowalski, R. G., Emerson, R. G., & Hirsch, L. J. (2004). Detection of electrographic seizures with continuous EEG monitoring in critically ill patients, *Neurology*, Vol.62, No. 10, (May 25 2004), pp. 1743-1748, ISSN 1526-632X

Clifton, G. L., Miller, E. R., Choi, S. C., Levin, H. S., McCauley, S., Smith, K. R., Jr., Muizelaar, J. P., Wagner, F. C., Jr., Marion, D. W., Luerssen, T. G., Chesnut, R. M., & Schwartz, M. (2001). Lack of effect of induction of hypothermia after acute brain injury, *N Engl J Med*, Vol.344, No. 8, (Feb 22 2001), pp. 556-563, ISSN 0028-4793

Colantonio, A., Escobar, M. D., Chipman, M., McLellan, B., Austin, P. C., Mirabella, G., & Ratcliff, G. (2008). Predictors of postacute mortality following traumatic brain injury in a seriously injured population, *J Trauma*, Vol.64, No. 4, (Apr 2008), pp. 876-882, ISSN 1529-8809

Cook, A. M., Peppard, A., & Magnuson, B. (2008). Nutrition considerations in traumatic brain injury, *Nutr Clin Pract*, Vol.23, No. 6, (Dec-2009 Jan 2008), pp. 608-620, ISSN 0884-5336

Cooper, D. J., Rosenfeld, J. V., Murray, L., Arabi, Y. M., Davies, A. R., D'Urso, P., Kossmann, T., Ponsford, J., Seppelt, I., Reilly, P., & Wolfe, R. (2011). Decompressive craniectomy in diffuse traumatic brain injury, *N Engl J Med*, Vol.364, No. 16, (Apr 21 2011), pp. 1493-1502, ISSN 1533-4406

Corrigan, J. D., Selassie, A. W., & Orman, J. A. (2010). The epidemiology of traumatic brain injury, *J Head Trauma Rehabil*, Vol.25, No. 2, (Mar-Apr 2010), pp. 72-80, ISSN 1550-509X

Czosnyka, M., Balestreri, M., Steiner, L., Smielewski, P., Hutchinson, P. J., Matta, B., & Pickard, J. D. (2005). Age, intracranial pressure, autoregulation, and outcome after brain trauma, *J Neurosurg*, Vol.102, No. 3, (Mar 2005), pp. 450-454, ISSN 0022-3085

Czosnyka, M., Smielewski, P., Kirkpatrick, P., Piechnik, S., Laing, R., & Pickard, J. D. (1998). Continuous monitoring of cerebrovascular pressure-reactivity in head injury, *Acta Neurochir Suppl*, Vol.71, No., 1998), pp. 74-77, ISSN 0065-1419 (Print)

Dewall, J. (2010). The ABCs of TBI. Evidence-based guidelines for adult traumatic brain injury care, *JEMS*, Vol.35, No. 4, (Apr 2010), pp. 54-61; quiz 63, ISSN 0197-2510

Dietrich, W. D., Alonso, O., Halley, M., & Busto, R. (1996). Delayed posttraumatic brain hyperthermia worsens outcome after fluid percussion brain injury: a light and electron microscopic study in rats, *Neurosurgery*, Vol.38, No. 3, (Mar 1996), pp. 533-541; discussion 541, ISSN 0148-396X

Dietrich, W. D., & Bramlett, H. M. (2007). Hyperthermia and central nervous system injury, *Prog Brain Res*, Vol.162, No., 2007), pp. 201-217, ISSN 0079-6123

Diringer, M. N., & Zazulia, A. R. (2004). Osmotic therapy: fact and fiction, *Neurocrit Care*, Vol.1, No. 2, 2004), pp. 219-233, ISSN 1541-6933

Donkin, J. J., & Vink, R. (2010). Mechanisms of cerebral edema in traumatic brain injury: therapeutic developments, *Curr Opin Neurol*, Vol.23, No. 3, (Jun 2010), pp. 293-299, ISSN 1473-6551

Dudley, R. R., Aziz, I., Bonnici, A., Saluja, R. S., Lamoureux, J., Kalmovitch, B., Gursahaney, A., Razek, T., Maleki, M., & Marcoux, J. (2010). Early venous thromboembolic event prophylaxis in traumatic brain injury with low-molecular-weight heparin: risks and benefits, *J Neurotrauma*, Vol.27, No. 12, (Dec 2010), pp. 2165-2172, ISSN 1557-9042

Eisenberg, H. M., Frankowski, R. F., Contant, C. F., Marshall, L. F., & Walker, M. D. (1988). High-dose barbiturate control of elevated intracranial pressure in patients with severe head injury, *J Neurosurg*, Vol.69, No. 1, (Jul 1988), pp. 15-23, ISSN 0022-3085

Eker, C., Asgeirsson, B., Grande, P. O., Schalen, W., & Nordstrom, C. H. (1998). Improved outcome after severe head injury with a new therapy based on principles for brain volume regulation and preserved microcirculation, *Crit Care Med*, Vol.26, No. 11, (Nov 1998), pp. 1881-1886, ISSN 0090-3493

Enriquez, P., & Bullock, R. (2004). Molecular and cellular mechanisms in the pathophysiology of severe head injury, *Curr Pharm Des*, Vol.10, No. 18, 2004), pp. 2131-2143, ISSN 1381-6128

Gabriel, E. J., Ghajar, J., Jagoda, A., Pons, P. T., Scalea, T., & Walters, B. C. (2002). Guidelines for prehospital management of traumatic brain injury, *J Neurotrauma*, Vol.19, No. 1, (Jan 2002), pp. 111-174, ISSN 0897-7151 (Print)

Gennarelli, T. A. (1993). Mechanisms of brain injury, *J Emerg Med*, Vol.11 Suppl 1, No., 1993), pp. 5-11, ISSN 0736-4679

Goodman, J. C., Valadka, A. B., Gopinath, S. P., Cormio, M., & Robertson, C. S. (1996). Lactate and excitatory amino acids measured by microdialysis are decreased by pentobarbital coma in head-injured patients, *J Neurotrauma*, Vol.13, No. 10, (Oct 1996), pp. 549-556, ISSN 0897-7151

Gopinath, S. P., Robertson, C. S., Contant, C. F., Hayes, C., Feldman, Z., Narayan, R. K., & Grossman, R. G. (1994). Jugular venous desaturation and outcome after head injury, *J Neurol Neurosurg Psychiatry*, Vol.57, No. 6, (Jun 1994), pp. 717-723, ISSN 0022-3050

Graham, D. I., Adams, J. H., & Gennarelli, T. A. (1988). Mechanisms of non-penetrating head injury, *Prog Clin Biol Res*, Vol.264, No., 1988), pp. 159-168, ISSN 0361-7742 (Print)

Gururaj G, Sastry Koeluri V, Chandramouli B, Subbakrishna D. 2004. Neurotrauma Registry in the NIMHANS. Bangalore, India: National Institute of Mental Health and Neurosciences.

Hartl, R., Gerber, L. M., Iacono, L., Ni, Q., Lyons, K., & Ghajar, J. (2006). Direct transport within an organized state trauma system reduces mortality in patients with severe traumatic brain injury, *J Trauma*, Vol.60, No. 6, (Jun 2006), pp. 1250-1256; discussion 1256, ISSN 0022-5282

Hartl, R., Gerber, L. M., Ni, Q., & Ghajar, J. (2008). Effect of early nutrition on deaths due to severe traumatic brain injury, *J Neurosurg*, Vol.109, No. 1, (Jul 2008), pp. 50-56, ISSN 0022-3085

Hays, A. N., Lazaridis, C., Neyens, R., Nicholas, J., Gay, S., & Chalela, J. A. (2011). Osmotherapy: use among neurointensivists, *Neurocrit Care*, Vol.14, No. 2, (Apr 2011), pp. 222-228, ISSN 1556-0961

Helmy, A., Vizcaychipi, M., & Gupta, A. K. (2007). Traumatic brain injury: intensive care management, *Br J Anaesth*, Vol.99, No. 1, (Jul 2007), pp. 32-42, ISSN 0007-0912

Hiler, M., Czosnyka, M., Hutchinson, P., Balestreri, M., Smielewski, P., Matta, B., & Pickard, J. D. (2006). Predictive value of initial computerized tomography scan, intracranial pressure, and state of autoregulation in patients with traumatic brain injury, *J Neurosurg*, Vol.104, No. 5, (May 2006), pp. 731-737, ISSN 0022-3085 (Print)

Hillered, L., Vespa, P. M., & Hovda, D. A. (2005). Translational neurochemical research in acute human brain injury: the current status and potential future for cerebral microdialysis, *J Neurotrauma*, Vol.22, No. 1, (Jan 2005), pp. 3-41, ISSN 0897-7151

Honda, M., Tsuruta, R., Kaneko, T., Kasaoka, S., Yagi, T., Todani, M., Fujita, M., Izumi, T., & Maekawa, T. (2010). Serum glial fibrillary acidic protein is a highly specific biomarker for traumatic brain injury in humans compared with S-100B and neuron-specific enolase, *J Trauma*, Vol.69, No. 1, (Jul 2010), pp. 104-109, ISSN 1529-8809

Howells, T., Elf, K., Jones, P. A., Ronne-Engstrom, E., Piper, I., Nilsson, P., Andrews, P., & Enblad, P. (2005). Pressure reactivity as a guide in the treatment of cerebral perfusion pressure in patients with brain trauma, *J Neurosurg*, Vol.102, No. 2, (Feb 2005), pp. 311-317, ISSN 0022-3085

Hutchinson, P. J., Gupta, A. K., Fryer, T. F., Al-Rawi, P. G., Chatfield, D. A., Coles, J. P., O'Connell, M. T., Kett-White, R., Minhas, P. S., Aigbirhio, F. I., Clark, J. C., Kirkpatrick, P. J., Menon, D. K., & Pickard, J. D. (2002). Correlation between cerebral blood flow, substrate delivery, and metabolism in head injury: a combined microdialysis and triple oxygen positron emission tomography study, *J Cereb Blood Flow Metab*, Vol.22, No. 6, (Jun 2002), pp. 735-745, ISSN 0271-678X

Illievich, U. M., Zornow, M. H., Choi, K. T., Scheller, M. S., & Strnat, M. A. (1994). Effects of hypothermic metabolic suppression on hippocampal glutamate concentrations after transient global cerebral ischemia, *Anesth Analg*, Vol.78, No. 5, (May 1994), pp. 905-911, ISSN 0003-2999

Jiang, J. Y., & Yang, X. F. (2007). Current status of cerebral protection with mild-to-moderate hypothermia after traumatic brain injury, *Curr Opin Crit Care*, Vol.13, No. 2, (Apr 2007), pp. 153-155, ISSN 1070-5295

Jiang, J., Yu, M., & Zhu, C. (2000). Effect of long-term mild hypothermia therapy in patients with severe traumatic brain injury: 1-year follow-up review of 87 cases, *J Neurosurg*, Vol.93, No. 4, (Oct 2000), pp. 546-549, ISSN 0022-3085

Johnston, A. J., Steiner, L. A., Chatfield, D. A., Coleman, M. R., Coles, J. P., Al-Rawi, P. G., Menon, D. K., & Gupta, A. K. (2003). Effects of propofol on cerebral oxygenation and metabolism after head injury, *Br J Anaesth*, Vol.91, No. 6, (Dec 2003), pp. 781-786, ISSN 0007-0912

Johnston, N. J., King, A. T., Protheroe, R., & Childs, C. (2006). Body temperature management after severe traumatic brain injury: methods and protocols used in the United Kingdom and Ireland, *Resuscitation*, Vol.70, No. 2, (Aug 2006), pp. 254-262, ISSN 0300-9572

Junpeng, M., Huang, S., & Qin, S. (2011). Progesterone for acute traumatic brain injury, *Cochrane Database Syst Rev*, No. 1, 2011), p. CD008409, ISSN 1469-493X

Juul, N., Morris, G. F., Marshall, S. B., & Marshall, L. F. (2000). Intracranial hypertension and cerebral perfusion pressure: influence on neurological deterioration and outcome in

severe head injury. The Executive Committee of the International Selfotel Trial, *J Neurosurg*, Vol.92, No. 1, (Jan 2000), pp. 1-6, ISSN 0022-3085

Kassell, N. F., Hitchon, P. W., Gerk, M. K., Sokoll, M. D., & Hill, T. R. (1980). Alterations in cerebral blood flow, oxygen metabolism, and electrical activity produced by high dose sodium thiopental, *Neurosurgery*, Vol.7, No. 6, (Dec 1980), pp. 598-603, ISSN 0148-396X

Kassell, N. F., Peerless, S. J., Drake, C. G., Boarini, D. J., & Adams, H. P. (1980). Treatment of ischemic deficits from cerebral vasospasm with high dose barbiturate therapy, *Neurosurgery*, Vol.7, No. 6, (Dec 1980), pp. 593-597, ISSN 0148-396X

Kleindienst, A., Meissner, S., Eyupoglu, I. Y., Parsch, H., Schmidt, C., & Buchfelder, M. (2010). Dynamics of S100B release into serum and cerebrospinal fluid following acute brain injury, *Acta Neurochir Suppl*, Vol.106, No., 2010), pp. 247-250, ISSN 0065-1419

Langlois JA, Rutland-Brown W, Thomas KE. 2006. Traumatic Brain Injury in the United States: Emergency Department Visits, Hospitalization and Deaths. Atlanta, GA: Centers for Disease Control and Prevention, National Center for Injury Control and Prevention.

Leker, R. R., & Shohami, E. (2002). Cerebral ischemia and trauma-different etiologies yet similar mechanisms: neuroprotective opportunities, *Brain Res Brain Res Rev*, Vol.39, No. 1, (Jun 2002), pp. 55-73

Letarte, P. B., Puccio, A. M., Brown, S. D., & Marion, D. W. (1999). Effect of hypocapnea on CBF and extracellular intermediates of secondary brain injury, *Acta Neurochir Suppl*, Vol.75, No., 1999), pp. 45-47, ISSN 0065-1419

Li, J., Li, X. Y., Feng, D. F., & Pan, D. C. (2010). Biomarkers associated with diffuse traumatic axonal injury: exploring pathogenesis, early diagnosis, and prognosis, *J Trauma*, Vol.69, No. 6, (Dec 2010), pp. 1610-1618, ISSN 1529-8809

Li, L. M., Timofeev, I., Czosnyka, M., & Hutchinson, P. J. (2010). Review article: the surgical approach to the management of increased intracranial pressure after traumatic brain injury, *Anesth Analg*, Vol.111, No. 3, (Sep 2010), pp. 736-748, ISSN 1526-7598

Liu-DeRyke, X., Collingridge, D. S., Orme, J., Roller, D., Zurasky, J., & Rhoney, D. H. (2009). Clinical impact of early hyperglycemia during acute phase of traumatic brain

Liu, W. G., Qiu, W. S., Zhang, Y., Wang, W. M., Lu, F., & Yang, X. F. (2006). Effects of selective brain cooling in patients with severe traumatic brain injury: a preliminary study, *J Int Med Res*, Vol.34, No. 1, (Jan-Feb 2006), pp. 58-64, ISSN 0300-0605

Lumpkins, K. M., Bochicchio, G. V., Keledjian, K., Simard, J. M., McCunn, M., & Scalea, T. (2008). Glial fibrillary acidic protein is highly correlated with brain injury, *J Trauma*, Vol.65, No. 4, (Oct 2008), pp. 778-782; discussion 782-774, ISSN 1529-8809

Manley, G. T., Fujimura, M., Ma, T., Noshita, N., Filiz, F., Bollen, A. W., Chan, P., & Verkman, A. S. (2000). Aquaporin-4 deletion in mice reduces brain edema after acute water intoxication and ischemic stroke, *Nat Med*, Vol.6, No. 2, (Feb 2000), pp. 159-163, ISSN 1078-8956

Marion, D. W. (2011). Decompressive craniectomy in diffuse traumatic brain injury, *Lancet Neurol*, Vol.10, No. 6, (Jun 2011), pp. 497-498, ISSN 1474-4465

Marion, D. W., Obrist, W. D., Carlier, P. M., Penrod, L. E., & Darby, J. M. (1993). The use of moderate therapeutic hypothermia for patients with severe head injuries: a

preliminary report, *J Neurosurg*, Vol.79, No. 3, (Sep 1993), pp. 354-362, ISSN 0022-3085

Marion, D. W., Penrod, L. E., Kelsey, S. F., Obrist, W. D., Kochanek, P. M., Palmer, A. M., Wisniewski, S. R., & DeKosky, S. T. (1997). Treatment of traumatic brain injury with moderate hypothermia, *N Engl J Med*, Vol.336, No. 8, (Feb 20 1997), pp. 540-546, ISSN 0028-4793

Marion, D. W., Puccio, A., Wisniewski, S. R., Kochanek, P., Dixon, C. E., Bullian, L., & Carlier, P. (2002). Effect of hyperventilation on extracellular concentrations of glutamate, lactate, pyruvate, and local cerebral blood flow in patients with severe traumatic brain injury, *Crit Care Med*, Vol.30, No. 12, (Dec 2002), pp. 2619-2625, ISSN 0090-3493

Marshall, G. T., James, R. F., Landman, M. P., O'Neill, P. J., Cotton, B. A., Hansen, E. N., Morris, J. A., Jr., & May, A. K. (2010). Pentobarbital coma for refractory intra-cranial hypertension after severe traumatic brain injury: mortality predictions and one-year outcomes in 55 patients, *J Trauma*, Vol.69, No. 2, (Aug 2010), pp. 275-283, ISSN 1529-8809

Martin, N. A., Patwardhan, R. V., Alexander, M. J., Africk, C. Z., Lee, J. H., Shalmon, E., Hovda, D. A., & Becker, D. P. (1997). Characterization of cerebral hemodynamic phases following severe head trauma: hypoperfusion, hyperemia, and vasospasm, *J Neurosurg*, Vol.87, No. 1, (Jul 1997), pp. 9-19, ISSN 0022-3085

McIntyre, L. A., Fergusson, D. A., Hebert, P. C., Moher, D., & Hutchison, J. S. (2003). Prolonged therapeutic hypothermia after traumatic brain injury in adults: a systematic review, *JAMA*, Vol.289, No. 22, (Jun 11 2003), pp. 2992-2999, ISSN 1538-3598

Meyer, M. J., Megyesi, J., Meythaler, J., Murie-Fernandez, M., Aubut, J. A., Foley, N., Salter, K., Bayley, M., Marshall, S., & Teasell, R. (2010). Acute management of acquired brain injury part I: an evidence-based review of non-pharmacological interventions, *Brain Inj*, Vol.24, No. 5, 2010), pp. 694-705, ISSN 1362-301X

Meyer, M. J., Megyesi, J., Meythaler, J., Murie-Fernandez, M., Aubut, J. A., Foley, N., Salter, K., Bayley, M., Marshall, S., & Teasell, R. (2010). Acute management of acquired brain injury part II: an evidence-based review of pharmacological interventions, *Brain Inj*, Vol.24, No. 5, 2010), pp. 706-721, ISSN 1362-301X (Electronic)

Mild therapeutic hypothermia to improve the neurologic outcome after cardiac arrest. (2002). *N Engl J Med*, Vol.346, No. 8, (Feb 21 2002), pp. 549-556, ISSN 1533-4406

Mishina, H., & Yabuki, A. (1994). Relationship of cerebral blood flow, cerebral metabolism, and electroencephalography to outcome in acute experimental compression ischemia--barbiturate effects on delayed brain swelling, *Neurol Med Chir (Tokyo)*, Vol.34, No. 6, (Jun 1994), pp. 345-352, ISSN 0470-8105

Muizelaar, J. P., van der Poel, H. G., Li, Z. C., Kontos, H. A., & Levasseur, J. E. (1988). Pial arteriolar vessel diameter and CO2 reactivity during prolonged hyperventilation in the rabbit, *J Neurosurg*, Vol.69, No. 6, (Dec 1988), pp. 923-927, ISSN 0022-3085

Narayan, R. K., Michel, M. E., Ansell, B., Baethmann, A., Biegon, A., Bracken, M. B., Bullock, M. R., Choi, S. C., Clifton, G. L., Contant, C. F., Coplin, W. M., Dietrich, W. D., Ghajar, J., Grady, S. M., Grossman, R. G., Hall, E. D., Heetderks, W., Hovda, D. A., Jallo, J., Katz, R. L., Knoller, N., Kochanek, P. M., Maas, A. I., Majde, J., Marion, D. W., Marmarou, A., Marshall, L. F., McIntosh, T. K., Miller, E., Mohberg, N.,

Muizelaar, J. P., Pitts, L. H., Quinn, P., Riesenfeld, G., Robertson, C. S., Strauss, K. I., Teasdale, G., Temkin, N., Tuma, R., Wade, C., Walker, M. D., Weinrich, M., Whyte, J., Wilberger, J., Young, A. B., & Yurkewicz, L. (2002). Clinical trials in head injury, *J Neurotrauma*, Vol.19, No. 5, (May 2002), pp. 503-557, ISSN 0897-7151

Nordstrom, C. H., Reinstrup, P., Xu, W., Gardenfors, A., & Ungerstedt, U. (2003). Assessment of the lower limit for cerebral perfusion pressure in severe head injuries by bedside monitoring of regional energy metabolism, *Anesthesiology*, Vol.98, No. 4, (Apr 2003), pp. 809-814, ISSN 0003-3022

Norwood, S. H., Berne, J. D., Rowe, S. A., Villarreal, D. H., & Ledlie, J. T. (2008). Early venous thromboembolism prophylaxis with enoxaparin in patients with blunt traumatic brain injury, *J Trauma*, Vol.65, No. 5, (Nov 2008), pp. 1021-1026; discussion 1026-1027, ISSN 1529-8809

Nurmohamed, M. T., van Riel, A. M., Henkens, C. M., Koopman, M. M., Que, G. T., d'Azemar, P., Buller, H. R., ten Cate, J. W., Hoek, J. A., van der Meer, J., van der Heul, C., Turpie, A. G., Haley, S., Sicurella, A., & Gent, M. (1996). Low molecular weight heparin and compression stockings in the prevention of venous thromboembolism in neurosurgery, *Thromb Haemost*, Vol.75, No. 2, (Feb 1996), pp. 233-238, ISSN 0340-6245

Nylen, K., Ost, M., Csajbok, L. Z., Nilsson, I., Blennow, K., Nellgard, B., & Rosengren, L. (2006). Increased serum-GFAP in patients with severe traumatic brain injury is related to outcome, *J Neurol Sci*, Vol.240, No. 1-2, (Jan 15 2006), pp. 85-91, ISSN 0022-510X

Ogden, Alfred T., Mayer, Stephan A., & E. Sander Connolly, Jr. (2005). Hyperosmolar agents in neurosurgical practice: the evolving role of hypertonic saline *Neurosurgery*, Vol.57, No. 2, 2005), pp. 207-215

Papadopoulos, M. C., & Verkman, A. S. (2008). Potential utility of aquaporin modulators for therapy of brain disorders, *Prog Brain Res*, Vol.170, No., 2008), pp. 589-601, ISSN 1875-7855

Pasantes-Morales, H., & Cruz-Rangel, S. (2010). Brain volume regulation: osmolytes and aquaporin perspectives, *Neuroscience*, Vol.168, No. 4, (Jul 28 2010), pp. 871-884, ISSN 1873-7544

Patel, H. C., Bouamra, O., Woodford, M., King, A. T., Yates, D. W., & Lecky, F. E. (2005). Trends in head injury outcome from 1989 to 2003 and the effect of neurosurgical care: an observational study, *Lancet*, Vol.366, No. 9496, (Oct 29-Nov 4 2005), pp. 1538-1544, ISSN 1474-547X

Petzold, A., Keir, G., Lim, D., Smith, M., & Thompson, E. J. (2003). Cerebrospinal fluid (CSF) and serum S100B: release and wash-out pattern, *Brain Res Bull*, Vol.61, No. 3, (Aug 15 2003), pp. 281-285, ISSN 0361-9230

Povlishock, J. T., & Wei, E. P. (2009). Posthypothermic rewarming considerations following traumatic brain injury, *J Neurotrauma*, Vol.26, No. 3, (Mar 2009), pp. 333-340, ISSN 1557-9042

Qiu, W., Shen, H., Zhang, Y., Wang, W., Liu, W., Jiang, Q., Luo, M., & Manou, M. (2006). Noninvasive selective brain cooling by head and neck cooling is protective in severe traumatic brain injury, *J Clin Neurosci*, Vol.13, No. 10, (Dec 2006), pp. 995-1000, ISSN 0967-5868

Qiu, W., Zhang, Y., Sheng, H., Zhang, J., Wang, W., Liu, W., Chen, K., Zhou, J., & Xu, Z. (2007). Effects of therapeutic mild hypothermia on patients with severe traumatic brain injury after craniotomy, *J Crit Care*, Vol.22, No. 3, (Sep 2007), pp. 229-235, ISSN 0883-9441

Raabe, A., Grolms, C., Keller, M., Dohnert, J., Sorge, O., & Seifert, V. (1998). Correlation of computed tomography findings and serum brain damage markers following severe head injury, *Acta Neurochir (Wien)*, Vol.140, No. 8, 1998), pp. 787-791; discussion 791-782, ISSN 0001-6268

Ritter, A. M., Gopinath, S. P., Contant, C., Narayan, R. K., & Robertson, C. S. (1996). Evaluation of a regional oxygen saturation catheter for monitoring SjvO2 in head injured patients, *J Clin Monit*, Vol.12, No. 4, (Jul 1996), pp. 285-291, ISSN 0748-1977

Robertson, C. S. (2001). Management of cerebral perfusion pressure after traumatic brain injury, *Anesthesiology*, Vol.95, No. 6, (Dec 2001), pp. 1513-1517, ISSN 0003-3022

Robertson, C. S., Gopinath, S. P., Goodman, J. C., Contant, C. F., Valadka, A. B., & Narayan, R. K. (1995). SjvO2 monitoring in head-injured patients, *J Neurotrauma*, Vol.12, No. 5, (Oct 1995), pp. 891-896, ISSN 0897-7151

Robertson, C. S., Valadka, A. B., Hannay, H. J., Contant, C. F., Gopinath, S. P., Cormio, M., Uzura, M., & Grossman, R. G. (1999). Prevention of secondary ischemic insults after severe head injury, *Crit Care Med*, Vol.27, No. 10, (Oct 1999), pp. 2086-2095, ISSN 0090-3493

Rockswold, S. B., Rockswold, G. L., Zaun, D. A., Zhang, X., Cerra, C. E., Bergman, T. A., & Liu, J. (2010). A prospective, randomized clinical trial to compare the effect of hyperbaric to normobaric hyperoxia on cerebral metabolism, intracranial pressure, and oxygen toxicity in severe traumatic brain injury, *J Neurosurg*, Vol.112, No. 5, (May 2010), pp. 1080-1094, ISSN 1933-0693

Ronne-Engstrom, E., & Winkler, T. (2006). Continuous EEG monitoring in patients with traumatic brain injury reveals a high incidence of epileptiform activity, *Acta Neurol Scand*, Vol.114, No. 1, (Jul 2006), pp. 47-53, ISSN 0001-6314

Rosenthal, G., Hemphill, J. C., 3rd, Sorani, M., Martin, C., Morabito, D., Obrist, W. D., & Manley, G. T. (2008). Brain tissue oxygen tension is more indicative of oxygen diffusion than oxygen delivery and metabolism in patients with traumatic brain injury, *Crit Care Med*, Vol.36, No. 6, (Jun 2008), pp. 1917-1924, ISSN 1530-0293

Rosenthal, G., Sanchez-Mejia, R. O., Phan, N., Hemphill, J. C., 3rd, Martin, C., & Manley, G. T. (2011). Incorporating a parenchymal thermal diffusion cerebral blood flow probe in bedside assessment of cerebral autoregulation and vasoreactivity in patients with severe traumatic brain injury, *J Neurosurg*, Vol.114, No. 1, (Jan 2011), pp. 62-70, ISSN 1933-0693

Rosomoff, H. L., & Holaday, D. A. (1954). Cerebral blood flow and cerebral oxygen consumption during hypothermia, *Am J Physiol*, Vol.179, No. 1, (Oct 1954), pp. 85-88, ISSN 0002-9513

Sacks, G. S., Brown, R. O., Teague, D., Dickerson, R. N., Tolley, E. A., & Kudsk, K. A. (1995). Early nutrition support modifies immune function in patients sustaining severe head injury, *JPEN J Parenter Enteral Nutr*, Vol.19, No. 5, (Sep-Oct 1995), pp. 387-392, ISSN 0148-6071

Schroder, M. L., Muizelaar, J. P., Bullock, M. R., Salvant, J. B., & Povlishock, J. T. (1995). Focal ischemia due to traumatic contusions documented by stable xenon-CT and

ultrastructural studies, *J Neurosurg*, Vol.82, No. 6, (Jun 1995), pp. 966-971, ISSN 0022-3085

Schwartz, M. L., Tator, C. H., Rowed, D. W., Reid, S. R., Meguro, K., & Andrews, D. F. (1984). The University of Toronto head injury treatment study: a prospective, randomized comparison of pentobarbital and mannitol, *Can J Neurol Sci*, Vol.11, No. 4, (Nov 1984), pp. 434-440, ISSN 0317-1671

Schwarz, S., Georgiadis, D., Aschoff, A., & Schwab, S. (2002). Effects of hypertonic (10%) saline in patients with raised intracranial pressure after stroke, *Stroke*, Vol.33, No. 1, (Jan 2002), pp. 136-140, ISSN 1524-4628

Seelig, J. M., Becker, D. P., Miller, J. D., Greenberg, R. P., Ward, J. D., & Choi, S. C. (1981). Traumatic acute subdural hematoma: major mortality reduction in comatose patients treated within four hours, *N Engl J Med*, Vol.304, No. 25, (Jun 18 1981), pp. 1511-1518, ISSN 0028-4793

Segatore, M. (1992). Fever after traumatic brain injury, *J Neurosci Nurs*, Vol.24, No. 2, (Apr 1992), pp. 104-109, ISSN 0888-0395

Selassie, A. W., Zaloshnja, E., Langlois, J. A., Miller, T., Jones, P., & Steiner, C. (2008). Incidence of long-term disability following traumatic brain injury hospitalization, United States, 2003, *J Head Trauma Rehabil*, Vol.23, No. 2, (Mar-Apr 2008), pp. 123-131, ISSN 0885-9701

Shiozaki, T., Sugimoto, H., Taneda, M., Yoshida, H., Iwai, A., Yoshioka, T., & Sugimoto, T. (1993). Effect of mild hypothermia on uncontrollable intracranial hypertension after severe head injury, *J Neurosurg*, Vol.79, No. 3, (Sep 1993), pp. 363-368, ISSN 0022-3085

Shohami, E., Beit-Yannai, E., Horowitz, M., & Kohen, R. (1997). Oxidative stress in closed-head injury: brain antioxidant capacity as an indicator of functional outcome, *J Cereb Blood Flow Metab*, Vol.17, No. 10, (Oct 1997), pp. 1007-1019, ISSN 0271-678X

Sirvent, J. M., Torres, A., El-Ebiary, M., Castro, P., de Batlle, J., & Bonet, A. (1997). Protective effect of intravenously administered cefuroxime against nosocomial pneumonia in patients with structural coma, *Am J Respir Crit Care Med*, Vol.155, No. 5, (May 1997), pp. 1729-1734, ISSN 1073-449X

Skandsen, T., Kvistad, K. A., Solheim, O., Strand, I. H., Folvik, M., & Vik, A. (2010). Prevalence and impact of diffuse axonal injury in patients with moderate and severe head injury: a cohort study of early magnetic resonance imaging findings and 1-year outcome, *J Neurosurg*, Vol.113, No. 3, (Sep 2010), pp. 556-563, ISSN 1933-0693

Smith, A. L., Hoff, J. T., Nielsen, S. L., & Larson, C. P. (1974). Barbiturate protection in acute focal cerebral ischemia, *Stroke*, Vol.5, No. 1, (Jan-Feb 1974), pp. 1-7, ISSN 0039-2499

Soares, H. D., Hicks, R. R., Smith, D., & McIntosh, T. K. (1995). Inflammatory leukocytic recruitment and diffuse neuronal degeneration are separate pathological processes resulting from traumatic brain injury, *J Neurosci*, Vol.15, No. 12, (Dec 1995), pp. 8223-8233, ISSN 0270-6474

Spiotta, A. M., Stiefel, M. F., Gracias, V. H., Garuffe, A. M., Kofke, W. A., Maloney-Wilensky, E., Troxel, A. B., Levine, J. M., & Le Roux, P. D. (2010). Brain tissue oxygen-directed management and outcome in patients with severe traumatic brain injury, *J Neurosurg*, Vol.113, No. 3, (Sep 2010), pp. 571-580, ISSN 1933-0693

Stein, S. C., Georgoff, P., Meghan, S., Mizra, K., & Sonnad, S. S. (2010). 150 years of treating severe traumatic brain injury: a systematic review of progress in mortality, *J Neurotrauma*, Vol.27, No. 7, (Jul 2010), pp. 1343-1353, ISSN 1557-9042

Stewart, L., Bullock, R., Rafferty, C., Fitch, W., & Teasdale, G. M. (1994). Propofol sedation in severe head injury fails to control high ICP, but reduces brain metabolism, *Acta Neurochir Suppl (Wien)*, Vol.60, No., 1994), pp. 544-546, ISSN 0065-1419

Stiefel, M. F., Spiotta, A., Gracias, V. H., Garuffe, A. M., Guillamondegui, O., Maloney-Wilensky, E., Bloom, S., Grady, M. S., & LeRoux, P. D. (2005). Reduced mortality rate in patients with severe traumatic brain injury treated with brain tissue oxygen monitoring, *J Neurosurg*, Vol.103, No. 5, (Nov 2005), pp. 805-811, ISSN 0022-3085

Tagliaferri, F., Compagnone, C., Korsic, M., Servadei, F., & Kraus, J. (2006). A systematic review of brain injury epidemiology in Europe, *Acta Neurochir (Wien)*, Vol.148, No. 3, (Mar 2006), pp. 255-268; discussion 268, ISSN 0001-6268 (Print)

Taya, K., Gulsen, S., Okuno, K., Prieto, R., Marmarou, C. R., & Marmarou, A. (2008). Modulation of AQP4 expression by the selective V1a receptor antagonist, SR49059, decreases trauma-induced brain edema, *Acta Neurochir Suppl*, Vol.102, No., 2008), pp. 425-429, ISSN 0065-1419

Taylor, S. J., Fettes, S. B., Jewkes, C., & Nelson, R. J. (1999). Prospective, randomized, controlled trial to determine the effect of early enhanced enteral nutrition on clinical outcome in mechanically ventilated patients suffering head injury, *Crit Care Med*, Vol.27, No. 11, (Nov 1999), pp. 2525-2531, ISSN 0090-3493

Tisdall, M. M., Tachtsidis, I., Leung, T. S., Elwell, C. E., & Smith, M. (2008). Increase in cerebral aerobic metabolism by normobaric hyperoxia after traumatic brain injury, *J Neurosurg*, Vol.109, No. 3, (Sep 2008), pp. 424-432, ISSN 0022-3085

Valadka, A. B., Furuya, Y., Hlatky, R., & Robertson, C. S. (2000). Global and regional techniques for monitoring cerebral oxidative metabolism after severe traumatic brain injury, *Neurosurg Focus*, Vol.9, No. 5, 2000), p. e3, ISSN 1092-0684

Valadka, A. B., Goodman, J. C., Gopinath, S. P., Uzura, M., & Robertson, C. S. (1998). Comparison of brain tissue oxygen tension to microdialysis-based measures of cerebral ischemia in fatally head-injured humans, *J Neurotrauma*, Vol.15, No. 7, (Jul 1998), pp. 509-519, ISSN 0897-7151

Valadka, A. B., & Robertson, C. S. (2007). Surgery of cerebral trauma and associated critical care, *Neurosurgery*, Vol.61, No. 1 Suppl, (Jul 2007), pp. 203-220; discussion 220-201, ISSN 1524-4040

Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. The Acute Respiratory Distress Syndrome Network. (2000). *N Engl J Med*, Vol.342, No. 18, (May 4 2000), pp. 1301-1308, ISSN 0028-4793

Vespa, P., Boonyaputthikul, R., McArthur, D. L., Miller, C., Etchepare, M., Bergsneider, M., Glenn, T., Martin, N., & Hovda, D. (2006). Intensive insulin therapy reduces microdialysis glucose values without altering glucose utilization or improving the lactate/pyruvate ratio after traumatic brain injury, *Crit Care Med*, Vol.34, No. 3, (Mar 2006), pp. 850-856, ISSN 0090-3493

Vespa, P. M., McArthur, D., O'Phelan, K., Glenn, T., Etchepare, M., Kelly, D., Bergsneider, M., Martin, N. A., & Hovda, D. A. (2003). Persistently low extracellular glucose correlates with poor outcome 6 months after human traumatic brain injury despite

a lack of increased lactate: a microdialysis study, *J Cereb Blood Flow Metab*, Vol.23, No. 7, (Jul 2003), pp. 865-877, ISSN 0271-678X

Vespa, P. M., Miller, C., McArthur, D., Eliseo, M., Etchepare, M., Hirt, D., Glenn, T. C., Martin, N., & Hovda, D. (2007). Nonconvulsive electrographic seizures after traumatic brain injury result in a delayed, prolonged increase in intracranial pressure and metabolic crisis, *Crit Care Med*, Vol.35, No. 12, (Dec 2007), pp. 2830-2836, ISSN 0090-3493

Vespa, P. M., Nuwer, M. R., Nenov, V., Ronne-Engstrom, E., Hovda, D. A., Bergsneider, M., Kelly, D. F., Martin, N. A., & Becker, D. P. (1999). Increased incidence and impact of nonconvulsive and convulsive seizures after traumatic brain injury as detected by continuous electroencephalographic monitoring, *J Neurosurg*, Vol.91, No. 5, (Nov 1999), pp. 750-760, ISSN 0022-3085

Vialet, R., Albanese, J., Thomachot, L., Antonini, F., Bourgouin, A., Alliez, B., & Martin, C. (2003). Isovolume hypertonic solutes (sodium chloride or mannitol) in the treatment of refractory posttraumatic intracranial hypertension: 2 mL/kg 7.5% saline is more effective than 2 mL/kg 20% mannitol, *Crit Care Med*, Vol.31, No. 6, (Jun 2003), pp. 1683-1687, ISSN 0090-3493

Vos, P. E., Lamers, K. J., Hendriks, J. C., van Haaren, M., Beems, T., Zimmerman, C., van Geel, W., de Reus, H., Biert, J., & Verbeek, M. M. (2004). Glial and neuronal proteins in serum predict outcome after severe traumatic brain injury, *Neurology*, Vol.62, No. 8, (Apr 27 2004), pp. 1303-1310, ISSN 1526-632X

Ward, J. D., Becker, D. P., Miller, J. D., Choi, S. C., Marmarou, A., Wood, C., Newlon, P. G., & Keenan, R. (1985). Failure of prophylactic barbiturate coma in the treatment of severe head injury, *J Neurosurg*, Vol.62, No. 3, (Mar 1985), pp. 383-388, ISSN 0022-3085

Waziri, A., Claassen, J., Stuart, R. M., Arif, H., Schmidt, J. M., Mayer, S. A., Badjatia, N., Kull, L. L., Connolly, E. S., Emerson, R. G., & Hirsch, L. J. (2009). Intracortical electroencephalography in acute brain injury, *Ann Neurol*, Vol.66, No. 3, (Sep 2009), pp. 366-377, ISSN 1531-8249

Weant, K. A., Martin, J. E., Humphries, R. L., & Cook, A. M. (2010). Pharmacologic options for reducing the shivering response to therapeutic hypothermia, *Pharmacotherapy*, Vol.30, No. 8, (Aug 2010), pp. 830-841, ISSN 1875-9114

Wintermark, M., van Melle, G., Schnyder, P., Revelly, J. P., Porchet, F., Regli, L., Meuli, R., Maeder, P., & Chiolero, R. (2004). Admission perfusion CT: prognostic value in patients with severe head trauma, *Radiology*, Vol.232, No. 1, (Jul 2004), pp. 211-220, ISSN 0033-8419

Woertgen, C., Rothoerl, R. D., & Brawanski, A. (2002). Early S-100B serum level correlates to quality of life in patients after severe head injury, *Brain Inj*, Vol.16, No. 9, (Sep 2002), pp. 807-816, ISSN 0269-9052

Young, N., Rhodes, J. K., Mascia, L., & Andrews, P. J. (2010). Ventilatory strategies for patients with acute brain injury, *Curr Opin Crit Care*, Vol.16, No. 1, (Feb 2010), pp. 45-52, ISSN 1531-7072

Zaloshnja, E., Miller, T., Langlois, J. A., & Selassie, A. W. (2008). Prevalence of long-term disability from traumatic brain injury in the civilian population of the United States, 2005, *J Head Trauma Rehabil*, Vol.23, No. 6, (Nov-Dec 2008), pp. 394-400, ISSN 1550-509X

Zemlan, F. P., Rosenberg, W. S., Luebbe, P. A., Campbell, T. A., Dean, G. E., Weiner, N. E., Cohen, J. A., Rudick, R. A., & Woo, D. (1999). Quantification of axonal damage in traumatic brain injury: affinity purification and characterization of cerebrospinal fluid tau proteins, *J Neurochem*, Vol.72, No. 2, (Feb 1999), pp. 741-750, ISSN 0022-3042

19

Clinical Neuroprotection Against Tissue Hypoxia During Brain Injuries; The Challenges and the Targets

Thomas I Nathaniel[1], Effiong Otukonyong[2], Sarah Bwint[3], Katelin Haley[3], Diane Haleem[3], Adam Brager[3] and Ayotunde Adeagbo[4]
[1]*Department of Biomedical Sciences, University of South Carolina School of Medicine - Greenville, Greenville*
[2]*Department of Health Sciences, East Tennessee State University, Johnson City*
[3]*Center for Natural and Health Sciences, Marywood University*
[4]*Department of Pharmacology and Physiology The Commonwealth Medical College, Scranton*
USA

1. Introduction

1.1 Understanding hypoxia tolerance and the challenge of clinical neuroprotection

Most of the existing research on brain hypoxia mainly focused mainly on understanding the mechanisms of neuronal death as the means of identifying targets for therapy. This approach has not been helpful in understanding how the brain of humans can be made to resist tissue hypoxia. This is a major factor that leads to neuronal death during stroke, for example. Hypoxia tolerance is a robust fundamental adaptation to low oxygen supply and represents a novel neuroscience problem with significance to mammalian physiology as well as human health. Physiological and molecular changes during hypoxia are critical to the prevention, management, and treatment of many important health conditions, such as stroke and cardiac arrest. However, the initiation and maintenance of physiological changes during hypoxia tolerance can be very difficult, and even those interventions that succeed in laboratory animals and controlled clinical trials do not always translate into clinical therapy. Transformative advances in the science of mammalian physiology, especially those that can connect mammalian physiological, molecular changes and diseases are urgently needed. In this review, we discussed major molecular and physiological adaptations during hypoxia tolerance that can be developed for the induction of clinical neuroprotection to tissue hypoxia during brain injuries.

2. Convergence between a specific neurotransmitter system and physiological mechanisms maybe critical for tissue hypoxia during brain injuries

Comparative studies of adaptive physiology demonstrated that hypoxia tolerant animal species represent potential sources of new strategies in our search for brain protection. This

is because studies on the neurons of these animals repeatedly reminded us that we are closer to understand how cells and tissues develop resistance to hypoxia. Hypoxia tolerant species are very valuable models for understanding oxygen signaling processes simply because the responses to hypoxia are well developed. The possibility of separating adaptive signaling or defense responses from injury is a major benefit of studying hypoxia tolerant cells. They also serve as models for the slow adaptation of tissues to hypoxia, which humans are clearly capable of, and which might be enhanced to improve adaptation to diseases involving oxygen deficits.

Studies on the pathophysiology of shock-induced disturbances in tissue homeostasis reveal that tissue hypoxia is a consequence of distressed microcirculation that worsens the diffusion geometry, such that tissue hypoxia induced significant physiological changes in brain cells. Measuring the targets that detect tissue hypoxia is known to reveal the immediate effect of the distressed microcirculation. Recent studies on hypoxia neurobiology research have advanced a considerable body of evidence supporting the hypothesis that convergence between neurotransmitter systems and physiological mechanisms is protective in hypoxia tolerant species. Establishing this protective phenotype in response to hypoxic stress depends on a convergence response at the genomic, molecular, and cellular and tissue levels (Singer, 2004, Jeffrey, 2006). At the cellular level, studies in mammalian hibernation that explore hypoxia tolerance capability reveal evidence of ion channel arrest, regulation of inhibitory neurotransmission and suppression of substrate oxidation as cellular physiological adaptations (Gentile et al., 1996, Wang et al., 2002). Furthermore, extracellular levels of GABA decline in the striatum during hibernation, while extracellular glutamate remains unchanged during steady-state torpor of hibernation when compared with euthermic animals (Zhou et al., 2001). A decrease in the tissue-specific depression of substrate oxidation is also thought to decrease oxygen consumption, and consequently attenuate cytotoxic events that lead to cell death (Barger et al., 2003). This effect was attributed to a decrease in ATP demand resulting in the maintenance of homeostasis of brain energy demand and supply. The central mechanism that underlies hypoxia preconditioning-induced tolerance, which maintains the homeostasis of brain energy demand and supply, remains unclear. Interestingly, a number of potential neurochemical induction pathways have been proposed to control hypoxia tolerance in natural genetic systems of hypoxia tolerance. Such pathways include neuroactive cytokines (Nawashiro et al., 1996), glutamate receptors (Ravid et al., 2007, Sivakumar et al., 2009), adenosine receptors (Perez-Pinzon et al., 2005), the ATP-sensitive potassium Channel (Reshef et al., 2000), nitric oxide (Gonzalez-Zulueta et al., 2000) and oxidative stress (Dalen et al., 2009).

Taken together, findings from the aforementioned studies indicate that neuroprotective mechanisms against hypoxic insults in natural genetic systems of hypoxia or ischemic tolerance maybe be hinged on the convergence between a specific neurotransmitter system and physiological mechanisms. Although only few of the existing studies have been demonstrated in humans, one of these few studies indicates that elucidation of the central neurochemical mechanism of hypoxia tolerance is in this area of interest because the tolerance has been experimentally induced by clinically approved drugs (Konstantin et al., 2003). In another human study, it was found that adenosine plasma levels strikingly increased, such that the adenosine flow lasted days after transient ischemic or hypoxia attack and weeks after stroke (Moncayo et al., 2000, Pasini et al., 2000). Our view openly acknowledges the existence of hypoxia tolerance capacity in human brains and a possible

central endogenous neuroprotective mechanism for hypoxia brain injuries in humans. In this context, considering the roles of adenosine as a molecule, it is possible that adenosine might represent a potential central neurotransmitter system that modulates physiological mechanisms during hypoxia protection. It is also important to emphasize that hypoxia itself could be the driving force for the convergence between a specific neurotransmitter system such as adenosine and physiological mechanisms during protection in hypoxia tolerant species. Since extensive studies have been done on adenosine system in the context of hypoxia protection over the past twenty years, we will now summarize the existing knowledge of specific roles of adenosine (A1) receptor in inducing survival during hypoxia.

3. Specific roles of A1 receptor during hypoxia tolerance

Survival in a severe hypoxic stress during which arterial oxyhemoglobin saturation is equal to 35% or less is connected with the ability of the brain to adapt to low oxygen supply and demand, and is thought to be regulated by a specific neurotransmitter system, such as adenosine (Blood et al., 2002). Studies in young sheep and adult rats indicate that intracerebral A1 concentrations increased during hypoxia. The specific role of A1 was linked to its ability to inhibit neuronal activity (Fowler et al., 1999). In vitro studies on hippocampal slices indicate that elevation of A1 receptors is associated with hypoxia (Jin and Fredholm, 1997), and severe asphyxia *in vivo* (Hunter et al., 2003c), following inhibition of neuronal activity. Studies in the fetal sheep further revealed that breathing movements can be inhibited by hypoxia and that such adaptation could be abolished by adenosine-receptor blockade at the level of the thalamus due to the inhibition of thalamic neurons (Chau and Koos, 1999). In a mouse knocked-out of A1 receptor, there is a significant decrease in tolerance to hypoxia (Johansson et al., 2001). Involvement of adenosine or adenosine triphosphate-sensitive potassium (K_{ATP}) channels in the development of tolerance has been suggested in global ischemia and hypoxia models (Kumral et al., 2010), cross-tolerance models (Xu et al., 2002) and *in vitro* studies (Perez-Pinzon et al., 2005). Activation of A1 receptors directly accelerate neuritogenesis in the primary neuronal precursor cells of rats(Canals et al., 2005). This finding suggests that A1 receptors may play an important role in myelinization and neuronal differentiation with the potential for clinical management of neuronal repair in hypoxic-induced brain injury.

By evaluating the action of hypoxia on synaptic transmission in hippocampal slices Sebastião and Ribeiro (2001) revealed that γ-aminobutyric acid (GABA), acetylcholine, and even glutamate may also have a neuroprotective role; however their action is evident only when activation of adenosine A_1 receptors is impaired. This finding indicates that adenosine A_1 receptors have a pivotal role of neuromodulating during hypoxia, though other substances can enhance adenosine actions when the nucleoside is not operative. A_1 receptors fine tuning neuromodulation is a very restrained change, similar to what *e.g.*, a pianist does, modulating a tune through introduction of another tune to modify the characteristics of the previous tune. A_1 receptors have specificity of interacting with receptors of other neurotransmitters and neuromodulators as well as with adenosine transport systems. A_1 receptors and other cellular elements involved in brain insults act via interconnections between the cellular elements and their secretions, such as the immune system(Ribeiro, 2005). In this manner, the nervous system can be highly regulated in normal physiology to induce neuroprotection against hypoxia. The fact that chemical

neuromodulators such as A1 receptors are already part of normal physiology, either during embryonic development or adulthood, implies that their activity can be modified by specific pharmacological agonists and antagonists to restore homeostasis or to promote the safe pathways that can lead to tissue hypoxia protection.

4. Cellular mechanisms that promote neuronal death can be manipulated to promote neuronal survival

It is well known that neuronal death or apoptosis may result from continued activation of damaging molecular processes or pathways set in motion by a series of hypoxic insults, with the ultimate breakdown of the cell as a unit. According to Lipton (1999) such neuronal death is a morphological one, during which the cell cannot recover to perform its anatomical function. The idea is that the study of molecular processes of neuronal death at this point provides an understanding of what leads to these drastic structural changes and what needs to be done to promote neuronal survival. An interesting question in this regard is how can the molecular mechanisms or pathways that promote hypoxia-induced neuronal death be manipulated to promote neuronal survival? By targeting the disruption of the mouse caspase 8 genes, it has been shown that caspase 8 can regulate the activities of death promoting receptor signaling within the TNFR superfamily. For example, the deletion of caspase 8 gene completely abrogated TNFR12 and Fas receptor-induced apoptosis that was enacted via generation of reactive oxygen species during hypoxia (Cobelens et al., 2007). In other studies that explore the mechanisms of hypoxia-induced cell death in primary cortical neurons, it was found that TNFalpha was responsible for inducing cell death in the cortical neurons of cultured rats (Reimann-Philipp et al., 2001). These investigations established that TNF receptors are responsible for neuronal apoptosis because of the formation of an intracellular protein complex induced by hypoxia.

Although TNFalpha is directly implicated in neuronal apoptosis, TNFalpha-induced neuronal death can be inhibited by nerve growth factors (Haviv and Stein, 1999). This finding indicates that that hypoxia-associated apoptotic effects of TNFalpha can be converted by trophic factors (NGFs), and that the survival-promoting effect of NGF is mediated by a specific pathway not shared by all tyrosine kinase receptors. This implies that the manipulation of caspases and NGFs during hypoxia-induced activation of TNFalpha in the cortical neurons can prevent apoptotic effect of TNFalpha during hypoxia.

Phosphorylation networks regulating JNK activity have evolved to enable swift and accurate responses, even in the face of hypoxia-induced cellular perturbations (Bakal et al., 2008). The JNK signaling network is thought to maintain cell and tissue integrity during hypoxia-induced cellular stress that involves stress-activated protein kinases (SAPKs), also known as JUN NH_2-terminal kinases (JNKs). Hypoxia-induced activation of JNK is an early response to hypoxic stress (Antoniou et al., 2009). When treated with CEP-1347, which inhibits JNK activation, the increase of cellular JNK activity was blocked, such that sympathetic and cortical neurons were saved from hypoxia-induced stress (Qi et al., 2009). Hypoxia-induced cell death can be averted by inhibiting JNK activation(Wardle, 2009). The explanation for this is that C-jun, a transcription factor that controls genes involved in cell death, is a constituent of another transcription factor called AP-1, and when phosphorylated by JNK, c-jun becomes activated and induces apoptosis, by withdrawing survival signals but when inactivated, metabolically vulnerable neurons can be saved from apoptosis.

Apart from JNK, there are other molecular systems that are able to induce apoptosis or neuronal death when in an active form, yet in an inactive form fail to do so. Molecular factors such as CREN, NF-kB (238) and FKHRL1 (Obexer et al., 2006), must be in its inactive form in order to refrain from inducing the expression of death genes in cerebellar granule neurons during hypoxia. These factors (CREN, NF-kB and FKHRL1) can be activated by Akt (Akt is a protein family of the kinases B (PKB) that is involved in cellular signaling to support their continued existence in brain cells to promote apoptosis (Park et al., 2007). Akt can also inhibit the cellular machinery that functions in killing cells. This is possible by phosphorylation at sites both upstream (BAD) and downstream (Caspase 9) of mitochondrial cytochrome c release (Dashniani et al., 2009). Such phosphorylation has been previously suggested to regulate glucose metabolism, thus, helping cells to live rather than die following hypoxic insults (Zhou et al., 2001). In summary, most of the aforementioned studies have been done in rats, exploring similar studies in hypoxia tolerating species will provide an in-depth-understanding of the activities of these molecular mechanisms or pathways during neuronal survival in hypoxia tolerating conditions.

Hormones are chemical substances produced by specialized glands with the primary function of regulating cellular activity. Levels of hormones in the brain demonstrate unique secretory characteristics that are linked to hypoxia. Leptin is a protein hormone with important effects in regulating metabolism functions. Most recent evidence has implicated leptin, the product of the obese gene derived from fat cells and placenta known to regulate body weight and food intake (Otukonyong et al., 2005), and synaptic plasticity during hypoxia neuroprotection (Shanley et al., 2001). More recently, the neuroprotective effects of leptin against tissue hypoxia have been explored (Perez-Pinzon and Born, 1999). This study revealed that leptin receptors are expressed in neurons of the hypothalamus. Another study (Guo et al., 2008),revealed the expression of leptin in the hippocampus and cerebral cortex. Endogenous synthesis and release of leptin by the brain may explain how localized leptin could protect neurons during hypoxia. For instance, cumulative evidence indicate that leptin could exert its neuroprotective effects to enhance neuronal survival both in vitro and in vivo by a mechanism involving stimulation of the Janus kinase (JAK)-signal transducers and activator of transcription (STAT) pathways. It has been shown that leptin protects neurons from neurotoxic 1-methyl-4-pyridinium (MPP+)-induced cell death in a dose dependent manner by activating the phosphatidylinositol 3-kinase (PI3-K)/Akt pathway; Jingnan et al., 2006). In mice model, systemic administration of leptin was shown to decrease infarct volume induced by focal cerebral hypoxia ischemia (Zhang et al., 2007). Apoptosis resulting from hypoxia or global ischemia is involved in the pathology of cerebral infarction and neuronal death. Leptin has been reported to inhibit apoptosis by removing growth factor from neuroblastoma cells utilizing JAK2-STAT3 and P13K/AKT signaling pathways (Guo et al., 2008). In seizures and epilepsy related hypoxia, leptin has been shown to protect hippocampal neurons against excitotoxicity in leptin deficient ob/ob mice, which are more prone to seizures (Erbayat-Atlay et al., 2006). Leptin has been reported as a hypoxic response gene whose transcription is induced by transcription factor HIF-1. Understanding the specific role of leptin in hypoxia conditioning can add leptin to the list of potential molecules for the treatment of hypoxia-associated brain injury.

Ghrelin is another peptide hormone that has been implicated in regulating glucose homeostasis (Andrews, 2011). The discovery of ghrelin was based on its ability to stimulate

growth hormone (GH) release by activating the GH secretagogue receptor (GHSR1a) widely distributed in the hypothalamus and the pituitary gland. The neuroprotective effect of ghrelin has been demonstrated in many animal models of hypoxic-induced brain injury and stroke (Donnan et al., 2008). Injection of ghrelin intraperitonealy or intravenously in rats (both in vivo and in vitro) neuroprotects the forebrain by reducing infarct volume and cell death (Liu et al., 2009). Ghrelin has also been shown to attenuate CAI and CA3 hippocampal neuronal loss by inhibiting casp 3 activation in the pilocarpine-model of epilepsy (Xu et al., 2009). It is also important to point out that the crosstalk in leptin and ghrelin secreting sites to contribute to neuroprotection during the period of hibernation. Precisely, during the winter months hibernating mammals, such as the Arctic ground squirrel undergo physiological and behavioral changes to cope with seasonal periods of food scarcity and high energy demand. Before going into hibernation, the Arctic ground squirrel eat a lot and accumulate body fat, such that leptin level increases resulting in the development of leptin resistance without which the process of adipose mass deposition will fail, and hibernation which is also characterized with hypoxia tolerance will be in jeopardy. When hibernating, the Arctic ground squirrels do not eat because food is scarce, body metabolism decreases and hypothermia sets in. Since ghrelin stimulates appetite, and the animals are able to eat after hibernation is over. Leptin resistance is known to allow fat to be stored in anticipation of another season. Therefore, we propose that the understanding of the interconnections between the leptin and ghrelin with the metabolic networks could open new windows on the treatment that identifies the role of leptin and ghrelin in the preservation of metabolically vulnerable neurons during tissue hypoxia following the onset of stroke.

5. Conclusion

Analysis of brain mechanisms that control hypoxia tolerance in natural systems indicate that the physiological and molecular mechanisms of hypoxia neuroprotection represent the core of our understanding of how the brain can be made to resist tissue hypoxic insults. Studies of mammalian hypoxia physiology revealed that hypoxia tolerating models have an intrinsic ability to resist hypoxia. The physiological and intracellular mechanisms underlying such protection are not fully understood. Transformative advances in the science of mammalian physiology, especially those that can connect mammalian physiological and molecular changes and diseases, such as stroke and cardiac arrest, are urgently needed. In this review, we suggest that the protective physiological and molecular mechanisms employed by hypoxia-tolerant species offer clues on strategies to adapt for the clinical management of brain injuries where oxygen demand fails to match the supply.

7. References

Andrews ZB (2011) The extra-hypothalamic actions of ghrelin on neuronal function. . Trends Neurosci 34:31-40.

Antoniou X, Sclip A, Ploia C, Colombo A, Moroy G, Borsello T (2009) JNK Contributes to Hif-1alpha Regulation in Hypoxic Neurons. Molecules 15:114-127.

Bakal C, Linding R, Llense F, Heffern E, Martin-Blanco E, Pawson T, Perrimon N (2008) Phosphorylation networks regulating JNK activity in diverse genetic backgrounds. Science 322:453-456.

Barger JL, Brand MD, Barnes BM, Boyer BB (2003) Tissue-specific depression of mitochondrial proton leak and substrate oxidation in hibernating arctic ground squirrels. . Am J Physiol Reg Integr Comp Physiol 284:1306 -1313.

Blood AB, Hunter CJ, Power GG (2002) The role of adenosine in regulation of cerebral blood flow during hypoxia in the near-term fetal sheep. J Physiol 543:1015–1023.

Canals M, Angulo E, Casadó V, Canela EI, Mallol J, Viñals F, Staines W, Tinner B, Hillion J, Agnati L, Fuxe K, Ferré S, Lluis C, Franco R (2005) Molecular mechanisms involved in the adenosine A and A receptor-induced neuronal differentiation in neuroblastoma cells and striatal primary cultures. . J Neurochem 92:337-348.

Chau A, Koos BJ (1999) Metabolic and cardiorespiratory responses to hypoxia in fetal sheep: adenosine receptor blockade. . Am J Physiol 276:1805–1811.

Cobelens PM, Kavelaars A, Heijnen CJ, Ribas C, Mayor F, Penela P (2007) Hydrogen peroxide impairs GRK2 translation via a calpain-dependent and cdk1-mediated pathway. . Cell Signal 19:269-277.

Dalen ML, Frøyland E, Saugstad OD, Mollnes TE, Rootwelt T (2009) Post-hypoxic hypothermia is protective in human NT2-N neurons regardless of oxygen concentration during reoxygenation. . Brain Res 1259:80-90.

Dashniani MG, Chkhikivishvili NT, Naneĭshvili TL, Burdzhanadze MA, Maglakelidze G, A. (2009) Regularities of the egocentric spatial memory development in children aged 24-60 months. Georgian Med News 174:65-72.

Donnan GA, Fisher M, Macleod M, Davis SM (2008) Stroke. Lancet 9624:1612-1623.

Otukonyong EE, Dube MG, Torto R, Kalra PS, Kalra SP (2005). Central leptin differentially modulates ultradian secretory patterns of insulin, leptin and ghrelin independent of effects on food intake and body weight. Peptides 26:2559-2566.

Erbayat-Atlay E, Yamada KA, Wong M (2006) Increased severity of pentylentetrazol-induced seizures in leptin deficient ob/ob mice. . Epilepsia 47:303-304.

Fowler JC, Partridge LD, Gervitz L (1999) Hydroxylamine blocks adenosine A1 receptor-mediated inhibition of synaptic transmission in rat hippocampus. . Brain Res 815:414–418.

Gentile NT, Spatz M, Brenner M, McCarron RM, Hallenbeck JM (1996) Decreased calcium accumulation in isolated nerve endings during hibernation in ground squirrels. Neurochem. . Neurochem Res 21:947 -954.

Gonzalez-Zulueta M, Feldman AB, Klesse LJ, Kalb RG, Dillman JF, Parada LF, Dawson TM, Dawson VL (2000) Requirement for nitric oxide activation of p21(ras)/extracellular regulated kinase in neuronal ischemic preconditioning. . Proc Natl Acad Sci USA 9:7436-7441.

Guo Z, Jiang H, Xu X (2008) leptin-mediated cell survival signaling in hippocampal neurons mediated by JAK STAT3 and mitochondrial stabilization. J Biol Chem 1754-1763.

Haviv R, Stein R (1999) Nerve growth factor inhibits apoptosis induced by tumor necrosis factor in PC12 cells. J Neurosci Res 55:269-277.

Hunter CJ, Blood AB, Power GG (2003c) Cerebral metabolism during cord occlusion and hypoxia in the fetal sheep: a novel method of continuous measurement based on heat production. . J Physiol 552:241–251.

Jeffrey MG (2006) Cerebral preconditioning and ischaemic tolerance

Nature Reviews Neuroscience 7:437-448.

Jin S, Fredholm BB (1997) Adenosine A1 receptors mediate hypoxia-induced inhibition of electrically evoked transmitter release from rat striatal slices. Eur J Pharmacol 329:107–113.

Jingnan L, Chang-Shin P, Sung-Keun L (2006) leptin inhibits 1-methyl-4- phenylpyridinium-induced cell death in SH-SY5Y cells. . Neurosci Lett 3:240- 243.

Johansson B, Halldner L, Dunwiddie TV, Masino SA, Poelchen W, Giménez-Llort L, Escorihuela RM, Fernández-Teruel A, Wiesenfeld-Hallin Z, Xu XJ, Hårdemark A, Betsholtz C, Herlenius E, Fredholm BB (2001) Hyperalgesia, anxiety, and decreased hypoxic neuroprotection in mice lacking the adenosine A1 receptor. Proc Natl Acad Sci U S A 98:9407-9412.

Konstantin P, A. S, K. R, Löwl D, Muselmann D, Victorov I, Kapinya K, Dirnagl U, Meisel A (2003) Hypoxia-Induced Stroke Tolerance in the Mouse Is Mediated by Erythropoietin. Stroke 34:1981-1986.

Kumral A, Yesilirmak DC, Aykan S, Genc S, Tugyan K, Cilaker S, Akhisaroglu M, Aksu I, Sutcuoglu S, Yilmaz O, Duman N, Ozkan H (2010) Protective Effects of Methylxanthines on Hypoxia-Induced Apoptotic Neurodegeneration and Long-Term Cognitive Functions in the Developing Rat Brain. Neonatology 98: 128-136.

Lipton P (1999) Ischemic cell death in brain neurons. Physiol Rev 79:1431-1568.

Liu Y, Chen L, Xu X, Vicaut E, Sercombe R (2009) Both ischemic preconditioning and ghrelin administration protect hippocampus from ischemia/reperfusion and upregulate uncoupling protein-2. BMC Physiol 9:17-20.

Moncayo J, de Freitas GR, Bogousslavsky J, Altieri M, van Melle G (2000) Do transient ischemic attacks have a neuroprotective effect? Neurology 54:2089-2094.

Nawashiro H, Tasaki K, Ruetzler CA, Hallenbeck JM (1996) TNF-alpha pretreatment induces protective effects against focal cerebral ischemia in mice. . J Cereb Blood Flow Metab 17:483-490.

Obexer P, Geiger K, Ambros PF, Meister B, Ausserlechner MJ (2006) FKHRL1-mediated expression of Noxa and Bim induces apoptosis via the mitochondria in neuroblastoma cells. Cell Death Differ 14:534-547.

Park MK, Kim CH, Kim YM, Kang YJ, Kim HJ, Kim HJ, Seo HG, Lee JH, Chang KC (2007) Akt-dependent heme oxygenase-1 induction by NS-398 in C6 glial cells: a potential role for CO in prevention of oxidative damage from hypoxia. Neuropharmacology 53:542-551.

Pasini FL, Guideri F, Picano E, Parenti G, Petersen C, Varga A, Perri TD (2000) Increase in plasma adenosine during brain ischemia in man: a study during transient ischemic attacks, and stroke. . Brain Res Bull 51:327−330.

Perez-Pinzon MA, Born JG (1999) 1999. Rapid preconditioning neuroprotection following anoxia in hippocampal slices: role of the K+ATP channel and protein kinase C. . Neuroscience 89:453-459.

Perez-Pinzon MA, Dave KR, Raval AP (2005) Role of reactive oxygen species and protein kinase C in ischemic tolerance in the brain. . Antioxid Redox Signal 7:1150-1157.

Qi D, Hu X, Wu X, Merk M, Leng L, Bucala R, Young LH (2009) Cardiac macrophage migration inhibitory factor inhibits JNK pathway activation and injury during ischemia/reperfusion. J Clin Invest 119:3807-3816.

Ravid O, Shams I, Ben Califa N, Nevo E, Avivi A, Neumann D (2007) An extracellular region of the erythropoietin receptor of the subterranean blind mole rat Spalax enhances receptor maturation. Proceedings of the National Academy of Sciences of the United States of America 104:14360-14365.

Reimann-Philipp U, Ovase R, Weigel PH, Grammas P (2001) Mechanisms of cell death in primary cortical neurons and PC12 cells. J Neurosci Res 64:654-660.

Reshef A, Sperling O, Zoref-Shani E (2000) Role of K(ATP) channels in the induction of ischemic tolerance by the "adenosine mechanism" in neuronal cultures. Adv Exp Med Biol. . Adv Exp Med Biol 486:217-221.

Ribeiro JA (2005) What can adenosine neuromodulation do for neuroprotection?

Ribeiro JA. Curr Drug Targets CNS Neurol Disord 4:325-329.

Sebastião AM, Ribeiro AJ (2001) Neuroprotection during hypoxic insults: Role of adenosine. Drug Development Research 52:291–295.

Shanley LJ, Irving A, Harvey J (2001) Leptin enhances NMDA receptor receptor function and modulates hippocampal synaptic plasticity. J Neurosci 21:186-190.

Singer D (2004) Metabolic adaptation to hypoxia: cost and benefit of being small. Respiratory Physiology & Neurobiology 141:215-228.

Sivakumar V, Ling EA, Lu J, Kaur C (2009) Role of glutamate and its receptors and insulin-like growth factors in hypoxia induced periventricular white matter injury. Glial 34:45-67.

Wang SQ, Lakatta EG, Cheng H, Zhou ZQ (2002) Adaptive mechanisms of intracellular calcium homeostasis in mammalian hibernators. . J Exp Biol 205:2957 -2962.

Wardle EN (2009) Programmed Cell Death: Apoptosis In: Guide to Signal Pathways in Immune Cells, pp 111-128: Humana Press.

Xu H, Aibiki M, Nagoya J (2002) Neuroprotective effects of hyperthermic preconditioning on infarcted volume after middle cerebral artery occlusion in rats: Role of adenosine receptors. . Crit Care Med 30:1126−1130.

Xu J, Wang S, Lin Y, Cao L, Wang R, Chi Z (2009) Ghrelin protects against cell death of hippocampal neurons in pilocarpine-induced seizures in rats. Neurosci Lett 453:58-61.

Zhang F, Wang SP, Signore AP (2007) Neuroprotective effects of leptin against ischemic injury induced by oxygen-glucose deprivation and transient cerebral ischemia. . Stroke 38:2329-2336.

Zhou F, Zhu X, Castellani JR, Stimmelmayr R, Perry G, Smith MA, Drew KL (2001) Hibernation, a Model of Neuroprotection. American Journal of Pathology. American Journal of Pathology 158:2146-2150.

20

Antioxidant Treatments: Effect on Behaviour, Histopathological and Oxidative Stress in Epilepsy Model

Rivelilson Mendes de Freitas
Federal University of Piaui
Brazil

1. Introduction

The aim of this chapter is to describe several studies which have attempted to measure/detect effects of antioxidant compounds (lipoic acid, ubiquinone, ascorbic acid and alpha-tocopherol) on behavioral alterations, neuronal damage and oxidative stress in hippocampus of rodents in epilepsy model induced by pilocarpine.

Epilepsy can cause an uncomfortable impact on social, educational and emotional development of affected people, especially in childhood and adolescence, but its diagnosis has had great progress over the last years. Nevertheless, among the diagnostic details requiring research, the precise localization and lateralization of epileptogenic focus remains unclear, despite the fact that it has been demonstrated that removal of cerebral cortex region may result in a state free of seizures. Moreover, epilepsy is considered a risk factor for depression and other psychological problems, whereas cognitive impairment may be related to behavioral troubles, particularly of conduct and attention deficit, hyperactivity and psychiatric disorders.

Neurotransmitter systems involved in the experimental model of epilepsy induced by pilocarpine are not fully defined yet. This seizure model in rodents is widely used to study the pathophysiology of convulsive process, since it reproduces behavioral, electroencephalographic (EEG) and neurochemical changes that are similar to those of the temporal lobe epilepsy in humans. The pilocarpine model is used to study the action mechanism of new drugs and antioxidant compounds during the installation and maintenance and/or propagation of epileptogenesis as well as to evaluate effects of new compounds isolated from medicinal plants on behavioral, histopathological, and other parameters relating neurochemical changes with epileptic activity (Costa Júnior et al, 2010).

Histopathological studies using this model have demonstrated neuronal damage in various brain regions. Some specific brain areas revealed typical histopathological changes, mainly hippocampus, striatum and frontal cortex, suggesting the involvement of these different areas during the establishment of the epileptic process. Among those areas, the cellular and structural modifications seen in hippocampus and striatum may be significantly related to the mechanisms of installation and propagation in epileptogenesis of limbic seizures.

The temporal lobe epilepsy is the most common form of epilepsy. It is characterized by spontaneous recurrent seizures that are often blocked by treatment with antiepileptic drugs.

Seizures can be characterized as clinical manifestations resulting from abnormal neuronal discharges, producing an imbalance between the mechanisms of inhibitory and excitatory neurotransmission. The mechanisms of activation, propagation and maintenance of seizures are widely studied but little understood. Many studies have been performed using the pilocarpine model to clarify the effects of new drug modulators on brain mechanisms of seizures and status epilepticus (Santos et al, 2010).

Status epilepticus is clinically defined as prolonged electrical and clinical seizure activity in which the patient does not regain consciousness to a normal alert state between repeated tonic-clonic attacks. The disorder is a neurological emergency associated with a mortality rate of 10-12% and an even greater morbidity. Status epilepticus can lead to permanent pathological damage and altered physiological function in certain brain regions and induces major changes in membrane phospholipids, massive increases in arachidonic acid concentrations, diacylglycerol-mediated activation of protein kinase C, calcium-mediated changes in calmodulin kinase II and possibly generation of free radicals that could play an essential role in mechanism of oxidative stress involved in neuronal damage. Status epilepticus can be characterized by a permanent change in neurotransmitter systems and oxidative stress that it is more facilitated in the brain rather than in other tissues because it contains large quantities of oxidizable lipids and metals (Freitas et al, 2010). Moreover, status epilepticus can produce considerable changes on the enzymatic activity of antioxidants systems according to the brain areas and the phase of the seizure studied.

The role of monoamines, amino acid and oxidative stress in pilocarpine model was investigated in hippocampus, striatum and frontal cortex of adult rats. The status epilepticus was induced by pilocarpine and the results correspond to its acute phase. The data obtained suggest that pilocarpine induced neurotransmitters and oxidative stress changes in brain regions which are similar to those found in human temporal lobe epilepsy (El-Etri et al, 1993, Freitas et al, 2003, Ferreira et al, 2009).

2. Pathophysiology of seizures in epilepsy model induced by pilocarpine

Epilepsies are complex neurobehavioral disorders resulting from increased excitability of neurons in several brain regions involving various neurotransmitters (Rauca et al, 2004). The cholinergic system plays an important role in generating EEG activity as well as regulating the vigilance states. Pilocarpine is a cholinergic agonist with a moderate affinity with M_1 muscarinic receptors and high affinity with M_5 ones. Muscarinic cholinergic agonists have effects on rapid eyes movement (REM) and slow wave sleep, playing a role in REM induction (MacGregor et al, 1997; Perlis et al, 2002). On the other hand, pilocarpine at a high dose (400 mg/kg, i.p.) makes seizures progress to a long-lasting status epilepticus (SE) within 1-2 h and induces behavioral and EEG alterations in rodents, which are similar to human temporal lobe epilepsy (TLE) (Marinho et al, 1998).

Pilocarpine-induced rodent models TLE might provide information regarding histopathological damage and oxidative stress consequences, as well as neurochemical changes associated with seizure activity in hippocampus of young and adult rats (Cavalheiro et al, 1991; Smith and Shibley, 2002, Freitas et al, 2003). TLE can be characterized by a permanent change in neurotransmitter systems and in development of the oxidative stress that is more facilitated in the brain rather than in other tissues because for several reasons, including a high consumption of oxygen, the presence of large quantities of oxidizable lipids and pro-oxidative metals, and its comparatively lower antioxidant capacity

(Frantseva et al, 2000; Naffah-Mazzacoratti et al, 2001). Neuronal cells continuously produce free radicals and reactive oxygen species (ROS) as part of their metabolic processes and during the establishment of convulsive process (Gilbert and Sawas, 1983; Halliwell and Gutteridge, 1999). The free radicals are very reactive and might produce oxidative damage in DNA, proteins and lipids (Peterson et al, 2002), leading to a sequence of outcomes that culminates in neuronal degeneration during the installation of seizures.

ROS can affect the ion transport, proteins and channels, via protein oxidation or via membrane phospholipids peroxidation, resulting in a deleterious change on the ionic homeostasis and the neuronal transmission (Rong et al, 1999; Sah et al, 2002). The ROS increment induces oxidative stress, which is defined as the excessive production of free radicals, such as superoxide (O_2^-), hydroxyl radical ($OH^\cdot$), nitric oxide (NO) and their metabolites (nitrate and nitrite) and others that can dramatically alter the neuronal function. Therefore, some researches have correlated the overproduction of these compounds with seizure-induced neuronal death and status epilepticus (MacGregor et al, 1997; Ferrer et al, 2000).

Several compounds can produce free radical such as H_2O_2, which in high concentration can react with O_2^- (Haber-Weiss reaction) or iron (Fenton reaction) producing highly reactive OH. The conversion of H_2O_2 to H_2O and O_2 is made by catalase and glutathione peroxidase (Michiels et al, 1994; Simonié et al, 2000). The formed $OH^\cdot$ radical is likely to react with non-radical molecules, transforming them into secondary free radicals. This reaction occurs during the lipid peroxidation producing hydroperoxides in brain epileptic (Vanhatalo and Riikonen, 1999). Nitric oxide (NO) can be estimated by their metabolites, which are associated with neurodegenerative diseases (Vanhatalo and Riikonen, 2001). Despite the fact that numerous studies clearly indicate the importance of antioxidant enzymatic activities in the epileptic phenomenon, the mechanisms by which these enzymes influence seizures and status epilepticus are not completely understood (Michiels et al, 1994; Simonié et al, 2000).

Status epilepticus is a severe form of continuous seizure attacks and a medical emergency associated with brain damage and significant mortality (Aminoff and Simon, 1980). The common sequels of status epilepticus include continuing recurrent seizures, permanent neurological deficit and brain injury. The status epilepticus can be induced by the administration of pilocarpine or lithium-pilocarpine (Hirsh et al, 1982; Freitas et al, 2004). Pilocarpine administration induces seizures with three distinct phases: [A] an acute period, which lasts 1-2 days and is associated to repetitive seizures and status epilepticus; [B] a seizure-free (silent period) characterized by a progressive return to normal EEG and behavior, which lasts 4 to 44 days; [C] a chronic period characterized by spontaneous recurrent seizures (SRS) that starts 5 to 45 days after pilocarpine administration and persists until the animal dies. In addition, systemic injection of pilocarpine induces status epilepticus in rodents associated to histopathological alterations, which are most prominent in limbic structures (Cavalheiro et al, 1991), such as hippocampus, striatum, frontal cortex and others (Tomé et al, 2010).

Pilocarpine is a muscarinic cholinergic agonist able to elicit seizures and status epilepticus in rodents, characterizing an experimental model frequently used to study SRS (Turski et al, 1983; Freitas et al, 2005). This seizure model resembles several phenomenological features of human temporal lobe epilepsy, including a particular resistance to anticonvulsant medication (Browne and Holmes, 2001). Tissue accumulation of free radicals can occur in many metabolic disorders, such as seizures. Affected patients present a variable degree of neurological dysfunction, including mental retardation, cognitive deficit and cerebral

edema. However, the exact mechanisms involved in these alterations remain poorly understood.

It has been described that the impairments in learning, memory and behavior observed in patients with epilepsy are caused, at least in part, by changes in cholinergic system function (Bruce and Baudry, 1995), since there are consistent evidence that high levels of acetylcholine (ACh) in the brain are associated with cognitive dysfunction (Brozek et al, 2000). Cholinergic transmission is mainly terminated after ACh hydrolysis cause by acetylcholinesterase enzyme (AChE).

In the brain, the phenomena of excitotoxicity has been related to an over production of free radicals by the tissue during pilocarpine-induced seizures and status epilepticus (Simonié et al, 2000) and in human epilepsy (Vanhatalo and Riikonen, 1999). The increase in ROS levels can be responsible for this neuropathology and can activate apoptosis processes (Rong et al, 1999). The free radicals in the convulsive process can be neutralized by an elaborate antioxidant defense system consisting of enzymes such as superoxide dismutase, glutathione peroxidase, catalase, and glutathione reductase, and numerous non-enzymatic antioxidants like reduced glutathione (GSH), indicating a neuronal response (Ferrer et al, 2000). Status epilepticus induces ROS production by protein oxidation measured by tyrosine nitration (Rong et al, 1999). It can also be determined by both the end-product of lipid peroxidation, malondialdehyde (MDA) levels (Bruce and Baudry, 1995), and the effectiveness of the antioxidant enzymatic responses (Dal-Pizzol et al, 2000). The hippocampus is the most affected area by pilocarpine-induced seizures. Other authors also characterized the neuropathology associated with this convulsive process in striatum, frontal cortex, thalamus and amygdala (Perlis et al, 2002; Freitas et al, 2004).

Seizures represent one of the most severe in vivo stimulatory stress that the brain is exposed to and generalized status epilepticus represents a very severe form of seizures. The international Classification of seizures has defined it as a condition characterized by an epileptic seizure that is so frequent or so prolonged as to create a fixed and lasting condition (Krug et al, 1981). Major motor status epilepticus can lead to permanent pathological damage and altered physiological function in certain brain regions. The pathophysiological changes seen in complex partial, simple partial and absence status epilepticus are much less clear (Freitas et al, 2005). SE can cause brain damage, but can also result from it, and it has been difficult to separate the two, particularly in humans (Lapin et al, 1998).

Status epilepticus has been widely studied in animal models. In status epilepticus, glutamate, aspartate, serotonin, dopamine and acetylcholine play major roles as excitatory neurotransmitters, and GABA as the dominant inhibitory neurotransmitter (Hort et al, 2000; Costa-Lotufo et al, 2002). However, the relation among brain excitatory and inhibitory neurotransmitters and status epilepticus cannot be perfectly established yet and deserves further studies with the purpose to clarify the pathophysiology of seizures.

The pathophysiology of epilepsy is not yet fully defined. The pilocarpine model of seizures in animals are widely used to study the pathophysiology of convulsive process (Ben-Ari et al, 1980), since it reproduces the behavioral and EEG changes that are similar to humans TLE (Ben-Ari et al, 1981). These models are used to study the involvement of neurotransmitter systems as modulators of epileptogenesis, but also to observe behavioral changes, histopathologic, and other neurochemical parameters related to seizure activity (Marinho et al, 1997; Costa- Lotufo et al, 2002, Freitas et al, 2006).

In general, pilocarpine-induced seizures seem to depend on activation of muscarinic receptor, the enzymatic activity changes in some systems (Simonié et al, 2000; Naffah-

Mazzacoratti et al, 2001; Liu et al, 2002), metabolism of fosfoinositídios (Marinho et al, 1998), as well as on the involvement of other neurotransmitter systems such as noradrenergic, dopamine (Kulkarni and George, 1996), serotonergic, GABAergic (Loup et al. 1999; Costa-Lotufo et al, 2002) and glutamatergic (Massieu et al. 1994; Chamberlain et al, 2000).

3. Behavioral alterations in epilepsy model

Status epilepticus is an emergency situation which requires prompt medical atentem. If it is severe permanent brain damage or death has to be prevented with pretreatment with antioxidant compounds. Status epilepticus often occurs in individuals with a history of seizures, in whom there are neuronal substrates already predisposed towards supporting seizure activity.

The pilocarpine model is an useful animal experimental to investigate the development of acute, silent and chronic phases (Cavalheiro et al, 1991). Immediately after pilocarpine administration, all animals persistently presented behavioral changes, including initial akinesia, ataxic lurching, peripheral cholinergic signs (miosis, piloerection, chromodacryorrhea, diarrhea and masticatory automatisms), stereotyped movements (continuous sniffing, paw licking, rearing and wet dog shakes that persisted for 10-15 min), clonic movements of forelimbs, head bobbing and tremors.

These behavioral changes progressed to motor limbic seizures as previously described by Tursky et al. (1983a). Limbic seizures lasted for 30-50 min evolving to status epilepticus for a period longer than 30 min. During 1 h of acute phase of seizures, no case of fatality was observed between the adult rats. However, during the 24 h observation of this phase, 63% of adult animals died (Cavalheiro et al, 1994; Todorova et al, 2004). Similarly, these results for the behavioral alterations in pilocarpine model were described previously by Marinho et al. (1998).

According to our previous studies (Freitas et al, 2004), few minutes after pilocarpine administration, the animals exhibited stereotyped oral and masticatory movements, hypokinesia, salivation, tremor and partial or generalized limbic seizures. Approximately 30 min after pilocarpine injection, the seizures evolved to status epilepticus lasting 12-18 h. During this period, 40% of animals died due to SE. This acute phase was followed by a silent period varying from 4 to 44 days (mean of 15 days) during which the animals displayed normal behavior. A chronic period of spontaneous and recurrent seizures (SRS) (3-4 seizures/week) was also observed and all animals which survived SE, displayed the chronic phase. During the interictal period, there were no behavioral alterations in the animals.

In epilepsy model, pilocarpine induced the first seizure to occur at 34.93 ± 0.70 min. All the animals that received pilocarpine injection (at a dose 400 mg/kg, i.p.) presented generalized tonic-clonic convulsions with status epilepticus, and 60% survived the seizures (Freitas et al, 2004).

4. Antioxidant compounds effects on behavioral alterations in epilepsy model

The nervous system contains some antioxidant enzymes, including superoxide dismutase and glutathione peroxidase that are expressed in higher quantities than catalase (Shivakumar et al, 1991). This spectrum of enzymatic defense suggests that the brain may efficiently metabolize superoxide, but it may have difficulties in eliminating the hydrogen peroxide produced by this reaction. The accumulation of hydrogen peroxide is of major

concern since the brain contains large quantity of iron and copper, which may catalyze the formation of hydroxil radical, which, in turn, can induce lipid peroxidation (Castagne et al, 1999).

The glutathione peroxidase is presented in large amounts during the Central Nervous System (CNS) development, but decreases in aged rats (Nanda et al, 1996). Nevertheless, other scavengers such as ascorbic acid and alpha-tocopherol also decrease the propagation of radical chain reaction. For these reasons, free radicals have been pointed as important molecules involved in the nervous system pathologies such as Huntington disease, Alzheimer, ischaemia and epilepsy (Jenner, 1998).

In epilepsy model induced by pilocarpine administration, we found that superoxide dismutase and catalase activities in the hippocampus are not altered during the acute phase of seizures. On the other hand, according to several authors, the augment of these enzymatic activities could decrease the O_2^- and H_2O_2 levels. Taken together, these results show that during the acute phase, the hippocampus of the adult animals in pilocarpine model after seizures is more vulnerable to oxidative stress.

In addition, high-levels of hydroperoxides were also observed in the same group of animals, which indicated that the lipid peroxidation could be dependent of disability of the antioxidant enzymatic (superoxide dismutase and catalase) activities. As the hydroperoxides are a class of compounds produced as the result of phospholipid peroxidation, its high concentration in the tissue suggests that the hippocampal cells are more vulnerable to damage during the acute period of seizures. Thus, the results described in literature about pilocarpine model suggest that the beneficial effects of antioxidants compounds in reducing the behavioral changes caused by seizures may be partly explained by their ability to remove free radicals, and prevent the formation of hydroperoxides in hippocampus of seized rats.

The need for animal models of epilepsy is driven by the constraints of studying human epileptic brain. Although a great deal has been learned through the study of human epileptic brain tissue throughout the past 100 years, and particularly based in recent experiments with pilocarpine model, our work was aimed at investigating the antioxidant effects of lipoic acid, ubiquinone, ascorbic acid and alpha-tocopherol in adult rats under pilocarpine-induced seizures. Our studies have demonstrated that all animals pretreated with the lipoic acid at the dose (10 or 20 mg/kg) during the first hour of acute phase of seizures induced by pilocarpine injection also manifested behavior alterations, such as peripheral cholinergic signs, tremors, staring spells, facial automatisms, wet dog shakes, rearing and motor seizures, which develop progressively within 1-2 h into a long-lasting status epilepticus. However, these behavioral changes occur at lower rates (Table 1). The findings also suggest that when administered 30 min before pilocarpine, lipoic acid reduces the percentage of animals that seized, increases latency to the first seizure and the survival percentage (Table 1).

Corroborating these data, other antioxidant compound evaluated in pilocarpine model when administered at the dose of 5 mg/kg before pilocarpine, ubiquinone had no effects on seized animals and survival percentage, but increased latency to the first seizure, when compared with the pilocarpine group (Table 1). On the other hand, at a dose of 10 or 20 mg/kg ubiquinone produced a higher reduction of seizures percentage, and a higher increase to that produced by dose of 5 mg/kg in latency to the first seizure and survival rate in pilocarpine model (Table 1).

Drugs	Dose (mg/kg)	Cholinergic Reactions (%)	Latency to first seizure (min)	Seizures (%)	Status epilepticus (%)	Survival rate (%)
Pilocarpine	400	100	35.00 ± 0.70	75	75	40
Ascorbic acid	250	100	80.00 ± 1.30*	33[a]	33[a]	100[a]
	500	100	189.19 ± 1.15*,**	25[a,b]	25[a,b]	100[a,b]
Alpha-tocopherol	200	100	103.00 ± 0.90*	32[a]	32[a]	68[a]
	400	100	158.10 ± 1.05*,**	16[a,b]	16[a,b]	70[a,b]
Ubiquinone	5	100	69.00 ± 0.65*	60[a]	60[a]	60[a]
	10	100	89.00 ± 0.83*,**	35[a,b]	35[a,b]	75[a,b]
Lipoic acid	10	100	106.13 ± 1.05*	00[a]	00[a]	75[a]
	20	100	119.00 ± 1.02*	10[a]	10[a]	90[a]

Rats were acutely treated with the drug doses shown in the table above and 30 min afterwards they received pilocarpine (400 mg/kg). Following, the animals were observed for 24 h for assessment of cholinergic reactions, motor seizures which develop progressively within 1-2 h into a long-lasting status epilepticus and survival rate. Results of latency to first seizure and latency to installation of status epilepticus are expressed in minutes (min) as mean ± S.E.M of the number of experiments shown in the experimental groups and the others in percentages *p<0.05, vs pilocarpine. **p<0.05 vs lower dose (ANOVA and *t*-Student Newman Keuls *post hoc* test); [a]p<0.05, vs pilocapine. [b]p<0.05, vs lower dose (χ^2 test).

Table 1. Effect of pretreatment with antioxidant drugs on behavioral alterations in pilocarpine-induced seizures and lethality in adult rats.

In preclinical practice, the animals pretreated with ascorbic acid (250 mg/kg) in pilocarpine model developed cholinergic reactions, 33% had seizures, 25% built up to status epilepticus and no animal died (Table 1). Ascorbic acid administration, 30 min before pilocarpine injections, increased the latency to the onset of the first seizure in 129% and latency of the status epilepticus in 195% (Table 1). In pilocarpine model, it is also shown that when administered at the smaller dose (200 mg/kg), 30 min before pilocarpine injection, alpha-tocopherol can be decrease by 43% the percentage of animals that seized, increased (194%) latency to the first seizure, and increased survival (41%) (Table 1).
Conversely, a higher dose (500 mg/kg) of the ascorbic acid in pilocarpine model, produced changes in behavior with lower intensity, such as peripheral cholinergic signs (100%), staring spells, facial automatisms, wet dog shakes, rearing and motor seizures (25%) were observed, which progressively developed (1-2 h) into long-lasting status epilepticus (25%), revealing a survival rate of 75% (Table 1). Other studies have revealed that alpha-tocopherol at a dose 400 mg/kg blocks all alterations in behavior, revealing only 16% of motor seizures, which progressively developed (1-2 h) into long-lasting status epilepticus in pilocarpine model and a survival rate of 84% from the seizures (Table 1).

5. Histopathological alterations in epilepsy model

Cholinergic mechanisms play an important role in the activation of limbic seizures, and dopaminergic, serotonergic, GABAergic and glutamatergic systems are responsible for the propagation and/or maintenance of seizures and status epilepticus induced by pilocarpine (Freitas et al, 2004). Previous studies have described a model of limbic seizures followed by brain damage produced by systemic injection of a high dose of pilocarpine in rats. The

evidence included temporal correlation among free radical generation, development of seizures and neuroprotective effects of antioxidant drugs against neuronal damage caused by seizures (Kabuto et al, 1998).

Other studies have shown pilocarpine-induced seizures and brain damage in various cerebral regions and a significant hippocampal injury in this epilepsy model (Turski et al, 1991; Curia et al, 2008). The anticonvulsant effect in the absence of anticholinergic drugs subsequent to the seizure onset suggests that muscarinic receptor activation is involved directly in the beginning of seizures by pilocarpine.

However, the oxidative stress might also play an essential role in the production of neuronal damage, which can be justified by neuroprotective actions of antioxidant compounds according to previous studies (Freitas et al, 2004; Xavier et al, 2007; Ayyildiz et al, 2007a). Previous research indicates that anticonvulsant effects of noradrenergic antagonist drugs have a fundamental role in the mechanisms responsible for beginning, severity and duration of seizure. In fact, the reduction of severity and duration of seizures are protective against neurotoxicity caused by seizures induced by chemical convulsants (e.g. pilocarpine, kainic acid and others). These data, in spite of confirming a pivotal role of anticonvulsant drugs in modulating seizure threshold and neuronal death, offer a novel target, which may be used to develop anticonvulsant and neuroprotective agents (Pizzanelli et al, 2009).

There are several indications that free radical plays a role in epileptogenesis. During seizures, the ROS concentration and brain lipid peroxidation increase (Curia et al, 2008). It is currently hypothesized that any pathological process such as status epilepticus, which releases dopamine and glutamate, activates D_2 and NMDA receptors.

This may lead to neuronal necrosis by elevating intracellular calcium and activating potentially destructive calcium-dependent enzymes, augmenting the production of free radicals during seizures induced by pilocarpine (Michotte et al, 1997).

The available experimental data suggest that convulsion generally accelerate brain damage. Limbic status epilepticus causes neuronal necrosis in hippocampus, amygdala, pyriform cortex, entorhinal cortex, thalamus, neocortex, striatum and substantia nigra (Ayyildiz et al, 2007b). The neuronal damage depends on synaptic activation (Vanin et al, 2003), probably via a glutamatergic calcium-medited mechanism (Marinho et al, 1998).

In the epilepsy model induced by a high dose of pilocarpine, we can observe neuronal loss in some brain areas, namely the hippocampus, striatum, amygdala, piriform cortex, entorhinal cortex, lateral septum, thalamus and substantia nigra, suggesting the involvement of these different areas during the establishment of the epileptic process (Honchar et al, 1983; Turski et al, 1983; Clifford et al, 1987; Marinho et al, 1997; Borelli et al, 2002; Freitas et al, 2006).

Among the areas in which neuronal damage occurs, the striatum and fronto-parietal cortex, besides being the most affected areas, may be related in important ways with the mechanism of propagation and/or maintenance (epileptogenesis) limbic seizures (Marinho et al, 1998). Barone and collaborators (1991) demonstrated that through intracerebral administration in the striatum of D_2 dopamine agonists, there was a protection with respect to the development of seizures in adult rats, suggesting the participation of this brain region in limbic seizures.

Histopathological examinations during the acute phase of seizures induced by pilocarpine show extensive hippocampal brain damage, pyriform, entorhinal, frontal, temporal and parietal cortices and in the striatum and amygdaloid nucleus (Marinho et al, 1997).

Cerebral lesions during the acute period are characterized by neuronal loss, gliosis and vacuolation, although there are contradictory data with respect to the severity and relative

distribution of brain damage (Mello et al, 1993). Brain necrosis is associated with the occurrence of seizures, although studies have demonstrated that this association is not obligatory, especially in the pilocarpine model (Peredery et al, 1992). The seizures induced by pilocarpine can be blocked by atropine, pointing towards involvement of the cholinergic system. On the other hand, atropine did not act after seizure onset, suggesting that others neurotransmitters and oxidative stress may participate in the maintenance and/or propagation of seizures and brain damage as well (Hirsh et al, 1982). Oxidative stress mediated by free radical produces lipid peroxidation, increases the nitrite content in the hippocampus, striatum and frontal cortex (Freitas et al, 2004) and may play a major role in the neuronal injury development after seizures induced by pilocarpine.

6. Antioxidant compound effects on histopathological alterations in epilepsy model

Hence, it could be expected that antioxidant drugs such as ascorbic acid and alpha-tocopherol, can be used as scavengers of free radicals, reducing brain injury induced by pilocarpine. In previous histopathological analyses, ascorbic acid and alpha-tocopherol antioxidants protected animals against seizures, status epilepticus and brain damage induced by pilocarpine (Figure 1) by decreasing the percentage of seizures, status epilepticus and death in relation to both doses tested.

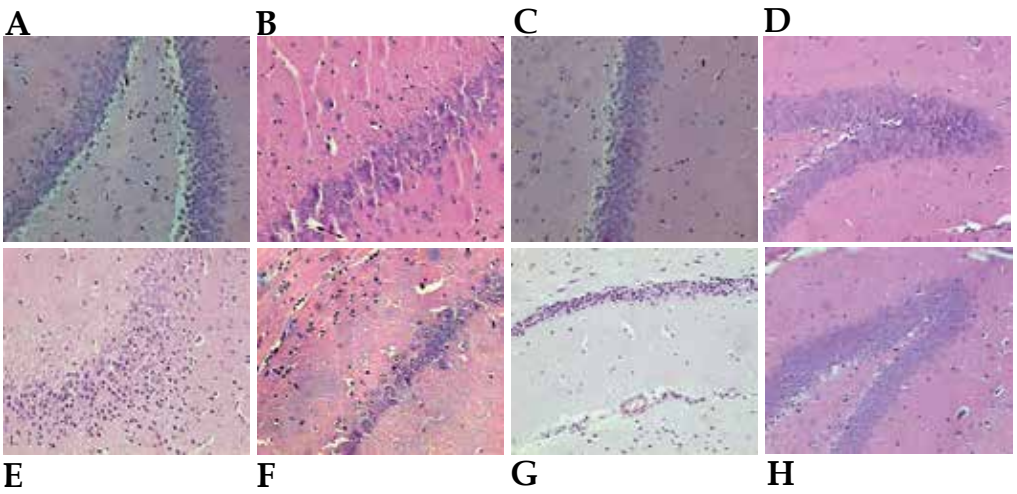


Fig. 1. Histopathological alterations in rat hippocampus treated with pilocarpine, ascorbic acid or their combinations. [**A**] Control group; [**B**] Pilocarpine group; [**C**] ascorbic acid 250 group; [**D**] ascorbic acid 250 plus Pilocarpine groups was treated with ascorbic acid (250 mg/kg) and 30 min before Pilocarpine; [**E**] ascorbic acid 500 group; [**F**] ascorbic acid 500 plus Pilocarpine group was treated with ascorbic acid (500 mg/kg) and 30 min before Pilocarpine. Severity of lesion was expressed as mean ± S.E.M. of scores of damage based in a scale from zero (none) to 100 (total) percent of structural involvement. Brain damage was considered positive if there was at least 50% hippocampal involvement. Hematoxylin & Eosin staining (H&E). Magnification, 100 X. One representative experiment with n=6 is shown.

A variety of epilepsy models reflect the effects of acid ascorbic and alpha-tocopherol and specify their action (Koza et al, 2007; Gaby, 2007). Previously, it had been demonstrated that these compounds reduced the frequency of penicillin-induced epileptiform activity (Ayyildiz et al, 2006; Ayyildiz et al, 2007b). In recent years, many roles of alpha-tocopherol have been discovered, including not only an antioxidant function, but also pro-oxidant, cell signaling, and gene regulatory functions. Some studies have reported that alpha-tocopherol is considered to be the main antioxidant substance in the human body, interfering with the production of hydroxyl radical and also with the oxygen in cell membranes, thereby reducing lipid peroxidation (Barros et al, 2007).

Our results demonstrated that seizure pattern and brain damage observed in pilocarpine-treated animals differ from those pretreated with alpha-tocopherol (400 mg/kg) plus pilocarpine (400 mg/kg). The latter reproduced the syndrome with lower intensity of histopathological changes and mortality rate, in comparison with the alpha-tocopherol (200 mg/kg) plus pilocarpine, corroborating the outcomes obtained by Ribeiro and collaborators (2005) and Ayyildiz and collaborators (2006). The percentage of status epilepticus (75%) that was found further corroborated prior investigations (Clifford et al, 1987; Marinho et al, 1997).

Ascorbic acid is probably the most important water-soluble antioxidant in the brain extracellular fluid, and it is essential in regenerating reduced alpha-tocopherol in membranes (Niki, 1991). Despite the fact that ascorbic acid has an antioxidant role to counter oxidative stress, ascorbic acid also form reactive oxidants, especially in the presence of transition metals. The evidence suggests that ascorbic acid participates in pro-oxidant reactions under certain conditions (Layton et al, 1998).

The outcomes confirm that ascorbic acid (250 or 500 mg/kg) decreased the frequency of pilocarpine-induced seizures, status epilepticus and brain lesions in rats. In addition, ascorbic acid decreases the severity of hippocampal lesions and mortality rate caused by pilocarpine. Yamamoto and collaborators (2002) demonstrated that the injection of ascorbate, 60 min before $FeCl_3$ administration, prevented the occurrence of epileptic discharges. Since there are wide variations of alpha-tocopherol and ascorbic acid doses used in different models of seizure, more detailed investigations are necessary before an ultimate conclusion on the effects of those compounds on pilocarpine-induced seizures can be achieved.

In conclusion, there is an accumulation of free radicals after status epilepticus induced by pilocarpine, and oxidative changes in other parameters during the acute phase. This finding suggests that seizures, status epilepticus and deaths induced by pilocarpine have a large participation in brain oxidative stress, which is closely related to the mechanism of propagation and/or maintenance of the epileptic focus by pilocarpine. These results suggest that free radicals as well as the muscarinic receptor activation seem to be involved in the genesis of seizures and brain damage obtained with pilocarpine. On the other hand, the muscarinic activation seems to play a major role in the neuronal damage produced by pilocarpine. Antioxidant compounds can exert neuroprotective function during acute phase of seizures, thereby decreasing the severity of hippocampal lesions. All these outcomes indicate the promising therapeutic potential of ascorbic acid and alpha-tocopherol in treatments for neurodegenerative diseases.

Brain tissue examinations of the animals pretreated with ascorbic acid (250 or 500 mg/Kg; Figure 1), alpha-tocopherol (200 or 400 mg/kg Figure 2), lipoic acid (10 or 20 mg/kg, Figure 3) or ubiquinone (5 or 10 mg/kg; Figure 4), did not reveal hippocampal and striatal

histopathological changes. Then again, pilocarpine-treated animals presented neuronal loss, gliosis, and typical vacuolar degeneration in hippocampus and striatum regions. Histopathological damage in hippocampus was observed in 50, 33, 33 and 17% of the animals co-administered with ascorbic acid (250 or 500 mg/kg) or alpha-tocopherol (200 or 400 mg/kg), and that 30 min after pretreatment received pilocarpine (400 mg/kg), respectively (Table 2). In addition, the analyses of histopathological damage in hippocampus of rats pretreated with lipoic acid (10 or 20 mg/kg) or ubiquinone (5 or 10 mg/kg), and that 30 min after pretreatment received pilocarpine (400 mg/kg) revealed a reduction of 52, 68, 52 and 100% in the number of animals with neuronal damage, respectively (Table 2).

Drugs	Dose (mg/kg)	Rats with lesion (%)	Severity of lesion (%)	Number of animals with lesion per group
Pilocarpine	400	83	59.92 ± 0.23	5
Ascorbic acid	250	00	00	0
	500	00	00	0
Ascorbic acid plus P400	250	50[a]	20.00 ± 0.32[a]	3
	500	33[a,b]	17.66 ± 0.33[a,b]	2
Alpha-tocopherol	200	00	00	0
	400	00	00	0
Alpha-tocopherol plus P400	200	33[a]	13.66 ± 0.33[a]	2
	400	17 [a,b]	5.97	1
Ubiquinone	5	00	00	0
	10	00	00	0
Ubiquinone plus P400	5	33[a]	12.00 ± 0.25[a]	2
	10	00	00	0
Lipoic acid	10	00	00	0
	20	00	00	0
Lipoic acid plus P400	10	33[a]	11.96 ± 0.12[a]	2
	20	17 [a,b]	5.97	1

Pilocarpine was administered in a single dose (400 mg/kg, P400, n=6), ascorbic acid groups with ascorbic acid (250 or 500 mg/kg), alpha-tocopherol (200 or 400 mg/kg, n=6), ubiquinone (5 or 10 mg/kg, n=6) and lipoic acid group with lipoic acid (10 or 20 mg/kg; n=6). The ascorbic plus P400 groups were treated with ascorbic acid (250 or 500 mg/kg, n=6) and 30 min before P400. The alpha-tocopherol plus P400 group was treated with alpha-tocopherol (200 or 400 mg/kg) and 30 min before P400. The ubiquinone plus P400 group was treated with ubiquinone (5 or 10 mg/kg) and 30 min before P400. The lipoic acid plus P400 group was treated with lipoic acid (10 or 20 mg/kg) and 30 min before P400. Severity of lesion was expressed as mean ± S.E.M. of scores of damage based on a scale from zero (none) to 100 (total) percent of structural involvement. Brain damage was defined as present if there was at least 50% hippocampal involvement. Results for % rats with brain lesion and % severity of lesion are expressed as percentages of the number of animals inside in parenthesis. [a]$p<0.05$ compared with P400 group (χ^2 test). [b]$p<0.05$ compared with ascorbic acid 250 plus P400 group or alpha-tocopherol 200 mg/kg plus P400 group or ubiquinone 5 mg/kg plus P400 group or lipoic acid 10 mg/kg plus P400 group (χ^2 test).

Table 2. Histopathological alterations in hippocampus of rats pretreated with antioxidant compounds after 24 h of phase acute of pilocarpine-induced seizures.

Histopathological damage in striatum was observed only in 33, 17, 17 and 17% of the animals co-administered with ascorbic acid (250 or 500 mg/kg), ubiquinone (5 mg/kg) and lipoic acid (10 mg/kg) and that 30 min after pretreatment received pilocarpine (400 mg/kg), respectively (Table 2). Moreover, the analyses of histopathological damage in striatum of rats pretreated with lipoic acid (20 mg/kg), alpha-tocopherol (200 or 400 mg/kg) or ubiquinone (10 mg/kg), and that 30 min after pretreatment received pilocarpine (400 mg/kg) revealed no neuronal damage (Table 2).

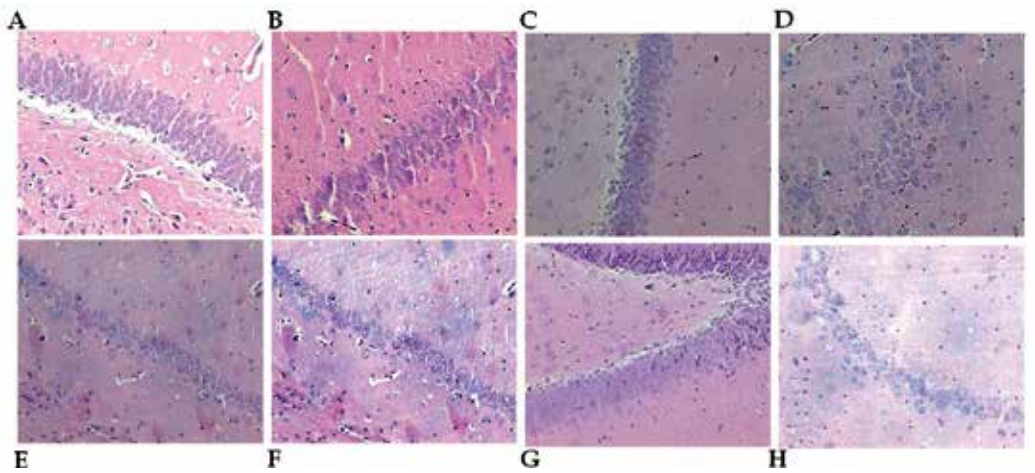


Fig. 2. Histopathological alterations in rat hippocampus treated with pilocarpine, atropine, alpha-tocopherol or their combinations. [**A**] Control group; [**B**] Pilocarpine group; [**C**] alpha-tocopherol 200 group; [**D**] alpha-tocopherol 200 plus Pilocarpine group was treated with alpha-tocopherol (200 mg/kg) and 30 min before P400; [**E**] alpha-tocopherol 400 group; [**F**] alpha-tocopherol 400 plus Pilocarpine group was treated with alpha-tocopherol (400 mg/kg) and 30 min before Pilocarpine. Severity of lesion was expressed as mean ± S.E.M. of scores of damage based in a scale from zero (none) to 100 (total) percent of structural involvement. Brain damage was considered positive if there was at least 50% hippocampal involvement. Hematoxylin & Eosin staining (H&E). Magnification, 100 X. One representative experiment with n=6 is shown.

7. Oxidative stress in epilepsy model

The lipid peroxidation level in the brain homogenates are increased in this model. During the acute phase of seizures induced by pilocarpine, increases in lipid peroxidation level, nitrite concentration and GSH content in striatum, frontal cortex and hippocampus have been verified in the same way (Freitas et al, 2004). The improved review demonstrates that status epilepticus induces different changes in superoxide dismutase activity according to the brain region, as that enzymatic activity remained unaltered in striatum and hippocampus but increased in frontal cortex. After the first hour of acute phase of seizures an increase is detected in several regions (striatum, hippocampus and frontal cortex). In addition, catalase activity was increased in striatum, hippocampus and frontal cortex in this epilepsy model (Freitas et al, 2003).

Drugs	Dose (mg/kg)	Rats with lesion (%)	Severity of lesion (%)	Number of animals with lesion per group
Pilocarpine	400	67	55.39 ± 0.52	4
Ascorbic acid	250	00	00	0
	500	00	00	0
Ascorbic acid plus P400	250	33[a]	25.42 ± 0.25[a]	2
	500	17[a,b]	13.46	1
Alpha-tocopherol	200	00	00	0
	400	00	00	0
Alpha-tocopherol plus P400	200	00	00	0
	400	00	00	0
Ubiquinone	5	00	00	0
	10	00	00	0
Ubiquinone plus P400	5	17[a]	14.31	1
	10	00	00	0
Lipoic acid	10	00	00	0
	20	00	00	0
Lipoic acid plus P400	10	17[a]	12.96 ± 0.22[a]	1
	20	00	00	0

Pilocarpine was administered in a single dose (400 mg/kg, P400, n=6), ascorbic acid groups with ascorbic acid (250 or 500 mg/kg), alpha-tocopherol (200 or 400 mg/kg, n=6), ubiquinone (5 or 10 mg/kg, n=6) and lipoic acid group with lipoic acid (10 or 20 mg/kg; n=6). The ascorbic plus P400 groups were treated with ascorbic acid (250 or 500 mg/kg, n=6) and 30 min before P400. The alpha-tocopherol plus P400 group was treated with alpha-tocopherol (200 or 400 mg/kg) and 30 min before P400. The ubiquinone plus P400 group was treated with ubiquinone (5 or 10 mg/kg) and 30 min before P400. The lipoic acid plus P400 group was treated with lipoic acid (10 or 20 mg/kg) and 30 min before P400. Severity of lesion was expressed as mean ± S.E.M. of scores of damage based on a scale from zero (none) to 100 (total) percent of structural involvement. Brain damage was defined as present if there was at least 50% straital involvement. Results for % rats with brain lesion and % severity of lesion are expressed as percentages of the number of animals inside in parenthesis. [a]$p<0.05$ compared with P400 group (χ^2 test). [b]$p<0.05$ compared with ascorbic acid 250 plus P400 group or alpha-tocopherol 200 mg/kg plus P400 group or ubiquinone 5 mg/kg plus P400 group or lipoic acid 10 mg/kg plus P400 group (χ^2 test).

Table 3. Histopathological alterations in striatum of rats pretreated with antioxidant compounds after 24 h of phase acute of pilocarpine-induced seizures.

Lipid peroxidation in a tissue is an index of irreversible biological damage of the cell membrane phospholipid, which in turn leads to inhibition of most of the sulphydryl and some nonsulphydryl enzymes (Gilbert and Sawas, 1983). Lipid peroxidation level increase and reduce, whereas glutathione decrease can be induced by many chemicals (e.g. kainic acid and pilocarpine) and by many tissue injuries, and has been suggested as a possible mechanism for the neurotoxic effects of epileptic activity (Sah et al, 2002). Our findings demonstrated that lipid peroxidation levels increase after the first hour and during 24 h of the acute phase of seizures induced by pilocarpine in hippocampus, striatum and frontal cortex.

In normal conditions, there is a steady state balance between the production of ROS and their destruction by cellular antioxidant system. It is demonstrated that nitrite content in striatum and frontal cortex is augmented after seizures and status epilepticus in adult rats, suggesting a possible increase in ROS level, which can be involved in neuronal damage induced status

epilepticus. Other studies have shown that nitrite and nitrate levels were not elevated in patients with cryptogenic west syndrome (Vanhatalo and Riikonen, 2001), but it is tempting to speculate that the seizure activity per se did not account for the whole increment observed in nitrite and nitrate levels, and other mechanisms may be associated with this parameter in this epilepsy model, as well as neuronal degeneration observed in human beings. However, new studies using antioxidants drugs during status epilepticus induced by pilocarpine can indicate whether lipid peroxidation, nitrite and glutathione reduced (GSH) concentrations are involved in the pathophysiology of status epilepticus in this model.

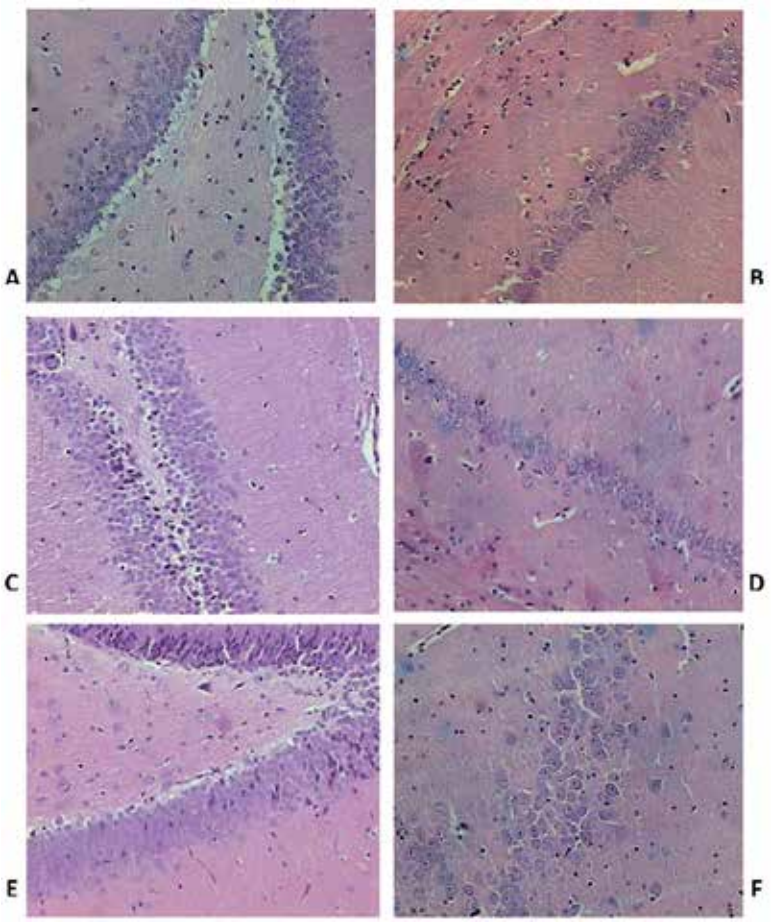


Fig. 3. Histopathological alterations in rat hippocampus pretreated with lipoic acid prior to pilocarpine-induced seizures. Severity of lesion was expressed as a mean ± S.E.M. of scores of damage based in a scale from zero (none) to 100 (total) percentage of hippocampus involvement. Brain damage was considered positive if there was at least 50% hippocampal involvement showed by Hematoxylin & Eosin staining (HE). Pictures (100 X) shown are from one representative experiment of n=8. **[A]:** Control group; **[B]:** Pilocarpine group; **[C]:** lipoic acid 10 group; **[D]:** lipoic acid 10 plus pilocarpine group; **[E]:** lipoic acid 20 group; **[F]:** lipoic acid 20 plus pilocarpine group.

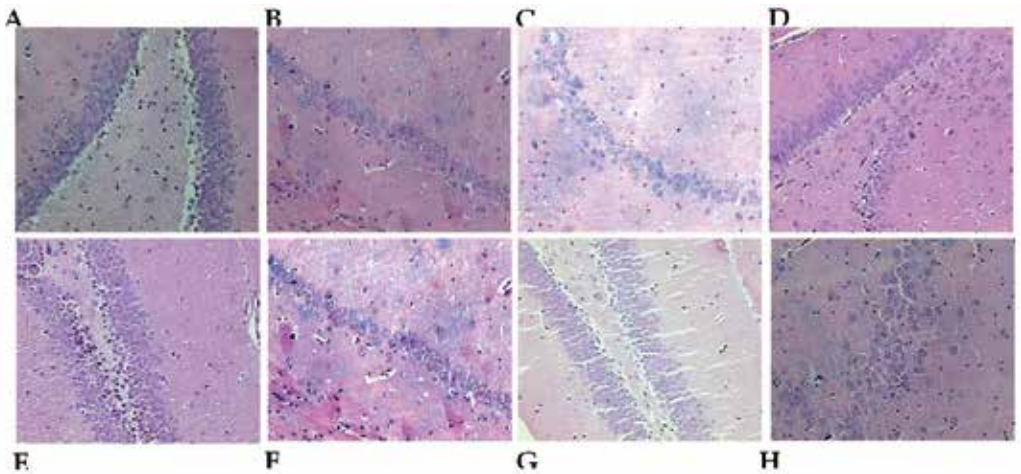


Fig. 4. Histopathological alterations in rat hippocampus pretreated with ubiquinone prior to pilocarpine-induced seizures. Severity of lesion was expressed as a mean ± S.E.M. of scores of damage based in a scale from zero (none) to 100 (total) percentage of hippocampus involvement. Brain damage was considered positive if there was at least 50% hippocampal involvement showed by Hematoxylin & Eosin staining (HE). Pictures (100 X) shown are from one representative experiment of *n*=8. **[A]:** Control group; **[B]:** Pilocarpine group; **[C]:** Ubiquinone 5 group; **[D]:** Ubiquinone 10 group; **[E]:** Ubiquinone 20 group; **[F]:** Ubiquinone 5 plus P400 group **[G]: U**biquinone 10 plus P400; **[H]:** Ubiquinone 20 plus P400.

Although there were no selective brain regions particularly vulnerable to oxidative stress, there were some regional variations in the amount of oxidative damage observed. In the regions studied, there were nearly equal elevations in lipid oxidative, nitrite content and GSH markers that persisted during the acute phase of seizures.

All living organisms can suffer oxidative damage, yet the animal brain is often said to be especially sensitive (Gilbert and Sawas, 1983; Bruce and Baudry, 1995). The experimental data demonstrate that pilocarpine administration and its resulting status epilepticus produce significant alterations in hippocampus, striatum and frontal cortex. We recorded alterations in superoxide dismutase activity in frontal cortex during the seizures, however, no alterations were observed in striatal superoxide dismutase activity of rats under the same conditions. It is likely that the unaltered superoxide dismutase activity in striatum might not be related to the mechanisms involved in installation and propagation of seizures and status epilepticus induced by pilocarpine, which produces several changes in parameters related to generation and elimination of oxygen free radicals in adult rats (Sawas and Gilbert, 1985). An increase in free radical formation can be accompanied by an immediate compensatory increase of free radical scavenging enzymatic (superoxide dismutase and catalase) activities and this action was observed during status epilepticus in brain regions. Nevertheless, a similar compensatory mechanism of scavenging was observed in catalase activity after status epilepticus, suggesting that the enzymatic function of different systems can be modified either during the acute phase of seizures or according to cerebral area investigated. Literature reports the involvement of catalase activity in hippocampus, striatum and frontal cortex after status epilepticus. An increase in catalase activity in these brain areas can be related to a long-term compensatory mechanism including modulation activity of enzymes from the ROS catabolism.

Moreover, the catalase activity might be one of the mechanisms capable to avoid the development of neurotoxic effects mediated by SE, indicating that basal-oxygen radical production can damage the cell and that its control is necessary (McCord, 1989; Naffah-Mazzacoratti, 2001).

Evidences for the role of free radicals in seizures has been found by using exogenously administered enzymatic and non-enzymatic antioxidants for protection against seizures and status epilepticus-induced neuronal damage (Kulkarni and George, 1996; Freitas et al, 2005). A steady state level of O_2^- and H_2O_2 is always present within cells as a result of a normal metabolism. Superoxide dismutase and catalase are responsible for degradation of O_2^- and H_2O_2, respectively. The balance between antioxidants enzymes, superoxide dismutase and catalase can be important during seizures and status epilepticus induced by pilocarpine. The present data indicate that pilocarpine treatment and its resulting status epilepticus induce neurochemical changes such as an increase in nitrite content and lipid peroxidation level, a decrease in GSH content as well as an activation of brain antioxidant mechanisms. The anatomic distribution of alterations observed in the enzymatic activities (superoxide dismutase and catalase) can suggest that the frontal cortex can be extensively involved in the propagation of epileptic activity and further studies should be carried out to ascertain that the catabolism of nitrite, ROS and GSH can be involved in the pathogenesis of status epilepticus.

The pilocarpine model is essential to investigate the mechanisms for initiation and propagation of seizures and status epilepticus. Additionally, it may be assumed that the increased generation of nitrite and lipid peroxidation levels after status epilepticus is not primary caused by an exhaustion of both the enzymatic and non-enzymatic defense systems measured. Adaptative mechanisms, as the induction of catalase activity, may be taken into consideration to counteract oxidative stress mediated by status epilepticus. However, the relation among brain structures, antioxidant systems, lipid peroxidation, nitrite concentration and status epilepticus cannot be perfectly established and deserves further investigation.

8. Antioxidant compound effects on oxidative stress in epilepsy model

Neurochemical alterations are observed in pilocarpine-induced seizures (Freitas et al, 2005), whose physiopathology is still poorly understood. However, there is data in literature suggesting that elevated reactive oxygen species concentrations and/or its metabolites are potentially neurotoxic (Freitas et al, 2004). We have demonstrated that lipoic acid reduces brain oxidative metabolism (Militão et al, 2010) and cannot inhibit Na^+, K^+-ATPase activity in rat hippocampus.

Animal models are useful to better understand the pathophysiology of seizures. In this context, the antioxidants compound effects in pilocarpine model were recently investigated (Mudd et al, 2001), revealing that they might produce a decrease in nitrite levels in rat hippocampus (Koçak et al, 2000; Augoustides-Savvopoulou et al, 2003). Animals exposed to lipoic acid treatment presented no differences in physical growth and brain, suggesting that lipoic acid ameliorates metabolic parameters in pilocarpine model (Mesulam et al, 2002).

By using this model, we investigated the effect of lipoic acid on spatial navigation tasks in the Morris water maze. Results have shown that seized rats did not present performance impairment neither in the acquisition phase nor on the time spent in target quadrant and in platform location nor in the latency to cross over the platform location in the reference memory task session . However, lipoic acid significantly impaired working memory

performance, since there was a significant effect with the 9-day interaction group and significant differences after days 2 and 5.

The biological effects of free radicals are controlled in vivo by a wide range of antioxidants, such as alpha-tocopherol, ascorbic acid, vitamin A, and glutathione reduced (Halliwell and Gutteridge, 1990; Ayyildiz et al, 2006). Acid ascorbic and alpha-tocopherol have many functions in the brain and in the neuronal microenvironment. They work as neuromodulators as well as antioxidant/free radical scavengers (Koza et al, 2007; Devi et al, 2008). It has been suggested that ascorbic acid and alpha-tocopherol have neuroprotective properties in some experimental models of excitotoxic neurological disorders, including seizure activity induced by pilocarpine (Gaby, 2007; Barros et al., 2007).

Systemic injection of pilocarpine, a cholinergic muscarinic agonist, induces status epilepticus in rodents, which is associated to histopathological and neurochemical changes as well as oxidative stress (Turski et al, 1983; Cavalheiro et al, 1994; Freitas et al, 2005). Elevated free radical products were observed during the status epilepticus. Free radicals are highly reactive chemical compounds due to the tendency of electrons to pair, which are normally associated with oxidative damage (Castagne et al, 1999).

Free radicals can be generated in the brain by several mechanisms such as inefficiency of the electron-carrying components of the mitochondrial transport chain, monoamines degradation, xanthine oxidase reaction or by metabolism of arachidonic acid. Nevertheless, the free radicals produced could be metabolized especially by antioxidant enzymes such as superoxide dismutases, catalase and glutathione peroxidase (Hussain et al, 1995; Meister, 1995; Frantseva et al, 2000; Ferreira et al, 2009). Then, the resulting free radicals are very likely to react with non-radical molecules and transform them into secondary free radicals, which are normally observed during the lipid peroxidation producing hydroperoxides (MacDonald et al, 1989). The lipid peroxidation and nitrite are increased in hippocampus rats during pilocarpine-induced seizures (Freitas, 2009; Militão et al, 2010). Thus, it is worthwhile assessing the role of antioxidant compounds in the prevention of these neurochemical alterations on oxidative stress during seizures.

The excitotoxicity has been shown to be related to over-production of free radicals in hippocampus of adult rats. The excessive release of excitatory amino acids such as glutamate may kill neurons via excessive activation of their receptors facilitating the installation and/or propagation of seizures (MacDonald et al., 1989; Cavalheiro et al., 1994; Meldrum, 1994).

Depending on neuronal maturity, the glutamate may induce either apoptotic or necrotic types of death (Ferrer et al, 1995) by impairing the Ca^{2+} homeostasis and inducting the oxidative stress (Leite et al, 1990; Murphy and Baraban, 1990). Based on this fact, it is important to evaluate the role of ubiquinone on oxidative stress in rat hippocampus during seizures since ubiquinone is a powerful antioxidant that prevents oxidative damage caused by free radicals, including oxidation of lipids within the mitochondrial membrane (Geromel et al., 2002).

Several antioxidant compounds, such as acid ascorbic (Xavier et al, 2007), lipoic acid (Freitas, 2009) and alpha-tocopherol (Barros et al, 2007) can protect the brain against oxidative stress in rat hippocampus caused by pilocarpine-induced seizures. Studies have suggested that ubiquinone (UQ) serves as an antioxidant by activating and increasing expression of mitochondrial uncoupling proteins which have antiapoptotic and antioxidant properties (Shults and Haas, 2005; Chaturvedi and Beal, 2008). Ubiquinol, a reduced form of ubiquinone, decreases lipid peroxidation directly by acting as a chain-breaking antioxidant

and indirectly by recycling alpha-tocopherol. Thus, it is important to investigate the neuroprotective effect of ubiquinone against hippocampal damage caused by oxidative stress observed during seizures.

In addition to preventing lipid peroxidation, UQ, as an effective antioxidant, also reacts with ROS (James et al., 2004). This study implies that ubiquinone may alter the oxidative stress in rat hippocampus caused by the seizures. To further attest this hypothesis, this study was aimed at evaluating the effects of ubiquinone on superoxide dismutase, catalase and glutathione peroxidase activities as well as in hydroperoxide concentration in the rat hippocampus during acute phase of seizures induced by pilocarpine.

The pilocarpine model could prove to be useful to delineate and understand the development of behavioral and neurochemical changes associated with temporal lobe epilepsy. Pilocarpine status may provide a model for studying the basic mechanisms responsible for refractory status epilepticus, amino acids and oxidative stress in humans and evaluating new drugs. The pilocarpine model may prove useful in the study of status epilepticus for number of reasons. First, these seizures accurately model human generalized epilepsy as it is seen from the anticonvulsant profile drugs. Secondly, the serve and refractory nature of this model indicates that it should be valuable in the development of new anticonvulsant agents. Finally, the prolonged and uniform degree of status epilepticus is useful for metabolic, neurochemical and neuroanatomical studies of the sequelae of prolonged seizure activity.

9. Acknowledgments

This work was supported by a research grant from the Brazilian National Research Council (CNPq). R.M.F. is fellow from CNPq. The technical assistance of Stênio Gardel Maia is gratefully acknowledged.

10. References

Aminoff, M.J. & Simon, R.P. (1980). Status epilepticus. Causes, clinical features and consequences in 98 patients, *The American Journal of Medicine* 12: 657-666.

Ayyildiz, M., Yildirim, M., & Agar, E. (2006). The effects of vitamin E on penicilin-induced epileptiform activity in rats, *Experimental Brain Research* 174: 109-113.

Ayyildiz, M., Coskun, S., Yildirim, M. & Agar, E. (2007a). The involvement of nitric oxide in the anticonvulsant effects of α-tocopherol on penicillin-induced epileptiform activity in rats, *Epilepsy Research* 73: 166-172.

Ayyildiz, M., Coskun, S., Yildirim, M. & Agar E. (2007b). The effects of ascorbic acid on penicillin-induced epileptiform activity in rats, *Epilepsia* 48: 1388-1395.

Augoustides-Savvopoulou, P., Luka, Z., Karyda, S., Stabler, S.P., Allen, R.H., Patsiaoura, K., Wagner, C. & Mudd, S.H. (2003). Glycine N-methyltransferase deficiency: a new patient with a novel mutation, *Journal of Inherited Metabolic Disease* 26: 745–759

Barone, P., Palma, V., Debartolomeis, A., Tedeschi, E., Muscettola, G. & Campanella, G. 1991. Dopamine D_1 and D_2 receptors mediate opposite functions in seizures induced by lithium-pilocarpine, *European Journal of Pharmacology* 95: 157-162.

Barros, D.O., Xavier, S.M.L., Barbosa, C.O., Silva, R.F., Maia, F.D., Oliveira, A.A. & Freitas, R.M. (2007). Effects of the vitamin E in catalase activities in hippocampus after status epilepticus induced by pilocarpine in Wistar rats, *Neuroscience Letters* 416: 227-230.

Ben-Ari, Y., Tremblay, E. & Ottersen, O.P. (1980). Injections of kainic acid into the amygdaloid complex of the rat: an electrographic, clinical and histological study in relation to the pathology of epilepsy, *Neuroscience* 5: 515-528.

Ben-Ari, Y., Tremblay, E., Riche, D., Ghilini, G. & Naquet, R. (1981). Electrographic, clinical and pathological alterations following systemic administration of kainic acid, bicuculdeoxyglucose or pentylenetetrazole: metabolite mapping using the deoxyglucose method with special reference to the pathology of epilepsy, *Neuroscience* 6: 1361-1391.

Borelli, E. & Bozzi, Y. (2002). Dopamine D_2 receptor signaling controls neuronal cell death induced by muscarinic and glutamtergic drugs, *Molecular and Cellular Neuroscience* 19: 263-271.

Brozek, G., Hort, J., Komarek, V., Langmeier, M. & Mares, P. (2000). Interstrain differences in cognitive functions in rats in relation to status epilepticus, *Behavioural Brain Research* 112: 77–83.

Browne, T.R. & Holmes, G.L. (2001). Epilepsy, *New Jersey Med.* 344*:* 1145–1451.

Bruce, A.J. & Baudry, M. (1995). Oxygen free radicals in rat limbic structures after kainate-induced seizures, *Free Radical Biology and Medicine* 18: 993-1002.

Castagne, V., Gastschi, M., Lefevre, K., Posada, A. & Clarke, P.G.H. (1999). Relationship between neuronal death and cellular redox status, focus on the developing nervous system, *Progress in Neurobiology* 59: 397–423.

Cavalheiro, E.A., Leite, J.P., Bortolotto, Z.A., Turski, W.A., Ikonomidou, C. & Turski, L. (1991). Long-term effects of pilocarpine in rats: structural damage of the brain triggers kindling and spontaneous recurrent seizures, *Epilepsia* 32: 778-782.

Cavalheiro, E.A., Fernandes, M.J., Turski, L. & Naffah-Mazzacoratti, M.G. (1994). Spontaneous recurrent seizures in rats: amino acid and monoamine determination in the hippocampus, *Epilepsia* 35*:* 1-11.

Chamberlain, M., Johnson, M.P. & Kelly G.M. (2000). Blockade of pilocarpine-induced cerebellar phosphoinositide hydrolysis with metabotropic glutamate antagonists: evidence for an indirect control of granulle cell glutamate release by muscarinic agonists, *Neuroscience Letters* 285: 71-75.

Chaturvedi, R.K. & Beal, M.F. (2008). Mitochondrial approaches for neuroprotection, *Annals of the New York Academy of Sciences* 1147: 395–412.

Clifford, D.B., Olney, J.W., Maniotis, A., Collins, R.C. & Zorumski, C.F. (1987). The functional anatomy and pathology of lithium-pilocarpine and high-dose pilocarpine seizures, *Neuroscience* 23: 953-968.

Costa Junior, J.S., Feitosa, C.M., Cito, A.M.G.L., Freitas, R.M., Henriques, J.A.P. & Saffi, J. (2010). Evaluation of Effects of Ethanolic Extract from *Platonia insignis* Mart. on Pilocarpine-induced Seizures, *Journal of Biological Sciences* 10*:* 747-753.

Costa-Lotufo, L.V., Fonteles, M.M.F., Lima, I.S.P., Oliveira, A.A., Nascimento, V.S., Bruin, V.M.S. & Viana, G.S.B. (2002). Attenuating effects of melatonin on pilocarpine-induced seizures in rats, *Comparative Biochemistry and Physiology Part C* 131: 521-529.

Curia, G., Longo, D., Biagini, G., Jones, R.S.G. & Avoli, M. (2008). The pilocarpine model of temporal lobe epilepsy, *Journal of Neuroscience Methods* 172: 143-157.

Dal-Pizzol, F., Klant, F., Vianna, M.M., Schroder, N., Quevedo, J., Benfato, M.S., Moreira, J.C. & Walz, R. (2000). Lipid peroxidation in hippocampus early and late after status epilepticus induced by pilocarpina of kainic acid in Wistar rats, Neuroscience Letters 291: 179-182.

Devi, P.U., Manocha, A. & Vohora, D. (2008). Seizures, antiepileptics, antioxidants and oxidative stress: an insight for researchers, *Expert Opinion on Pharmacotherapy* 9: 3169-3177.

El-Etri, M.M., Ennis, M., Jiang, M. & Shipley, M.T. (1993). Pilocarpine-induced convulsions in rats: evidence for muscarinic receptor-mediated activation of locus coeruleus and norepinephrine release in cholinolytic seizure development, *Experimental Neurology* 121: 24- 39.

Ferrer, I., Martin, F., Reiriz, J., Perez-Navarro, E., Alberch, J., Macaya, A. & Planas, A.M. (1995). Both apoptosis and necrosis occur following intrastriatal administration of excitotoxins, *Acta Neuropathologica* 90: 504–510.

Ferrer, I., Lopez, E., Blanco, R., Rivera, R., Krupinski, J. & Marti, E. (2000). Differential c-Fos and caspase expression following kainic acid excitotoxicity, *Acta Neuropathologica* 99: 245-256.

Ferreira, P.M.P., Militão, G.C.G. & Freitas, R.M. (2009). Lipoic acid effects on lipid peroxidation level, superoxide dismutase activity and monoamines concentration in rat hippocampus, *Neuroscience letters* 464*:* 131-134.

Frantseva, M.V., Perez, V.J.L., Hwang, P.A. & Carlen, P.L. (2000). Free radical production correlates with cell death in an in vitro model of epilepsy, *European Journal of Neuroscience* 12: 1431–1439.

Freitas, R.M., Sousa, F.C.F., Vasconcelos, S.M.M., Viana, G.S.B. & Fonteles, M.M.F. (2003). Acute alterations of neurotransmitters levels in striatum of young rat after pilocarpine-induced status epilepticus, *Arquivos de Neuropsiquiatria* 61: 430-433.

Freitas, R.M., Sousa, F.C.F., Vasconcelos, S.M.M., Viana, G.S.B. & Fonteles, M.M.F. (2004). Pilocarpine-induced seizures in adult rats: lipid peroxidation level, nitrite formation, GABAergic and glutamatergic receptor alterations in the hippocampus, striatum and frontal cortex, *Pharmacology Biochemistry and Behavior* 78: 327-332.

Freitas, R.M., Aguiar, L.M.V., Vasconcelos, S.M.M., Sousa, F.C.F., Viana, G.S.B. & Fonteles, M.M.F. (2005). Modifications in muscarinic, dopaminergic and serotonergic receptors concentrations in the hippocampus and striatum of epileptic rats, *Life Sciences* 78: 253-258.

Freitas, R.M., Sousa, F.C.F., Viana, G.S.B. & Fonteles, M.M.F. (2006). Acetylcholinesterase activities in hippocampus, frontal cortex and striatum of Wistar rats after pilocarpine-induced status epilepticus, *Neuroscience Letters* 399: 76-78.

Freitas, R.M. (2009). The evaluation of effects of lipoic acid on the lipid peroxidation, nitrite formation and antioxidant enzymes in the hippocampus of rats after pilocarpine-induced seizures, *Neuroscience letters* 455: 140–144.

Freitas, R.M., Jordan, J. & Feng D. (2010). Lipoic acid effects on monoaminergic system after pilocarpine-induced seizures, *Neuroscience Letters* 477: 129-133.

Gaby, A.R. (2007). Natural approaches to epilepsy, *Alternative Medicine Review* 12: 9-24.

Geromel, V., Rotig, A., Munnich, A. & Rustin, P. (2002). Coenzyme Q10 depletion is comparatively less detrimental to human cultured skin fibroblasts than respiratory chain complex deficiencies, *Free Radical Research* 36: 375–379.

Gilbert, J.C. & Sawas, A.H. (1983). ATPase activities and lipid peroxidation in rat cerebral cortex synaptosomes, *Archives internationales de pharmacodynamie et de thérapie* 263: 189-196.

Halliwell, B. & Gutteridge, J.M.C. (1990). The antioxidants of human extracellular fluids, *Archives of Biochemistry and Biophysics* 280: 1-8.

Halliwell, B. & Gutteridge, J.M.C. (1999). Free radicals in biology and medicine, Oxford Science Publications, London.

Hirsh, E., Baran, T.Z. & Snead, O.C. (1982). Ontogenic study of lithium-pilocarpine induced status epilepticus in rats, *Brain Research* 583: 120-126.

Honchar, M.P., Olney, J.W. & Sherman, W.R. (1983). Systemic cholinergic agents induce seizures and brain damage in lithium-treated rats, *Science* 220: 323-325.

Hort, J., Brozek, G., Komárek, V., Langmeier, M. & Mares, P. (2000). Interstrain differences in cognitive functions in rats in relation to status epilepticus, *Behavioral Brain Research* 112*:* 77-83.

Hussain, S., Slikker, W. & Ali, S.F. (1995). Age-related changes in antioxidant enzymes, superoxide dismutase, catalase, glutathione peroxidase and glutathione in different regions of mouse brain, *International Journal of Developmental Neuroscience* 13: 811–817.

James, A.M., Smith, R.A. & Murphy, M.P. (2004). Antioxidant and prooxidant properties of mitochondrial coenzyme Q, *Archives of Biochemistry and Biophysics* 423: 47–56.

Jenner, P. (1998). Oxidative mechanisms in nigral cell death in Parkinson's disease, *Movement Disorders* 13: 24-34.

Kabuto, H, Yokoi, I. & Ogawa, N. (1998). Melatonin inhibits iron-induced epileptic discharges in rats by suppressing peroxidation, Epilepsia 30: 237-243.

Koçak, G., Aktan, F., Canbolat, O., Ozogul, C., Elbeg, S., Yildizoglu-Ari, N. & Karasu, C. (2000) Alpha-lipoic acid treatment ameliorates metabolic parameters, blood pressure, vascular reactivity and morphology of vessels already damaged by streptozotocin-diabetes, *Diabetes, Nutrition & Metabolism* 13: 308-318.

Kozan, R., Ayyildiz, M., Bas, O., Kaplan, S. & Agar, E. (2007). The influence of ethanol intake and its withdrawal on the anticonvulsant effect of α-tocopherol in the penicillin-induced pileptiform activity in rats, *Neurotoxicology* 28: 463-470.

Krug, M.; Brodemann, R. & Ott, T. (1981). Identical responses of the two hippocampal theta generators to physiological and pharmacological activation, *Brain Research Bulletin* 6: 5-11.

Kulkarni, S.K. & George, B. (1996). Protective effects of GABAergic drugs and other anticovulsivants in lithium-pilocarpine-induced status epilepticus, *Methods & Findings in Experimental & Clinical Pharmacology* 18*:* 335-340.

Lapin, I.P., Mirzaev, S.M., Ryzov, I.V. & Oxenkrug, G.F. (1998). Anticonvulsant activity of melatonin against seizures induced by quinolinate, kainite, glutamate, NMDA, and pentylenetetrazole in mice, *Journal of Pineal Research* 24: 215-218.

Layton, M.E., Samson, F.E. & Pazdernik, T.L. (1998). Kainic acid causes redox changes in cerebral cortex extracellular fluid: NMDA receptor activity increases ascorbic acid whereas seizure activity increases uric acid, *Neuropharmacology* 37: 149-157.

Leite, J.P., Bortolotto, Z.A. & Cavalheiro, E.A., 1990. Spontaneous recurrent seizures in rats: an experimental model of partial epilepsy, *Neuroscience & Biobehavioral Reviews* 14: 511–517.

Liu, K.J.; Liu, S.; Morrow, D. & Peterson S.L. (2002). Hydroethidine detection of superoxide production during the lithium-pilocarpine model of status epilepticus, *Epilepsy Research* 49: 226-238.

Loup, F., Fritschy, J.M., Kiener, T. & Bouilleret, V. (1999). GABAergic neurons and $GABA_A$-receptors in temporal lobe epilepsy, *Neurochemistry International* 34: 435-445.

MacDonald, J.F., Mody, I. & Salter, M.W. (1989). Regulation of N-metyl-D-aspartate receptor revealed by intracellular dialysis of murine neurones in culture, *The Journal of Physiology* 414: 17–34.

MacGregor, D.G., Graham, D.I. & Stone, T.W. (1997). The attenuation of kainate-induced neurotoxicity by chlormethiazole and its enhancement by dizocilpine, muscimol, and adenosine receptor agonists, *Experimental Neurology* 148: 110-123.

Marinho, M.M.F., Sousa, F.C.F., Bruin, V.M.S., Aguiar, L.M.V., Pinho, R.S.N. & Viana, G.S.B. (1997). Inhibitory action of a calcium channel blocker (nimodipine) on seizures and brain damage induced by pilocarpine and lithium-pilocarpine in rats, *Neuroscience Letters 235*: 13-16.

Marinho, M.M.F., Sousa, F.C.F., Bruin, V.M.S., Vale, M.R. & Viana, G.S.B. (1998). Effects of lithium, alone or associated with pilocarpine, on muscarinic and dopaminergic receptors and on phosphoinositide metabolism in rat hippocampus and striatum, *Neurochemisty International* 33*:* 299-306.

Massieu, L., Rivera, A. & Tapia, R. (1994). Convulsions and inhibition of glutamate decarboxylase by pyridoxal phosphate-γ-glutamyl hydrazone in the developing rat, *Neurochemical Research* 19*:* 183-187.

McCord, J.M. (1989). Superoxide radical: controversies, contradiction and paradoxes, Proceedings of the Society for Experimental Biology and Medicine 209: 112–117.

Meister, A. (1995). Glutathione biosynthesis and its inhibition, *Methods in Enzymology* 252: 26-30.

Meldrum, B.S. (1994). The role of glutamate in epilepsy and other CNS disorders, *Neurology* 44: 14–23.

Mello, L.E.A.M., Cavalheiro, E.A., Tan, A.M., Kupfer, W. R., Pretorius, J.K., Babb, T.L. & Finch, D.M. (1993). Circuit Mechanisms of Seizures in the Pilocarpine Model of Chronic Epilepsy: Cell Loss and Mossy Fiber Sprouting, *Epilepsia* 34: 985-995.

Mesulam, M-M., Guillozet, A., Shaw, P., Levey, A., Duysen, E.G. & Lockridge, O. (2002) Acetylcholinesterase knockouts establish central cholinergic pathways and can use butyrylcholinesterase to hydrolyze acetylcholine, *Neuroscience* 170: 627–639.

Michiels, C., Raes, M., Toussaint, O. & Remacle, J. (1994). Importance of Se-glutathione peroxidase, catalase, and Cu/Zn-SOD for cell survival against oxidative stress, *Free Radical Biology & Medicine* 17: 235-248.

Michotte, Y., Ebinger, G., Manil, J., Khan, G.M. & Smolders, I. (1997). NMDA receptor-mediated pilocarpine-induced seizures: characterization in freely moving rats by microdialysis, *British Journal of Pharmacology* 121: 1171-1179.

Militão, G.C.G., Ferreira, P.M.P. & Freitas, R.M. (2010) Effects of lipoic acid on oxidative stress in rat striatum after pilocarpine-induced seizures, *Neurochemistry International* 56*:* 16-20.

Mudd, S.H., Levy, H.L. & Kraus, J.P. (2001). Disorders of trans sulfuration. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic and molecular bases of inherited disease, 8 th edn. McGraw-Hill, New York, pp 2007-2056.

Murphy, T.H. & Baraban, J.M. (1990). Glutamate toxicity in immature cortical neurons precedes development of glutamate receptor current, *Developmental Brain Research* 57: 146–150.

Naffah-Mazzacoratti, M.G., Cavalheiro, E.A., Ferreira, E.C., Abdalla, D.S.P., Amado, D. & Bellissimo, M.I. (2001). Pilocarpine-induced status epilepticus increases glutamate release in rat hippocampal synaptosomes, *Epilepsy Research* 46: 121-128.

Nanda, D., Tolputt, J. & Collard, K.J. (1996). Changes in brain glutathione levels during postnatal development in the rat, *Developmental Brain Research*. 94: 238–241.

Niki, E. (1991). Action of ascorbic acid as a scavenger of active and stable oxygen radicals, *The American Journal of Clinical Nutrition* 54: S1119-S1124

Peredery, O., Blomme, M.A. & Parker, G. (1992). Absence of maternal behaviour in rats with lithium/pilocarpine seizure induced brain damage: support of macleans triune brain theory, *Physiology and Behavior*, 52: 665-671.

Perlis, M.L., Smith, M.T., Orff, H.J., Andrews, P.J., Gillin, J.C. & Giles, D.E. (2002). The effects of an orally administered cholinergic agonist on REM sleep in major depression, *Biological Psychiatry* 51: 457-462.

Peterson, S.L., Morrow, D., Liu, S. & Liu, K.J. (2002). Hydroethidine detection of superoxide during lithium-pilocarpine model of status epilepticus, *Epilepsy Research* 49: 226-238.

Pizzanelli, C., Lazzeri, G., Fulceri, F., Giorgi, F.S., Pasquali, L., Cifelli, G., Murri, L. & Fornai, F. (2009). Lack of alpha 1b-adrenergic receptor protects against epileptic seizures, *Epilepsia* 50: 59-64.

Rauca, C., Wiswedel, I., Zerbe, R., Keilhoff, G. & Krug, M. (2004). The role of superoxide dismutase and α-tocopherol in the development of seizures and kindling induced by pentylenetetrazol - influence of the radical scavenger a-phenyl-N-tert-butyl nitrone, *Brain Research* 1009: 203-212.

Ribeiro MCP, Avila DS, Scheneider CYM, Hermes, F.S., Furian, A.F., Oliveira, M.S., Rubin, M.A., Lehmann, M., Krieglstein, J. & Mello, C.F. (2005). α-tocopherol protects against pentylenetetrazol- and methylmalonate-induced convulsions, *Epilepsy Research* 66: 185-194.

Rong, Y., Doctrow, S.R., Tocco, G. & Baudry, M. (1999). EUK-134, a synthetic superoxide dismutase and catalase mimetic, prevents oxidative stress and attenuates kainate-induced neuropathology, *Proceedings of the National Academy of Sciences,* 96: 9897-9902.

Sah, R., Galeffi, F., Ahfens, R., Jordan, G. & Schartz-Bloom, R.D.J. (2002). Modulation of the GABA(A)-gated chloride channel by reactive oxygen species, *Journal of Neurochemistry* 80: 383 - 391.

Santos, I.M.S., Freitas, R.L.M., Silva, E.P., Feitosa, C.M., Saldanha, G.B., Souza, G.F., Tomé, A.R., Feng D. & Freitas, R.M. (2010). Effects of ubiquinone on hydroperoxide

concentration and antioxidant enzymatic activities in the rat hippocampus during pilocarpine-induced seizures, *Brain Research* 1315: 33-40.

Sawas, A.H. & Gilbert, J.C. (1985). Lipid peroxidation as a possible mechanism for the neurotoxic and nephrotoxic effects of a combination of lithium carbonate and haloperidol, *Archives internationales de pharmacodynamie et de thérapie* 276: 301–312.

Shivakumar, B.R., Anandatheerthavarada, H.K. & Ravindranath, V. (1991). Free radical scavenging system in developing rat brain, *International Journal of Developmental Neuroscience* 9: 181-185.

Shults, C.W. & Haas, R., 2005. Clinical trials of coenzyme Q10 in neurological disorders, *Biofactors* 25: 117–126.

Simonié, A., Laginja, J., Varljen, J., Zupan, G. & Erakovié, V. (2000) Lithium plus pilocarpine induced status epilepticus – biochemical changes, *Neuroscience Research* 36: 157-166.

Smith, B.N. & Shibley, H. (2002). Pilocarpine-induced status epilepticus results in mossy fiber sproutng and spontaneous seizures in C57BL/6 and CD-1 mice, *Epilepsy Research* 49: 109-120.

Todorova, V.K., Harms, S.A., Kaufmam, Y., Luo, S., Luo, K.Q., Babb, K. & Klimberg, V.S. (2004). Effect of dietary glutamine on tumor glutathione levels and apoptosis-related proteins in DMBA-induced breast cancer of rats, *Breast Cancer Research and Treatment* 88: 247-256.

Tomé, A.R., Ferreira, P.M.P. & Freitas, R.M. (2010). Inhibitory action of antioxidant (ascorbic acid or α-tocopherol) on seizures and brain damage induced by pilocarpine in rats, *Arquivos de Neuropsiquiatria* 68: 355-361.

Turski, W.A., Cavalheiro, E.A., Schwarz, M., Czuczwar, S.J., Kleironk, Z. & Turski, L. (1983). Limbic seizures produced by pilocarpine in rats: behavioural, eletroencephalographic and neuropathological study, *Behavioral Brain Research* 9: 315–336.

Turski, L., Cavalheiro, E.A., Leite, J.P., Bortolotto, Z.A., Turski, W.A. & Ikonomidou, C. (1991). Long-term effects of pilocarpine in rats: structural damage of the brain triggers kindling and spontaneous recurrent seizures, *Epilepsia* 32: 778-782.

Vanhatalo, S. & Riikonen, R. (1999). Markedly elevated nitrate/nitrite levels in the cerebrospinal fluid of children with progressive encephalopathy with edema, hypsarrhythmia and optic atrophy (PEHO syndrome), *Epilepsia* 40: 210-212.

Vanhatalo, S. & Riikonen, R. (2001). Nitric oxide metabolites, nitrates and nitrites in the cerebrospinal fluid in children with west syndrome, *Epilepsy Research* 46: 3-13.

Vanin, A., Vitskova, G., Narkevich, V. & Bashkatova, V. (2003). The influence of anticonvulsant and antioxidant drugs on nitric oxide level and lipid peroxidation in the rat brain during penthylenetetrazole-induce epileptiform model seizures, *Prog Neuro-Psychopaharm Biol Psychiatry* 27: 487-492.

Xavier, S.M.L., Barbosa, C.O., Barros, D.O., Silva, R.F., Oliveira, A.A. & Freitas, R.M. (2007). Vitamin C antioxidant in hippocampus of adult Wistar rats after seizures and status epilepticus induced by pilocarpine, *Neuroscience Letters* 420: 76-79.

Yamamoto, N., Kabuto, H., Matsumoto, S., Ogawa, N. & Yokoi, I. (2002). α-tocopheryl-L-ascorbate-2-O-phosphate diester, a hydroxyl radical scavenger, prevents the occurrence of epileptic foci in a rat model of post-traumatic epilepsy, *Pathophysiology* 8: 205-214.

21

Growth Hormone and Kynesitherapy for Brain Injury Recovery

Jesús Devesa[1,2], Pablo Devesa[1,2], Pedro Reimunde[2] and Víctor Arce[1]
[1]*Department of Physiology, School of Medicine, Santiago de Compostela*
[2]*Medical Center "Proyecto Foltra", Teo*
Spain

1. Introduction

Acquired brain injury is a medical and social reality of growing magnitude and extraordinary severity requiring an increasingly specialised response to the extent permitted by technological advances and research. Although there are no accurate or reliable statistical data regarding the number of people affected by brain injury, scientific opinion maintains that brain injury represents one of the largest health problems in developed countries, both in terms of the number of deaths caused and the high number of people left suffering from some sort of functional and cognitive disability as a result of the sequelae caused by the damage occurred in the brain.

The most frequent causes of acquired brain injury are pre/perinatal hypoxia/ischemia or post-natal infections (for example, meningitis), cranial traumas, stroke. One of the first consequences of acquired brain injury is the loss of consciousness; the duration and degree of it are one of the most significant indicators of the severity of the injury. After the progressive recovery of the level of consciousness and the spatial orientation, most patients suffer a wide range of cognitive and motor sequelae; the nature and severity of them depend on the location and extent of brain damage, and the age of the patient as well.

The multiple functional and social impairments caused by the brain injury and the motor impairments affecting memory, speech or behaviour require a multi-disciplinary treatment approach from the critical-acute stage, calling for the highest level of specialisation until the patient can readjust back into the community. There is consensus among specialists regarding the damaged brain's ability to recover part of its functions spontaneously, a process that may take several months or even years. Experts also agree on the need for early neurorehabilitation to improve these natural mechanisms and achieve the best possible functional and social recovery, considering the complexity of the sequelae (motor, cognitive, emotional) caused in varying combinations by brain injury. However, access to rehabilitation facilities specialized in brain injury is marked by the shortage of public or private resources, with a huge difference in the availability of such facilities among the different countries. This shortage, scarcity or non-existence of public or private rehabilitation facilities in many countries, together with the enormous cost of these services offered by private centres, make access to rehabilitation and recovery difficult or even impossible for many people. Therefore, as a result of not seeing the benefit of potential recovery and social integration, this leads to a greater dependence and burden on the family. Therefore, acquired brain injury is a major public health, and yet

medical science has little to offer for the persistent symptoms that prevent many of these individuals from fully re-entering society.

Until few years ago it was thought that recovery from a brain injury would occur through the reinforcement of the mechanisms of neural plasticity. Establishing new synaptic connections between surviving neurons would partially allow to recover lost functions. There are evidences that environmental enrichment induces epigenetic changes that facilitate synaptogenesis and memory in models of brain plasticity. On this basis special units for brain recovery should be the closest correpondence for an enriched environment for patients with brain injury. Anything that can be done to optimize the hospital and rehabilitation environment should be beneficial. No study was shown to what extent the beneficial effect is due to specific rehabilitation strategies, to the time spent in physiotherapy and occupational therapy, and a non-specific effect of a more stimulating environment with competent staff that can encourage and support the patients and family members, but all these factors are likely to be important. In all, it is likely that admission to an acute rehabilitation unit would benefit the recovery after a brain damage (Johansson, 2011).

Today we know that appart of brain plasticity, the development of any brain injury quickly leads to enhanced proliferation of neural stem cells. From the damaged cerebral areas a number of cytokines would be released for activating the migration and differentiation of newborn cells. It has been recently identified a P2Y-like receptor GPR17 that is a sensor of brain damage and a new target for brain repair (Lecca et al., 2008). Upon brain injury, the extracellular concentrations of nucleotides and cysteinyl-leukotrienes (cysLTs), two families of endogenous signaling molecules, are markedly increased at the site of damage, suggesting that they may act as "danger signals" to alert responses to tissue damage and start repair. In brain telencephalon, GPR17, a recently deorphanized receptor for both uracil nucleotides and cysLTs (e.g., UDP-glucose and LTD4), is normally present on neurons and on a subset of parenchymal quiescent oligodendrocyte precursor cells. Induction of brain injury using an established focal ischemia model in the rodent induces profound spatiotemporal-dependent changes of GPR17. In the lesioned area, an early and transient up-regulation of GPR17 in neurons expressing the cellular stress marker *Heat shock protein 70* (Hsp70, *Heat shock protein 70)* is observed. Magnetic resonance imaging in living mice showed that the *in vivo* pharmacological or biotechnological *knock down* of GPR17 markedly prevents brain infarct evolution, suggesting GPR17 as a mediator of neuronal death at this early ischemic stage. At later times after ischemia, GPR17 immuno-labeling appeared on microglia/macrophages infiltrating the lesioned area to indicate that GPR17 may also acts as a player in the remodeling of brain circuitries by microglia. At this later stage, parenchymal GPR17+ oligodendrocyte progenitors started proliferating in the periinjured area, suggesting initiation of remyelination. The *in vitro* exposure of cortical pre-oligodendrocytes to the GPR17 endogenous ligands UDP-glucose and LTD4 promoted the expression of myelin basic protein, confirming progression toward mature oligodendrocytes. Thus, GPR17 may act as a "sensor" that is activated upon brain injury on several embrionic distinct cell types, and may play a key role in both inducing neural death inside the ischemic core and in orchestrating the local/remodeling repair response (Lecca et al., 2008).

According to these concepts two different mechanisms are involved in trying to repair brain damage: 1) quick proliferation of neural precursors, and 2) development of neural plasticity. Both are independent but complementary of each other. Neural stem cells proliferation and brain plasticity require the intervention of neurotrophic factors. This concept is showed in Figure 1.

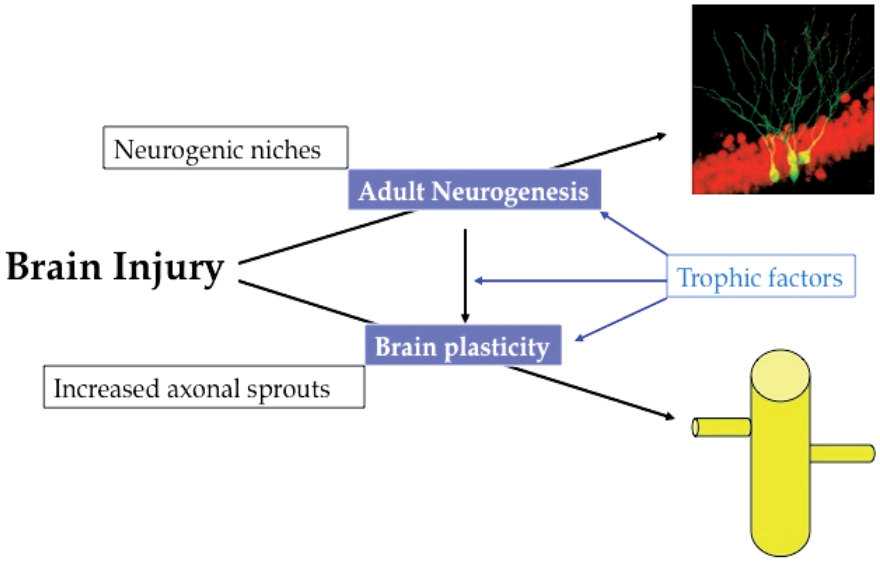


Fig. 1. Physiological responses to a brain injury. After a brain injury adult neurogenesis starts in neurogenic niches. Later, adequate rehabilitation and rich environment facilitate the development of brain plasticity. Both responses require the intervention of neurotrophic factors.

The knowledge of these and other events occurring after brain injury led to investigate how to enhance and improve mechanisms involved in brain repair.
In this chapter we will analyze current concepts about brain stem cells proliferation for brain repair and the effect of administering a neurotrophic and neuroprotective agent, growth hormone together with specific kynesitherapy, on the recovery of different kind of patients with acquired brain injury.

2. Adult neurogenesis

The complexity and specialization of neural functions increase as animal species ascend in the zoological scale. It is clear that the degree of specialization achieved, with so many and so complex interactions and functions, requires a long period of time for morphogenesis, in which not only cell proliferation and differentiation occurs, but also apoptosis must take place in a perfectly scheduled sequential expression of many different genes; later, after birth, the morphogenetic development of central nervous system is modulated by learning phenomena and the environment. However, this classical concept about neural development establishing that no new neurons are formed after birth has been changed when it was reported that new neurons are produced daily in several niches of the adult brain in many mammal species, including humans (Kuhn et al., 1996; Eriksson et al., 1998; Kempermann et al., 2004). This production is particularly important in the subventricular zone of the lateral ventricles and the subgranular zone of the dentate gyrus of the hippocampus, where neural progenitors reside primarily in the subgranular layers (Gould et al., 1999; Fukuda et al., 2003; Kronenberg et al., 2003).

The paradigm about a central nervous system unable to replace the daily loss of neurons began to be questioned in the mid-60's. However, the pioneer studies carried out in these years were not taken into account mainly due to technical and methodological issues (Altman & Gas, 1965; Kaplan & Hinds, 1977). Later, the notorious advance of the immunohistochemistry, cytology and molecular biology changed the dominant paradigm. Researchers, as Goldman (Goldman & Nottebohm, 1983) suggested that the generation of new neurons, neurogenesis, could be a normal phenomenon in the adult canary brain. The same group demonstrated later that the new formed neurons were incorporated into functional circuits (Paton et al., 1984). In the early 90's, cells with properties of neural precursors were isolated in the striatum of mice (Reynolds & Weiss, 1992). Soon the subventricular zone was identified as a niche were neural precursors were formed (Lois & Alvarez-Buylla, 1993). Lastly, at the end of 90's, it was described that adult neurogenesis is a phenomenon that also occurs in higher primates, including humans (Eriksson et al., 1998; Gould et al., 1999), a concept now widely accepted.

2.1 Where adult neurogenesis occurs and what is its functional significance?

Constitutive neurogenesis takes place in the rodent adult mammalian brain, and particularly in the subventricular zone. This zone harbors a population of stem cells that proliferate and give rise to neurons and glial cells. Neuroblasts formed here migrate long distances along the rostral migratory stream toward the olfactory bulb, where they differentiate into GABA and dopaminergic interneurons, involved in odor discrimination (Betarbet el al. 1996; Gheusi et al., 2000). Following cerebral injuries, such as ischemia, epileptogenesis, or focal neuronal degeneration, neurogenesis increases in the subventricular zone and the newly formed neurons are able to repopulate damaged areas (Parent, 2002; Romanko et al., 2004; Zhang et al., 2004). Appart of the subventricular zone and the subgranular zone of the dentate gyrus, recent data indicate that adult neurogenesis can also occur in other brain areas. It is thought that in the adult mammal brain physiological anti-neurogenic influences can be removed in pathological conditions or after any specific injury. This has been recently demonstrated in a model of unilateral vestibular neurectomy (UVN) that mimics human pathology in adult cats (Dutheil et al., 2011). UVN promoted an intense reactive cell proliferation in the deafferented vestibular nuclei located in the brainstem. The new cells survived up to one month, differentiated into glial cells - microglia or astrocytes - or GABAergic neurons, so highlighting a GABAergic neurogenesis. Surprisingly, post-UVN reactive cell proliferation contributed successfully to fine restoration of vestibular posturo-locomotor functions. Moreover, following brain injury, glia outside known neurogenic niches acquire or reactivate stem cell potential as part of reactive gliosis (Robel et al., 2011). A comparison of molecular pathways activated after injury with those involved in the normal neural stem cell niches highlights strategies that could overcome the inhibition of neurogenesis outside the stem cell niche and instruct parenchymal glia towards a neurogenic fate. This new view on reactive glia therefore suggests a widespread endogenous source of cells with stem cell potential, which might potentially be harnessed for local repair strategies (Robel et al., 2011).

Evenmore interesting is the fact that in the dentate gyrus of the adult hippocampus a continuous incorporation of new neurons exists. These new neurons slowly integrate into the existing dentate gyrus network: inmature adult-born neurons appear to function as pattern integrators of temporally adjacent events, thereby enhancing pattern separation for events separated in time; whereas maturing adult-born neurons it is likely that contribute to

pattern separation by being more amenable to learning new information, leading to dedicated groups of granule cells, the principal projection neurons of the dentate gyrus, that respond to experienced environments (Aimone et al., 2010). This continuous neurogenesis is important for hippocampal function. At different stages in its maturation, each new neuron has different properties and, at any given time, the dentate gyrus population consists of excitatory granule cells of many different ages. The youngest, apparently more excitable in the network, could complement the pattern separation function of matures by adding a degree of similarity between events experienced close in time. Several models suggest that directing plasticity towards maturing neurons can preserve the representations of old memories in the dentate gyrus while maintaining its capacity to learn new information (Aimone et al. 2010).

Thus, although the role of this continuous adult neurogenesis remains to be fully established, a number of data suggest that it contributes to promote brain repair after injuries (Abdipranoto et al., 2008; Abdipranoto-Cowley et al., 2009). On the other side, it has been suggested that neurogenesis may be impaired in neurodegenerative disorders such as Parkinson's disease and Alzheimer's disease, and this might contribute to the pathogenesis of these chronic neurodegenerative disorders (Abdipranoto et al., 2008; Yu et al., 2008).

2.2 How adult neurogenesis is regulated?

The knowledge about how adult neurogenesis is regulated may provide adequate therapeutic tools for trying to repair the brain after an injury. However this is a difficult task, because many factors seem to be involved, directly or indirectly, in this process. We will analyze here the role of some of these putative neurogenic factors for trying later to establish then a relationship between them and the effects of growth hormone on neurogenesis.

Since some years ago we know that adult neurogenesis can be modulated by several physiological processes including learning, exercise, environmental enrichment, or stress (Kempermann et al., 1997; Nilsson et al., 1999; van Praag et al., 1999; Trejo et al., 2001; Lledo et al., 2006; Zhao et al., 2008). Many, if not all, of these effects are related to signals mediated by hormones or growth factors.

2.2.1 Gonadal steroids and adult neurogenesis

In adult songbirds, integration and survival of new neurons in the vocal control nucleus is modulated by the gonadal steroids testosterone and estradiol (Nordeen EJ & Nordeen KW, 1989; Rasika et al., 1994; Hidalgo et al., 1995; Johnson & Bottjer, 1995). In mammals, estrogen has been implicated in hippocampal function, with sex differences observed in long-term potentiation (Maren et al., 1994) and performance of hippocampal-dependent tasks (Roof & Havens, 1992; Roof et al., 1993; Galea et al., 1996). During proestrus, a time when estrogen levels are high, cell proliferation in the subgranular zone of the dentate gyrus increases, compared with estrus and diestrus, when estrogen levels are lower (Tanapat et al, 1999). Estradiol exerts extensive influence on brain development and is a powerful modulator of hippocampal structure and function. Surprisingly, the remarkable increase in ovarian hormones existing during the first and third trimester of pregnancy has no effect on cell proliferation in the subgranular zone of the dentate gyrus, suggesting that concomitants changes in oher factors, perhaps glucocorticoids, may counterbalance the positive regulation of cell proliferation by estradiol.

There is a sex difference in rates of cell genesis in the developping hippocampus of the laboratory rats, most likely occurrying because of the effects of estradiol on brain during critical periods of neural development (Bowers et al., 2010). Males generate more new cells than females. A recent study shows that exogenous estradiol treatment promotes cell proliferation and survival in the neonatal female but not the male hippocampus, whereas antagonizing endogenous estradiol synthesis or action reduces cell proliferation in the male but not in the female hippocampus (Bowers et al., 2010). Moreover, in the adult female hippocampus, estradiol stimulates cell proliferation and survival and increases dendritic synapse density, while the adult male hippocampus is insensitive to the spinogenesis or cell genesis inducing effects of estradiol treatment. However, inhibiting aromatase activity or blocking estrogen receptor binding reduces cell proliferation in the developing male but not in the female hippocampus (Bowers et al., 2010). Both the amount of endogenous estradiol and aromatase activity in the developing hippocampus are very low compared to the hypothalamus and do not appear to be sexually dimorphic (Konkle & McCarthy, 2010), suggesting that hippocampal sensitivity to estradiol is high and differs between sexes.

The possibility exists that the effects of estradiol are not mediated directly at the hippocampus but, instead, were secondary to changes in other brain areas projecting to the hippocampus (Bowers et al., 2010). Cholinergic neurons of the medial septum/diagonal band of Broca are essential for estradiol-induced spinogenesis in adult CA1 hippocampus (Lam & Leranth, 2003) and cholinergic input modulates maturation and integration of adult born dentate gyrus granule cells (Campbell et al., 2010). Gonadally intact males release more acetylcholine into the hippocampus than females during locomotor tasks and this sex difference is organized by estradiol during development (Mitsushima et al., 2009a; Mitsushima et al., 2009b). The cholinergic system matures relatively early and more new septal cholinergic neurons are born in males during a brief period of gestation but the sex difference does not persist into adulthood (Schaevitz & Berger-Sweeney, 2005). Nonetheless, it is possible that the effects observed in the study of Bowers et al. are the results of estradiol-induced acetylcholine release into the neonatal hippocampus during the early postnatal period. It is also possible that estradiol is acting outside the central nervous system (Bowers et al., 2010). At this time it is important to remark that acetylcholine is a powerful inducer of pituitary growth hormone release (Devesa et al., 1992).

With regard to progesterone, it seems that enhances the survival of newborn neurons, rather than its proliferation, via the Src-ERK and PI3K pathways (Zhang et al., 2010). In fact, progesterone attenuates the estradiol-induced enhancement of cell proliferation (Galea et al., 2006).

2.2.2 Adrenal steroids and adult neurogenesis

Antidepressants increase adult hippocampal neurogenesis in animal models, an effect already described by Elizabeth Gould soon after discovering that adult neurogenesis occurs in non human primates (Gould et al. 1999); however, the underlying molecular mechanisms responsible for this effect of antidepressants were not known. Recent sudies have suggested that glucocorticoids are involved in the neurogenic action of antidepresssants (David et al., 2009; Huang & Herbert 2006). Glucocorticoids release is the main response of the organism to acute and chronic stress. Chronic exposure to stress results in a reduction of hippocampal neurogenesis and of hippocampal volume.

The antidepressant-induced changes in neurogenesis are dependent on the glucocorticoid receptor. Specifically, the selective serotonin reuptake inhibitor antidepressant, sertraline,

increases neuronal differentiation and promotes neuronal maturation of human hippocampal progenitor cells via a glucocorticoid receptor-dependent mechanism that is associated with glucorticoid receptor phosphorylation via protein kinase A signaling, therefore increasing cytosolic cAMP levels. Interestingly, this effect is only observed when sertraline is present during the proliferation phase, and it is accompanied by exit of cells from the cell cycle, as shown by reduced proliferation and increased glucocorticoid receptor-dependent expression of the cyclin-dependent kinase 2 inhibitors, $p27^{Kip1}$ and $p57^{Kip2}$ (Anacker et al., 2011). These effects of antidepressants are only seen in the presence of glucocorticoids; however, the molecular processes that lead to increased neuronal differentiation are activated directly by antidepressants alone and do not require glucocorticoids (Anacker at al., 2011). That is, regulation of neurogenesis by antidepressants is complex; it involves different glucocorticoid receptor-dependent mechanisms that lead to enhanced cell proliferation without changes in neuronal differentiation, or enhanced neuronal differentiation in the presence of decreased cell proliferation.

The fact that antidepressants induce adult neurogenesis via glucocorticoid receptor-dependent mechanism is not in contradiction with previous studies demonstrating that adrenal steroids inhibit adult neurogenesis by supressing cell proliferation in the hippocampal subgranular zone (Lennington et al., 2003). Aged rats and monkeys exhibit diminished cell proliferation in the subgranular zone as well as elevated levels of circulating glucocorticoids (Kuhn et al, 1996; Sapolsky, 1992). Removal of adrenal steroids by adrenalectomy increases cell proliferation in the subgranular zone in both aged and young adult (Cameron & McKay, 1999). Moreover, the number of new subgranular zone cells in adrenalectomized aged rats was threefold higher than the number in young control rats, indicating that adrenalectomized aged rats have rates of proliferation that surpass those normally found in young adults (Cameron & McKay, 1999). Moreover, a study on human post-mortem brain tissue has found that antidepressants increase the number of neural progenitor cells in patients with major depression to levels above those present in controls, and depressed patients are generally characterized by elevated endogenous levels of glucocorticoids (Boldrini et al., 2009). In the study of Anacker et al. (Anacker et al., 2011) the cell cycle-promoting genes, CCND1 and HDM2, were upregulated only by sertraline and dexamethasone co-treatment, the only condition which increases cell proliferation. In contrast, dexamethasone increased expression of the cell cycle-inhibiting genes, FOXO1 and GADD45B, which may explain the reduced cell proliferation and reduced neuronal differentiation with this treatment.

Experience has shown that therapy using music for therapeutic purposes has certain effects on neuropsychiatric disorders (both functional and organic disorders). However, the mechanisms of action underlying music therapy remain unknown, and scientific clarification has not advanced. The results of past studies have clarified that music influences and affects cranial nerves in humans from fetus to adult. The effects of music at a cellular level have not been clarified, and the mechanisms of action for the effects of music on the brain have not been elucidated. It has been proposed that listening to music facilitates the neurogenesis, the regeneration and repair of cerebral nerves by adjusting the secretion of steroid hormones, ultimately leading to cerebral plasticity. Music affects levels of such steroids as cortisol, testosterone and oestradiol, and it is likely that music also affects the receptor genes related to these substances, and related proteins (Fukul & Toyoshima, 2008).

2.2.3 Pituitary hormones and adult neurogenesis

Prolactin is a hormone that increases durig the pregnancy and also at postpartum, signaling lactation. The study of Shingo et al. (Shingo et al., 2003) showed that neurogenesis rates increase in the subventricular zone during pregnancy by 65% and again after delivery. In addition to observing cell proliferation, this study tracked integration of new neurons in the olfactory bulb, indicating that olfactory discrimination is critical for recognition and rearing of offspring. A doubling of olfactory interneurons may thereby enhance olfactory function following pregnancy, providing the mother with enhanced olfactory capability (Shingo et al., 2003). A similar mechanism has been recently described in male mice. Paternal-adult offspring recognition behavior in mice is dependent on postnatal offspring interaction and is associated with increased neurogenesis in the paternal olfactory bulb and hippocampus. Newly generated paternal olfactory interneurons are preferentially activated by adult offspring odors, but disrupting prolactin signaling aboslishes increased paternal neurogenesis and adult offspring recognition (Mak & Weiss, 2010).

Prolactin is a regulator of the stress response and stimulator of neurogenesis in the subventricular zone, but also protects neurogenesis in the dentate gyrus of chronically stressed mice and promotes neuronal fate (Torner et al. 2009). Neural stem and progenitors cells express the prolactin receptor and prolactin signals in these cells via ERK 1/2, however *in vitro* studies did not observe any effect of prolactin on these neural precursors proliferation, differentiation or survival, suggesting that prolactin action on *in vivo* neurogenesis occurs via an indirect mechanism (Wagner et al. 2009).

The effects of growth hormone on adult neurogenesis will be widely analyzed later in this chapter.

Recently it has been described that Oxytocin, but not Vasopressin, stimulates adult neurogenesis in the hippocampus of rats, even in animals subjected to glucocorticoid administration or cold water swim stress, indicating that the hormone stimulates neuronal growth and may protect against the suppressive effects of stress hormones on hippocampal plasticity (Leuner et al., 2011).

2.2.4 Growth factors and adult neurogenesis

When dissociated from the adult subventricular zone, neural stem cells require either epidermal growth factor (EGF) or basic fibroblast growth factor (FGF2) for self-renewal and long-term survival in culture (Reynolds & Weiss, 1992; Kuhn et al., 1997). Analysis of EGF and FGF2 responsiveness in the developing telencephalon indicates that early growth factor choice is temporally regulated (Tropepe et al., 1999; Maric et al., 2003). In the adult, the vast majority of subventricular zone cells expressing EGFR also express FGFR1 supporting the finding that most EGF-responsive cells can also be stimulated by FGF2 (Gritti et al., 1999). However, EGF and FGF2 appear to differ in their mechanisms of support, with EGF promoting faster expansion of the stem cell-like pool (symmetric division) compared to FGF2 (Gritti et al., 1999). This may be the result of differential control of cell cycle length by each growth factor, with SVZ stem-like cells cycling faster in the presence of EGF (Gritti et al., 1999). Alternatively, since the subventricular zone has two subsets of mitotically active cells, the neural stem cells (a relatively quiescent population with a cell cycle length up to 28 days) (Morshead & van der Kooy, 1992; Morshead et al., 1994), and the transitory amplifying progenitor (TAP) cells (cell cycle length approximately 12 h), these two growth factors may preferentially target one cell type. The latter appears to be supported by the work of Kuhn et al. (Kuhn et al., 1997) and Doetsch et al. (Doetsch et al., 2002). Kuhn et al.

(Kuhn et al., 1997) found that intracerebroventricular infusion of FGF2 into the lateral ventricle resulted in increased numbers of new neurons in the olfactory bulbe, while EGF infusion reduced the number of neurons reaching the olfactory bulbe, but substantially increased generation of astrocytes in the olfactory bulbe and the neighboring striatum. Extension of this study by Doetsch et al. (Doetsch et al., 2002) suggests that TAP cells are EGF receptive and these cells become invasive and glia-like, diverting neurogenesis to gliogenesis.

Recent studies using long-term ventricular infusion of EGF demonstrate intense cell proliferation around the ventricular wall, implicating the presence of EGF-reactive cells also outside the classical neurogenic lateral niche. Intraventricular injection of EGF induces within minutes CREB and ERK phosphorylation in astrocyte-like progenitor cells (type B cells) and EGF receptor-expressing transit-amplifying progenitor cells-both in the striatal and septal ventricular walls (Gampe et al., 2011). EGF infusion for 6 days induced continued CREB and ERK activation in nestin+ cells paralleled by intense periventricular cell proliferation. In addition, the ependyma became EGF receptor-immunoreactive, revealed intense CREB phosphorylation and underwent partial de-differentiation. These results demonstrate that intraventricular application of EGF induces CREB and ERK phosphorylation along the entire ventricular walls and thus permits a direct identification of EGF-responsive cell types. They further support the notion that not only the striatal ventricular wall where the subependimal zone is located but also the septal ventricular wall carries latent potential for the formation of neurons and glial cells (Gampe et al., 2011). Basal activity of CREB is required for the mitogenic signaling of EGF in neural stem cells at a level between ERK activation and SRE-mediated transcriptional activation (SRE: serum response element, a promoter sequence regulating c-fos gene expression) (Iguchi et al., 2011).

Interestingly, recent findings have shown that cells derived from subventricular zone Type-B cells (neural stem cells in the subventricular zone) actively respond to EGF stimulation becoming highly migratory and proliferative. A subpopulation of these EGF-activated cells expresses markers of oligodendrocyte precursor cells (OPCs). When EGF administration is removed, subventricular zone-derived OPCs differentiate into myelinating and pre-myelinating oligodendrocytes in the white matter tracts of corpus callosum, fimbria fornix and striatum. In the presence of a demyelinating lesion, OPCs derived from EGF-stimulated subventricular zone progenitors contribute to myelin repair. Given their high migratory potential and their ability to differentiate into myelin-forming cells, subventricular zone neural stem cells represent an important endogenous source of OPCs for preserving the oligodendrocyte population in the white matter and for the repair of demyelinating injuries (Gonzalez-Perez & Alvarez-Buylla, 2011). Of interest here is the fact that growth hormone induces EGF and EGFR expression in many territories (Pan et al., 2011) and activates EGFR by tyrosine phosphorilation as an essential element leading to MAP kinase activation and gene expression (Yamauchi et al., 1998).

Another growth factor involved in neurogenesis is the hematopoietic growth factor erythropoietin (EPO). mRNA and protein of EPO and its receptor (EPOR) are detected in a number of brain areas during brain development as well as *in vitro* in neurons, astrocytes, oligodendrocytes, microglia and cerebral endothelial cells. Expression of EPO and EPOR in the adult brain is stress-reponsive and is regulated by oxygen supply; both are upregulated after hypoxia or ischemia. Other stimuli such as hypoglycemia and IGF-I activate hypoxia-inducible factor and lead to increased expression of EPO (Byts & Sirén, 2009). The tissue protective functions EPO are independent of its action on erythropoiesis. Peripherally

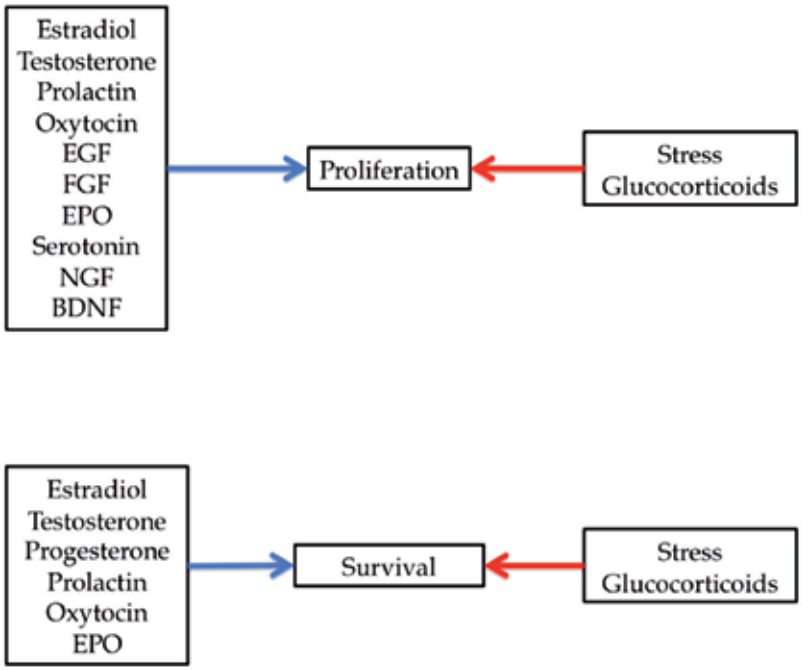
administered EPO crosses the blood-brain barrier and activates in the brain anti-apoptotic, anti-oxidant and anti-inflammatory signaling in neurons, glial and cerebrovascular endothelial cells and stimulates angiogenesis and neurogenesis. These mechanisms underlie its potent tissue protective effects in experimental models of stroke, cerebral hemorrhage, traumatic brain injury, neuroinflammatory and neurodegenerative disease (Byts & Sirén, 2009). Brain specific blockade of EPOR leads to deficits in neural cell proliferation and neuronal survival in the embryonic brain and in post-stroke neurogenesis in the adult brain (Chen et al., 2007; Tsai et al., 2006). Moreover, *in vivo* and *in vitro* data indicate hat EPO amplifies stroke-induced oligodendrogenesis that could facilitate axonal remyelination and lead to functional recovery after stroke (L. Zhang et al., 2010). Of interest here is that growth hormone induces EPO release from kidneys.

Brain injuries such as ischemia affect adult neurogenesis in adult rodents as both global and focal ischemic insults enhance the proliferation of progenitor cells residing in neurogenic niches. The ischemic insult increases the number of progenitor cells in monkey subgranular and subventricular zones, and causes gliogenesis in the ischemia prone hippocampal CA1 sector (Tonchev, 2011). The analysis of the expression at protein level of a panel of potential regulatory molecules, including neurotrophic factors and their receptors revealed that a fraction of mitotic progenitors were positive for the neurotrophin receptor Tyrosine kinase B (TrkB), while immature neurons expressed the neurotrophin receptor Tyrosine kinase A (TrkA). Astroglia, ependymal cells and blood vessels in subventricular zone were positive for distinctive sets of ligands/receptors indicating that a network of neurotrophic signals operating in an autocrine or paracrine manner may regulate neurogenesis in adult primate subventricular zone (Tonchev, 2011). The analysis of microglial and astroglial proliferation in postischemic hippocampal CA1 sector showed that proliferating postischemic microglia in adult monkey CA1 sector express the neurotrophin receptor TrkA, while activated astrocytes were labeled for nerve growth factor (NGF), ligand for TrkA, and TrkB, a receptor for brain derived neurotrophic factor (BDNF). These results implicate NGF and BDNF as regulators of postischemic glial proliferation in adult primate hippocampus (Tonchev, 2011). Figure 2 summarizes how these hormones and growth factors act on adult neurogenesis.

2.2.5 Growth hormone/IGF-I system and adult neurogenesis

GH is a pleiotropic hormone expressed not only in the pituitary but in almost any tissue (Devesa et al., 2010b). Thus, far beyond of its classical actions on body growth and intermediate metabolism, GH exerts an important role in the regulation of cell proliferation and survival in several tissues, including the CNS (Costoya et al., 1999; Sanders et al., 2009; McLenachan et al., 2009; Aberg et al., 2009).

The hypothesis that Growth Hormone (GH) and IGF-I play a role on brain repair after an injury has been postulated years ago. The existence of GH expression within the CNS has been reported by several authors, however its physiological role and, in particular, its possible contribution in the reparation of neurologic injuries remain poorly understood despite of that the positive effects of GH treatment on adult neurogenesis have been demonstrated in laboratory animals (McLenachan et al., 2009; Christophidis et al., 2009; Svensson et al., 2008; P. Devesa et al., 2011), and recent data from our group and others (Devesa et al., 2009; High et al., 2010; Reimunde et al., 2010; Reimunde et al., 2011; Devesa et al., 2011) suggest that the hormone may play a similar role in humans. GH-driven neurogenesis may also depend on the local production of GH (P. Devesa et al., 2011), the so called perypheral GH system that may be activated under both physiological and pathologic conditions (Devesa et al., 2010b).



Blue lines indicate stimulation of neural precursors cells proliferation and/or survival, while red lines indicate inhibition.

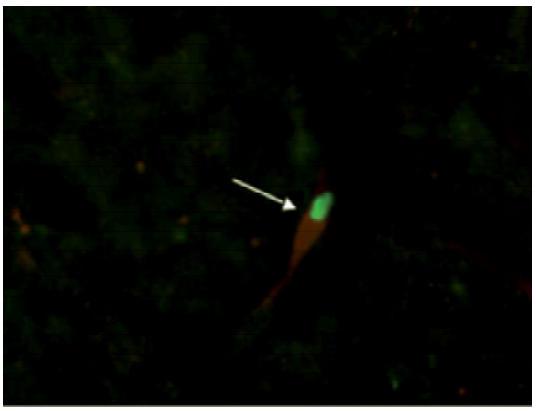
Fig. 2. Factors involved in adult neurogenesis control.

In keeping with previous reports, we have found that hippocampal cells express GH under basal conditions and, more interestingly, the number of GH-expressing cells seems to increase after brain injury induced by kainate acid administration in rats (P. Devesa et al., 2011). Furthermore, using a double-labeling immunofluorescence we were able to notice that almost all GH-positive cells also showed BrdU immunoreactivity, thus suggesting the existence of a strong correlation between GH expression and cell proliferation (P. Devesa, 2011) (Figure 3).

Interestingly, data from Katakowski et al. (Katakowski et al., 2003) demonstrate that the activation of PI3K/Akt signal transduction pathway mediates the migration of neuroblasts to damaged brain areas after stroke, most likely for inducing regeneration. PI3K/Akt is a key signaling pathway for the intracellular effects of GH (Costoya et al., 1999). Moreover, the group of Scheepens demonstrated that the expression of GH and its receptor is strongly upregulated after brain injury and specifically associated with stressed neurons and glia (Scheepens et al., 2000). More recently, the same group demonstrated that during recovery from an ischemic brain injury, a cerebral growth hormone axis is activated; the level of GHR immunoreactivity in the ipsilateral SVZ was significantly increased 5 days after injury vs. the contralateral SVZ, coinciding both spatially and temporally with injury-induced neurogenesis. The population of GHR immunopositive cells in the ipsilateral SVZ at this time was found to include proliferating cells, neural progenitor cells and post-proliferative migratory neuroblasts (Christophidis et al., 2009).

As indicated before, several lines of evidence support a role for GH in neurogenesis. The GH receptor is expressed in regions of the brain in which neurogenesis occurs during embryonic brain development (García-Aragón et al., 1992; Turnley et al., 2002) and in neurogenic

regions of the postnatal rat brain (Lobie et al., 1993). Growth hormone itself is also found in cells of the ventricular zone during embryonic neurogenesis (Turnley et al., 2002), and is produced endogenously within the postnatal hippocampus (Donahue et al., 2002; Donahue et al., 2006; Sun et al., 2005a; Sun et al., 2005b). Interestingly, GH gene expression within the hippocampus is increased by some factors known to increase neurogenesis (Parent, 2003), including learning (Donahue et al., 2002) and estrogen (Donahue et al., 2006).



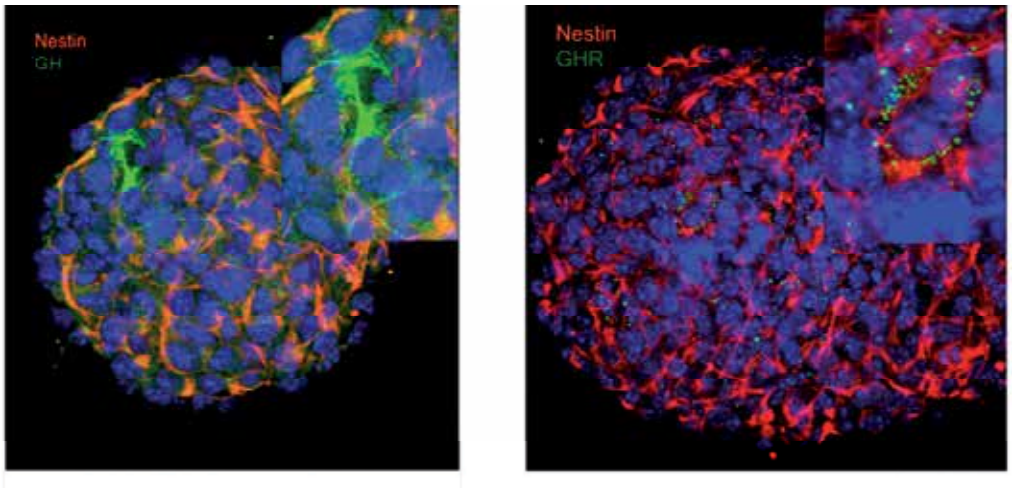
The detection of irBrDU in the nucleus of the cell (green) indicated by the arrow demonstrates that it is a newly born cell in rat hippocampal CA3 area showing irGH (brown), after brain injury induced by kainate acid administration and GH treatment, in its cytoplasm. Newly born neurons formed in the subgranular layer of the dentate gyrus migrate to the CA3 zone after commencing to maturate in the granular area. Thus, it is feasible that the detection of irGH in CA3 cells may be related to a trophic and survival role of the hormone. Taken from Pablo Devesa, Doctoral thesis, 2011. University of Santiago de Compostela, Spain.

Fig. 3. Immunofluorescence (60x). Colocalization of GH and BrdU in a newly born hippocampal cell.

However, the role of GH in the hippocampal dentate gyrus has been related to neuronal survival rather than generation, as altered hippocampal GH levels have no effect on cell proliferation but do affect the survival of immature neurons (Sun et al., 2005b; Sun et al., 2007; Lichtenwalner et al., 2006). Studies of the effects of GH on embryonic rat cerebral cortical (Ajo et al., 2003) and hippocampal (Byts et al., 2008) neuronal cultures found that it induces the proliferation and differentiation of these cells. Overall, these findings indicate that GH may facilitate the proliferation, differentiation and survival of new neurons in response to brain injury. Further studies will reveal whether or not GH is "called" for brain repair after GPR17 activation.

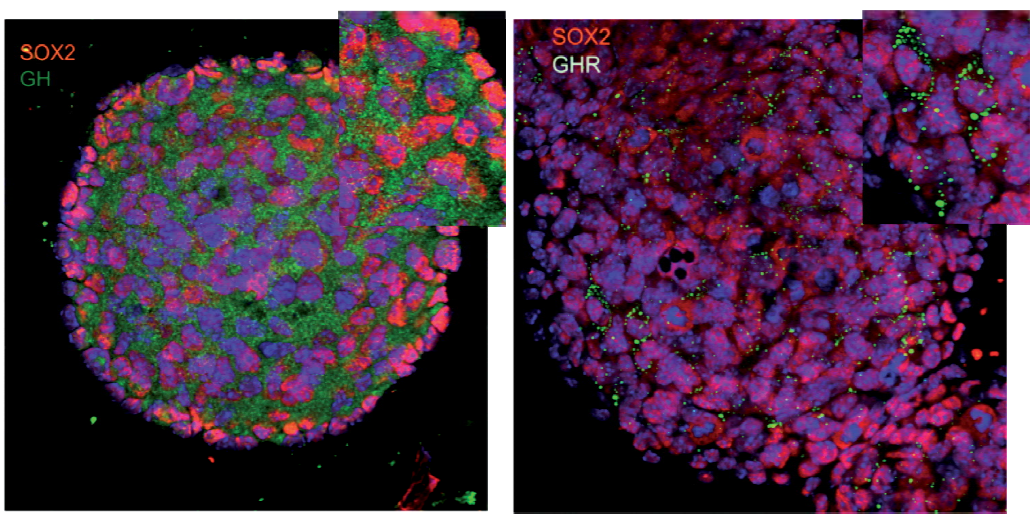
On these bases, we explored a potential role for GH and its receptor on the proliferation, differentiation and survival of neural stem cells (NSCs) *in vitro*. NSCs were isolated from the SGZ of the DG from 9 days old C57/BL6 mice and cultured as neurospheres.

Western blot and immunofluorescence studies showed that neurospheres derived from these NSCs were demonstrated to express both GH and its receptor either in conditions of proliferation and differentiation showing colocalization with nestin (Figure 4) and SOX2 (Figure 5), markers of undifferentiated cells or cells in development. Migrating cells from neurospheres also showed irGHR (Figure 6). In all, these results indicate that GH and its receptor seem to be needed for the physiological neurogenesis.



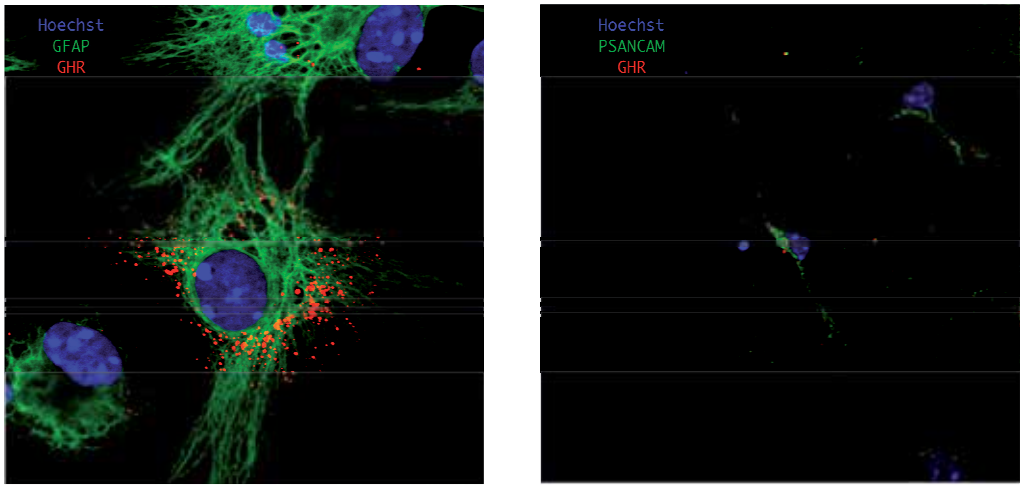
Neurospheres derived from NSCs obtained from the dentate gyrus of 9days old mice in proliferation medium showing irGH (left, green) and irGHR (right, green dots). The detection of ir for Nestin (red) indicates that cells in neurospheres are undifferentiated or in development. Taken from Pablo Devesa, Doctoral thesis, 2011. University of Santiago de Compostela, Spain.

Fig. 4. Confocal microscopy. Colocalization of GH and GHR with Nestin in mice neurospheres.



Neurospheres derived from NSCs obtained from the dentate gyrus of 9days old mice in proliferation medium showing irGH (left, green) and irGHR (right, green dots). The detection of ir for SOX2 (red) indicates that cells in neurospheres are undifferentiated or in development. Taken from Pablo Devesa, Doctoral thesis, 2011. University of Santiago de Compostela, Spain.

Fig. 5. Confocal microscopy. Colocalization of GH and GHR with SOX2 in mice neurospheres.



Detection of irGHR (red dots) in different cells migrating from neurospheres formed from NSCs obtained from the dentate gyrus of 9days old mice. The detection of irGFAP (left, green) indicates that these cells are astrocytes, while the detection of irPSANCAM (right, green) indicates that this cell is a migrating neuron. Cells nuclei are stained in blue. Taken from Pablo Devesa, Doctoral thesis, 2011. University of Santiago de Compostela, Spain.

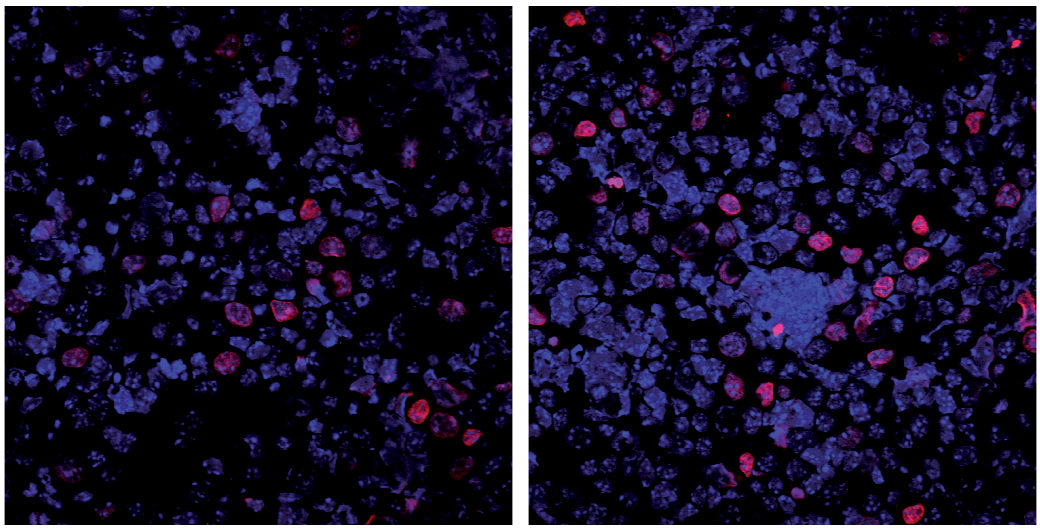
Fig. 6. Confocal microscopy. Detection of GHR in cells migrating from mice neurospheres.

However, from these studies we could not be able for establishing the exact role that the GH−GHR system plays on this mechanism. This is why we explored what would happen when adding the hormone to the culture media either in conditions of proliferation and differentiation.

As reported in other studies (Christophidis et al., 2009) treating NSCs with GH in the presence of BrdU significantly increased the proportion of cells incorporating BrdU almost doubling it (Figure 7). That is, despite of the fact that NSCs express the hormone, the addition of exogenous GH increased stem cells proliferation. This effect is similar to that we observed in animals with kainate acid-induced brain injury (P. Devesa et al., 2011).

It has been suggested that the GHR may actually play a greater role in the survival, migration or differentiation of neurons generated in response to hypoxia/ischemia than in proliferation (Christophidis et al., 2009). Consistent with a role for GHR in survival, GH protects both mature neurons (Möderscheim et al., 2007; Byts et al., 2008; Silva et al., 2003) and primary neurospheres derived from embryonic mouse NSCs (van Marle et al., 2005) from death *in vitro*. Also, the survival of newborn neurons in the subgranular zone of adult rat dentate gyrus is impaired as a result of GH deficiency (Lichtenwalner et al., 2006), and elevated GH levels within the hippocampus reduce apoptosis (Sun et al., 2007). A role for GH in neuronal differentiation is supported by a recent study that found that physiological concentrations of human GH stimulate neurite initiation and arborization of embryonic hippocampal neurons by activating the PI3K/Akt signalling pathway (Byts et al., 2008). Other studies have found that high concentrations of human GH reduce neuronal proliferation but enhance differentiation of these cells instead (Ajo et al., 2003; Lyuh et al., 2007). Indeed, it appears that GH promotes proliferation of neural cells at the expense of their differentiation, as neurosphere cultures derived from GHR *knockout* mice proliferate less than those of *wt* mice due to their inability to respond to autocrine GH, yet they exhibit

accelerated neuronal differentiation (McLenachan et al., 2008). Interestingly, treatment of neurospheres derived from newborn or adult mouse neural stem cells with 100 ng/mL rat GH, which stimulates proliferation (Christophidis et al., 2009), significantly reduced neuronal differentiation (Turnley et al., 2002; Scott et al., 2006).



GH treatment increased the number of proliferating stem cells in proliferation conditions, as irBrdU (red) shows. Left: control, saline treated cells. Right: GH treated cells. Taken from Pablo Devesa, Doctoral thesis, 2011. University of Santiago de Compostela, Spain.

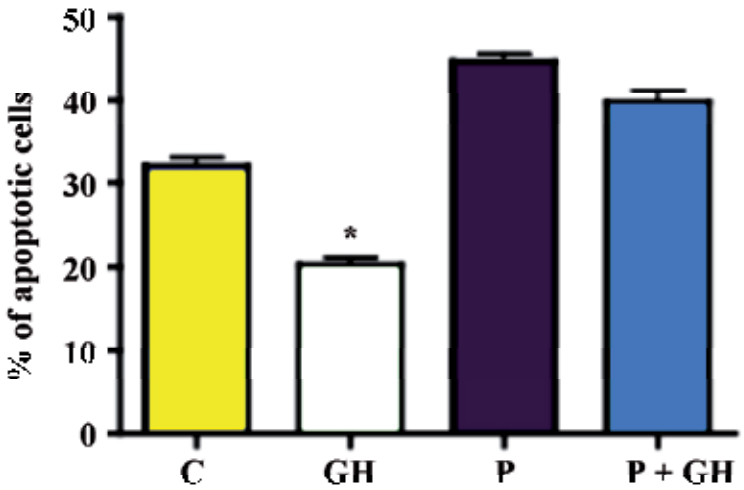
Fig. 7. *In vitro* effects of GH treatment on proliferating neural stem cells.

Overall, it appears that the effect of GH on neuronal differentiation depends upon the concentration of GH and proliferative potential of the cells used.

We also analyzed the role of GH on NSCs survival. In basal conditions there is a physiological rate of apoptosis that is significantly reduced by adding GH to the culture media in differentiation conditions. The antiapoptotic role of the hormone was clearly demonstrated by treating the cells with the GH agonist pegvisomant. This drug binds to the GHR but is unable to induce any kind of intracellular response because inhibits the dimerization of the GHR needed for intracellular signalling. In these conditions, basal apoptosis was significantly increased indicating that the autocrine interplay GH—GHR is key for NSCs survival. Moreover, treating the cells with GH did not modify the increased apoptosis elicited by pegvisomant (Figure 8).

GH effects are mediated via the GHR, which is a member of the cytokine receptor superfamily. The critical step in initiating GH signaling is the activation of receptor-associated Janus kinase 2 (JAK2), which induces cross-phosphorylation of tyrosine residues in the kinase domain of JAK2 and GHR. Phosphorylated residues on JAK2 and GHR form docking sites for the members of the STAT family of transcription factors (Perrini et al., 2008). Phosphorylation of the STATs by JAK2 results in their dissociation from the receptor and translocation to the nucleus, with subsequent binding to DNA and regulation of gene expression. Among the target genes, GH regulates the expression of suppressor of cytokine signaling (SOCS), a family of negative regulators that terminate the GH signaling cascade (Hansen et al., 1999). SOCS proteins bind to phosphotyrosine residues on the GHR or JAK2

and suppress GH signaling by inhibiting JAK2 activity, competing with STATs for binding to the GHR, or inducing degradation of the GHR complex.



GH administration significantly reduced basal apoptosis in cultured NSCs cells (*p < 0.01 vs. control). Treatment with pegvisomant abolished the antiapoptotic effect of GH. C: control; P: pegvisomant. Taken from Pablo Devesa, Doctoral thesis, 2011. University of Santiago de Compostela, Spain.

Fig. 8. *In vitro* effects of GH treatment on the survival of mice neural stem cells.

The RAS/mitogen-activated protein kinase (MAPK) pathway has also been shown to be activated by GH. The GHR–JAK2 complex has been shown to recruit the adapter protein SHC, resulting in SHC tyrosine phosphorylation and binding to GRB2, and activation of RAS, RAF, MAPK/extracellular-regulated protein kinase (MEK), and ERK-1/2 (Vanderkuur et al., 1997). Alternatively, it has been suggested that GH might also regulate the activation of ERK by a SRC-dependent, JAK2-independent mechanism which involves phopholipase D (Zhu et al., 2002). This JAK2- independent mechanism has been recently demonstrated *in vivo* (Barclay et al., 2010). Tyrosine phosphorylation of a GRB2-binding site in the epidermal growth factor receptor could also be involved in GH-mediated MAPK activation (Yamauchi et al., 1997).

GH signaling via SHC and ERK can be interrupted after blocking insulin receptor substrate-1 (IRS-1) (Wang et al., 2009). IRS-1 is a docking protein tyrosine phosphorylated in response to insulin, IGF-I, GH, and other cytokines. IRS-1 greatly enhances GH-induced ERK.

GH has also been shown to stimulate the PI3K pathway through JAK2-mediated tyrosine phosphorylation of the insulin receptor substrates (IRS-1 to IRS-3), leading to their association with PI3K regulatory subunits (Zhu et al., 2001). In addition, direct binding of the p85 and p85β subunits to phosphotyrosine residues in the carboxyl terminus domain of the GHR has also been demonstrated (Moutoussamy et al., 1998).

As stated before, GH stimulation of PI3K is linked to the stimulation of the antiapoptotic serine protein kinase B or Akt (Costoya et al., 1999). Akt activation has been shown to be dependent on the presence of the JAK2-binding region of GHR, and to promote cell survival through the inhibition of the proapoptotic protein caspase 3 (Sanders et al., 2006). The role of PI3K/Akt on cell survival is well known after the pioneer study of our group (Costoya et al.,

1999). Inhibiting PI3K/Akt with a potent inibitor of phosphoinositide 3-kinases led to a significant increase in apoptosis in NSCs, however GH treatment was able to clearly decrease the rate of apoptosis observed by blocking PI3K/Akt signaling. This indicates that the hormone is able for signaling through other different pathways acting on cell survival (P. Devesa, 2011). We then studied the effects of inhibiting another cell survival pathway such as ERK. Inhibiting ERK phosphorylation significantly increased NSCs apoptosis in differentiation conditions, but again the treatment with GH was able to revert the proapoptotic effect of blocking ERK signaling.

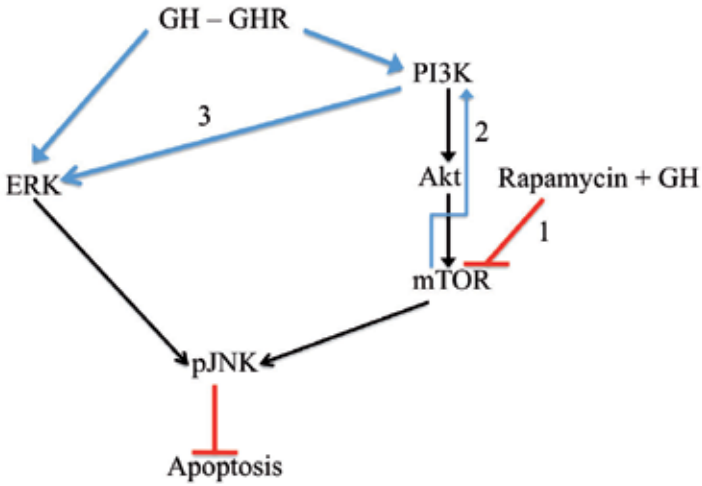
Since GH was able to revert the propapoptotic effects of the inhibition of both PI3K/Akt and ERK, we decided to study signalling pathways located upstream, specifically mTOR. This is a master regulator of cell mass and metabolism, which is in part regulated by growth factor signalling through the canonical RTK (receptor tyrosine kinase)-PI3K/Akt axis and by nutrient (through class III PI3Ks), hypoxia or AMP.

Rapamycin is an allosteric mTOR inhibitor. As expected, treating NSCs with rapamycin led to a significant increase in apoptosis, but once more this effect on cellular death was reverted when GH was added together with rapamycin. This seems to be surprising given the importance of mTOR in signalling cell survival, and again indicated that the hormone uses different pathways for promoting cell survival. However, evidence exists showing that mTOR inhibition can lead to pathway reactivation: abrogation of the negative-feedback loop which is normally initiated by the direct substrate p70S6K (p70 S6 kinase) on insulin receptor substrate proteins can lead to strong PI3K/Akt pathway reactivation, most likely producing ERK pathway reactivation in a PI3K/Akt dependent manner (Carracedo et al., 2008). For a better understanding of such a concept see the scheme shown in Figure 9.

Thus, while PI3K/Akt inhibition would allow GH to act through ERK pathway, ERK inhibition would not impede PI3K/Akt to be the survival-signalling pathway. Of interest here is the fact that both kind of inhibitions only were reverted when exogenous GH was added. This means that the autocrine production of the hormone by NSCs is not enough for blocking intense cell death signals.

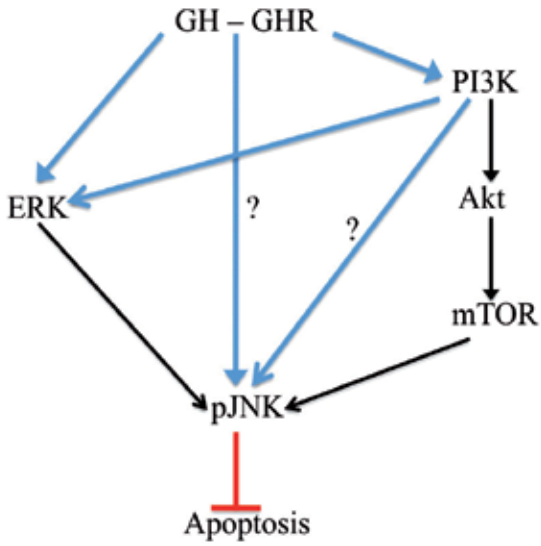
We then blocked simultaneously ERK and mTOR activation. Once more the intense apoptosis observed after pharmachological blockade of these pathways was reverted when GH was added together with them. The only explanations for this result is that the hormone may act directly at a upper level in the stream of signaling pathways for cell survival or the effect is due to the overactivation of PI3K occurred as a result of blocking those two other signaling pathways. To test the first possibility we used SP600125, an anthrapyrazolone inhibitor of Jun N-terminal kinase (Bennett et al., 2001). That is we were acting at the terminal level in the stream. In this case the clear increase in apoptosis produced by the inhibitor could not be reverted by administering GH together with the drug. It suggests either a direct effect of the hormone on JNK activation for cell survival or a direct effect of overactivated PI3K on JNK (as postulated by Zhu et al., 1998).

In summary, our results (P. Devesa, 2011), together with other studies previously described, demonstrate that GH is able for promoting NSCs proliferation, and also plays a key role on the survival of these cells. With regard to the antiapoptotic role of GH, it seems to be exerted trough different signaling pathways: PI3K/Akt, ERK and pJNK (Figure 10). Since culture media contain insulin our results do not allow us to conclude whether the effect of GH on pJNK is a direct effect or it results from the known crosstalk between GH and insulin at the cellular level (Xu & Messina, 2009) or from the overactivation of PI3K directly enhancing JNK activity (Zhu et al., 1998). In any case GH effects a very strong effect on the survival of NSCs.



As showed in the scheme, rapamycin treatment inhibits mTOR, thus leading to a feedback activation of the PI3K/Akt pathway [2]. Physiologically this would lead to increased ERK activation [3], but this not occurs unless exogenous GH is given together with rapamycin [1]. Red lines indicate inhibition; blue lines indicate activation. Taken from Pablo Devesa, Doctoral thesis, 2011. University of Santiago de Compostela, Spain.

Fig. 9. Schematic representation of the cell survival signaling pathways studied.



The scheme shows how GH may act on cell survival in NSCs according to the results we obtained. The possibility of a direct effect of the hormone on JNK phosphorylation could not be demonstrated here; another possibility is that the activation of JNK occurs because of overactivation of PI3K after mTOR and ERK blockade. Red line indicates inhibition; blue lines indicate activation. Taken from Pablo Devesa, Doctoral thesis, 2011. University of Santiago de Compostela, Spain.

Fig. 10. Schematic representation about the putative role of GH in NSCs survival.

A number of studies indicate that the neurobiological consequences of the decline in GH/IGF-I, that occurs physiologically during aging, include decreased neurogenesis in the dentate gyrus of the hippocampus (Lichtenwalner et al., 2001; Lichtenwalner et al., 2006), where IGF-I appears to affect primarily the survival of newborn neurons, but also may influence the maturation and differentiation of newborn cells (Aberg et al., 2000; Darnaudery et al., 2006).

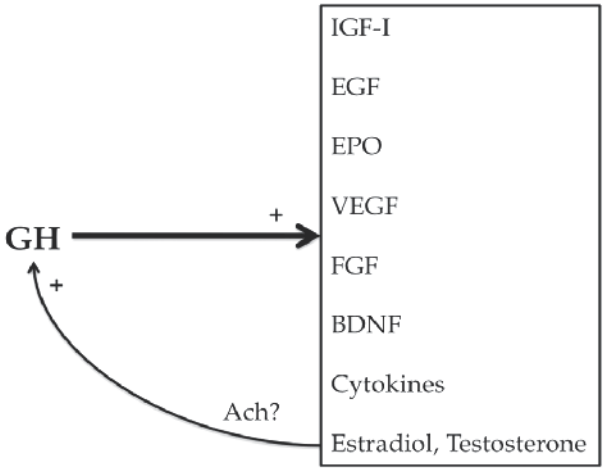
Despite that neurogenesis is dramatically reduced during senescence, newborn granule cells in the aged dentate gyrus retain the capacity for participation in functional hippocampal networks (Marrone et al., 2011); this is in agreement with the aforementioned hypothesis, suggesting that it is the age-associated lack of neurotrophic factors (GH/IGF-I?) the cause of decreased neurogenesis. In fact, growth hormone prevents neuronal loss in the aged rat hippocampus (Azcoitia et al., 2005).

GH induces IGF-I expression in liver and other tissues; however it is not the only factor responsible for it. In fact, there are a number of pathological situations in which a clear divergence exists between GH production and plasma levels of IGF-I. This is the case, for instance, of Anorexia Nervosa; in these patients, while large burst of pituitary GH are released into the blood, IGF-I plasma levels are consistently lower than normal. The opposite situation can be observed in obese children; GH secretion is deficient in these children, but plasma IGF-I levels are normal and growth velocity is above the mean for age (Devesa et al., 1992). The common link between these two situations seems to be plasma glucose levels, low in Anorexia Nervosa and showing a tendency to hyperglycemia in obesity. Thus, it has been postulated that glucose is needed for the hepatic production of IGF-I; more particularly a product of intracellular metabolism of glucose, since 2-deoxiglucose (who can not be metabolized) is unable to induce hepatic IGF-I expression. Therefore, although GH usually is the factor responsible for the hepatic production of IGF-I, in terms of neural effects both hormones may follow different paths. In fact, studies in rats submitted to moderate and severe hypoxia showed that the spatial distribution of the neuroprotection conveyed by growth hormone correlates with the spatial distribution of the constitutive neural growth hormone receptor, but not with the neuroprotection offered by IGF-I treatment in this model. These results suggest that some of the neuroprotective effects of growth hormone are mediated directly through the growth hormone receptor and do not involve IGF-I induction (Scheepens et al., 2001).

The effects of the GH/IGF-I axis on cell turnover in the adult brain probably are not limited to neuronal progenitors, since IGF-I can promote proliferation of oligodendrocyte progenitor cells (OPCs) and differentiation and survival of oligodendrocytes (McMorris & McKinnon, 1996; Mason et al., 2000; Aberg et al., 2007; Pang et al., 2007), an effect also induced by EGF (Gonzalez-Perez & Alvarez-Buylla, 2011) whose expression and that of its receptor may also be induced by GH (Pan et al., 2011). Thus, it is likely that the aging-related decline in GH/IGF-I and dependent changes in oligodendrocyte genesis and/or maturation may contribute to impaired remyelination in the central nervous system of aged individuals (Gilson & Blakemore, 1993; Shields et al., 1999; Franklin et al., 2002; Sim et al., 2002) and to a decline in normal cognitive function. In fact, GH has positive cognitive effects when given to GH-deficient patients.

In all, the system GH/IGF-I exerts both neuroprotective and regenerative effects at the CNS. In line with this a recent study in human patients describes that a high serum IGF-I during the rehabilitation phase of stroke correlates to better recovery of long-term function (Aberg et al., 2011).

It is likely that some of the positive effects of GH at central level are not directly exerted by the hormone but they are mediated trough the induction and release of a number of neurotrophic factors, as shown in Figure 11.



Some of the neurotrophic effects of GH *in vivo* could be mediated and/or enhanced through the induction of the expression and release of a number of neurotrophic factors. GH induces the expression of IGF-I, EGF, EPO; VEGF and FGF. The GH-effects on the induction of BDNF expression have not been tested yet but it is likely that they occur, directly or indirectly. GH induces an acute and strong release of neutrophiles from the bone marrow, then allowing the release of neurotrophic cytokines. GH has trophic effects on the gonads, thus facilitating estradiol and testosterone production. In turn, it is likely that the neurotrophic effects of these sexual steroids may be partially due to a positive effect on the pituitary GH release mediated by Ach and noradrenaline (Devesa et al., 1992).

Fig. 11. GH induction of the expression and/or release of a number of factors with known neurotrophic activity.

3. Effects of GH treatment combined with kynesitherapy on the recovery of patients with brain injuries

3.1 Cerebral palsy children

Cerebral palsy (CP) is a catastrophic acquired disease, occurring during development of the fetal or infant brain. It mainly affects the motor control centres of the developing brain, but can also affect cognitive functions, and is usually accompanied by a cohort of symptoms including lack of communication, epilepsy, and alterations in behavior.

Major causes for CP include abnormal intrauterine developments, due to fetal-maternal infections, asphyxia before birth, hypoxia during delivery, brain trauma during labor and delivery, and complications in the perinatal period. Apart from these, prematurity is responsible for 40%–50% of cases of CP. Periventricular leucomalacia (PVL) and parenchymal venous infarction complicating germinal matrix/intraventricular hemorrhage have long been recognized as the two significant white matter diseases responsible for the majority of cases of cerebral palsy in survivors of preterm birth.

However, in more recent studies using magnetic resonance imaging to assess the preterm brain, two new appearances have been documented, adding to the spectrum of white matter

disease of prematurity: punctate white matter lesions, and diffuse excessive high signal intensity. These appear to be more common than PVL but less significant in terms of their impact on individual neurodevelopment. They may, however, be associated with later cognitive and behavioral disorders known to be common following preterm birth.

Most CP children often have poor linear growth during childhood, resulting in a diminished final adult height. However the number of studies in which it has been reported whether or not GH secretion is impaired in CP is quite limited. These studies reflect that GH provocative testing induced a GH deficient secretion. In a recent study it was indicated that diminished circulating IGF-1 and GH concentrations may explain why children with CP are smaller than normally growing children (Ali et al., 2007a). On the other hand, osteopenia is a common finding in children with CP, and seems to be associated with decreased IGF-1 and IGFBP3 plasma levels, usual markers of deficient GH secretion. The large percentage of CP children with GH deficiency (GHD) has been reported to be noteworthy. However, given the complexity of GH neurorregulation (Devesa et al., 1992) it seems to be logical that severe brain damage may affect a number of neurotransmitter pathways involved in GH control, thus affecting the normal secretion of the hormone.

Other possible causes of decreased growth in CP include psychosocial deprivation and suboptimal nutritional status, but these are also involved in subnormal GH secretion (Devesa et al., 1992).

We studied whether GH secretion was affected in 46 CP children (28 males, 18 females; aged 3 to 11 years old). Our results indicated that 70% of the patients seemed to have deficient GH secretion (Devesa et al, 2010a), therefore they were candidates to benefit from GH replacement therapy.

In few studies have the benefits of GH-replacement therapy in children with CP been reported, and most of these studies only reflect the increased growth observed during the treatment period with the hormone (Coniglio & Stevenson, 1995; Shim et al., 2004; Ali et al., 2007b).

Neuropsychological assessments have demonstrated that GHD is associated with reduced cognitive performance; specifically, in the majority of studies it has been found that GHD can lead to clinically relevant changes in memory, processing speed, attention, vocabulary, perceptual speed, spatial learning, and in reaction time tests. Cognitive dysfunction appears to be specifically related to GH deficiency; this hypothesis is supported by the positive correlations between serum IGF-I concentration and IQ, whereas poorer emotional well-being and reduced perceptual-motor performance are attributed to other pituitary hormone deficiencies.

In a recent study we described (Devesa et al., 2011) the positive effects of GH therapy together with psychomotor and cognitive stimulation in 11 children (7 males, 4 females; aged 3 to 7 years old) with CP and GHD. Cognitive performances in the children studied were assessed by using the Battelle Developmental Inventory Screenning Test (BDIST) (Newborg et al., 1988). Tests were performed at admission, two months after commencing psychomotor and cognitive stimulation and two months after adding GH replacement therapy to psychomotor and cognitive stimulation. Before admission all these CP children had been intensively stimulated, most of them since they were 1 year old. Psychomotor and cognitive stimulation were adapted to the specific needs of each patient. Psychomotor stimulation involved tasks aimed at improving tonic–postural control, laterality, breathing and relaxation, static and dynamic balance, motor coordination and dissociation, body image, oculomotor coordination, spatial and temporal orientation, and gross and fine motor

skills. Cognitive stimulation involved tasks directed at improving interaction with the environment, communication, attention, perception, memory, reasoning, and concept learning. These therapies were carried out for 45 minutes per day, 5 days per week during 4 months. Recombinant human GH was given subcutaneously, 30 μg/kg/day, 5 days/week, during 2 months (after 2 months of psychomotor and cognitive stimulation).

Patients did not improve their psychomotor and cognitive status during the pretreatment period, during which only psychomotor and cognitive stimulation were performed. However, significant improvements in all BDIST domains were observed when GH was administered together with psychomotor and cognitive stimulation; specifically, patients improved in personal and social skills, adaptive behavior, gross motor skills and total psychomotor abilities, receptive and total communication, cognitive skills, and in the total score of the scale ($p < 0.01$), and in fine motor skills and expressive communication ($p < 0.02$). As expected, plasma IGF-1 and IGFBP3 significantly increased after GH treatment.

These results led us to conclude that the combined therapy involving GH replacement and psychomotor and cognitive stimulation is useful for the appropriate neurodevelopment of children with CP and GHD (Devesa et al., 2011). Since these children had received an intensive neurostimulation without significant improvements before being treated with GH, it seems to be clear that the hormone, and/or IGF-1 was the main factor responsible for the results obtained. Moreover, since exogenous GH combines with locally produced GH for repairing brain injuries (P. Devesa et al., 2011) it is feasible to assume that GH treatment may be used too in CP children without GHD.

In another study (Reimunde et al., 2011), we assessed the effects of growth hormone treatment (30 μg/kg/day) combined with physical rehabilitation in the recovery of gross motor function in children with GHD and CP (four males and six females, mean age 5.63 ± 2.32 years) as compared with that observed in a similar population of CP children (five males, five females, mean age 5.9 ± 2.18 years) without growth hormone deficiency treated only with physical rehabilitation for two months. The Gross Motor Function Measure (GMFM-88) and Modified Ashworth Scale were performed before commencing the treatment and after completion thereof. The GMFM-88 is a scale constructed for evaluation of change in gross motor function in children with cerebral palsy and consists of 88 items grouped into five dimensions, ie, dimension A (lying and rolling, 17 items), dimension B (sitting, 20 items), dimension C (crawling and kneeling, 14 items), dimension D (standing, 13 items) and dimension E (walking, running, and jumping, 24 items).

Scores for each dimension are expressed as a percentage of the maximum score for that dimension, adding the scores for all dimensions, and dividing by 5 to obtain the total score. The reliability, validity, and responsiveness of the GMFM-88 scores are documented for children with cerebral palsy (Palisano et al., 2000). The Modified Ashworth Scale is used for measuring spasticity in spastic patients (Bohannon & Smith MB, 1987).

In children with CP and GHD, Dimension A ($p < 0.02$), dimension B ($p < 0.02$), and dimension C ($p < 0.02$) of the GMFM-88, and the total score of the test ($p < 0.01$) significantly improved after the treatment; dimension D and dimension E did not increase, and four of five spastic patients showed a reduction in spasticity. However, in children with cerebral palsy and without growth hormone deficiency, only the total score of the test improved significantly after the treatment period. Plasma IGF-I values (previously low in GHD CP children) were similar in both groups at the end of the treatment period, indicating that growth hormone replacement therapy was responsible for the large differences observed between both groups in response to physical rehabilitation.

According to these results it is likely that GH administration plays a key role in recovering from a brain injury, independently or not that a GH deficiency exists. In this regard, voluntary physical exercise is known to be a factor increasing neurogenesis and also enhancing learning and memory (Beckinstein et al., 2011). However few positive effects are obtained in CP chidren undergoing exhaustive daily physical work, as our data also reflect. Exercise is a powerful stimulus for endogenous growth hormone release, most likely through enhanced central NA tone (Devesa et al., 1992). It has been demonstrated that inhibiting PI3K/Akt signaling, one of the pathways by which GH acts (Costoya et al., 1999), blocks exercise-mediated enhancement of adult neurogenesis and synaptic plasticity in rats (Bruel-Jungerman et al., 2009). The possibility exists that the lack of significant improvements in CP children submitted to intensive physical work is due to an impaired GH secretion, as it seems to occur whenever a brain injury exists, independently that current provocative tests do not reflect the existence of a GH deficiency.

As described before, blindness is a common finding in CP children. We studied 20 children with CP occurring as a consequence of prematurity leading to Periventricular Leukomalacia (11 males, 9 females, aged 2,05 ± 1,43 years; mean ± SD of the mean) with critical impairment of vision and marked pallor of the optic nerve. They were treated with GH and visual stimulation performed with a tachistoscope (repetitive white light flashes, 100-150 ms, carried out in 10 phases lasting 1 min each one, 80 flashes/min; 5 days/week). Stimulation was performed in a dark isolated room. Visual evoked potentials (VEP) were recorded before commencing the treatment and after finishing it. Treatment lasted for 5 ± 2.65 months. The rationale for interrupting visual stimulation and GH treatment was based on clinical observations (for example, visual interaction with the environment, looking at objects or relatives, taking objects with the hands...).

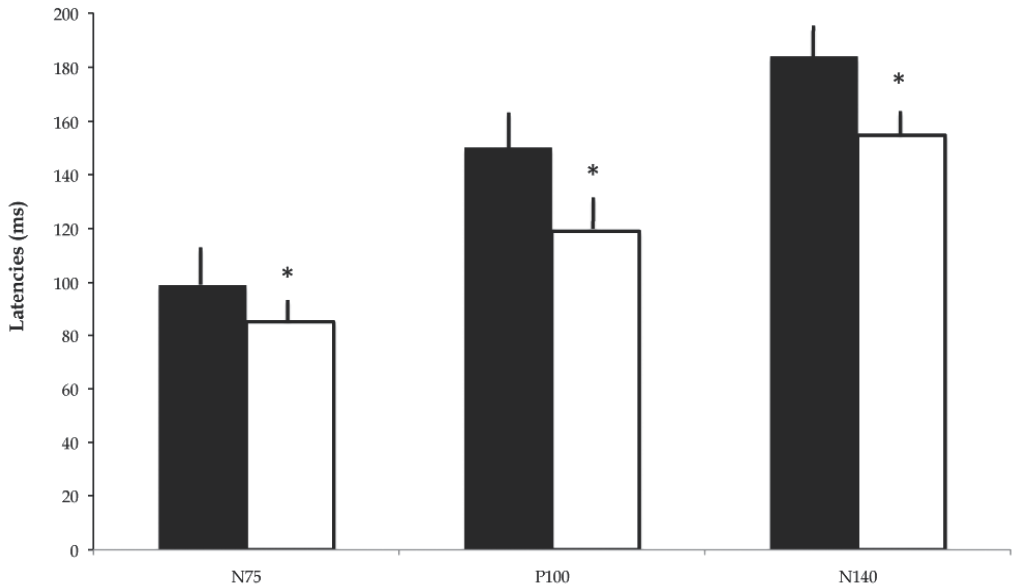
Results from this study (unpublished data) show that the children we studied presented severe visual deficiencies, characterized by a delayed conduction from the retina to the occipital cortex, as VEP showed, but these were corrected at the end of treatment period, as Figure 12 shows. Latencies were significatly decreased, while no significant changes were observed in amplitudes.

Delayed conduction from the retina to the occipital cortex means a deficient myelination. This may be related to a delayed maturation of the CNS, but it is unlikely that this was the reason in these CP children since they suffered brain injuries produced by prematurity leading to Periventricular Leukomalacia in which cerebral white matter is consistently affected. Moreover, some of these children were older than 2 years old, a period of time from which it is considered that a brain damage is already established and significant improvements are unlikely to appear. Injuries involving the optic radiations, such as it happens in Periventricular Leukomalacia, have a bad prognosis (Hoyt, 2003) and most of these children are expected to remain visually handicapped.

We utilized a tachistoscope as a stimulation method of both visual hemifields, but it is unlikely that visual stimulation alone could be responsible for the clinical and VEP changes observed after the treatment. Despite the fact that there was not a control group in our study and this could lead to missinterpretating the results obtained, most of these children had been receiving intense stimulation previously without achieving significant results.

A recent study describe that plasma levels of GH/IGF-I influence glial turnover in the white matter (Hua et al., 2009). It is not clear whether the maintenance of Oligodendrocyte Precursor Cells (OPCs) and oligodendrocyte turnover in the adult brain serves normal function or only provides a rapidly recruitable population of cells for myelin repair

following damage. Proliferation of oligodendrocyte precursors and recruitment of new, myelinating oligodendrocytes from immature precursors contribute to myelin repair following demyelinating lesions. Following demyelination, proliferation of OPCs and commitment, differentiation and survival of adult-born oligodendrocytes all appear to be targets of regulation by inflammatory cytokines and growth factors.



The figure shows changes observed in the latencies of Visual Evoked Potential waves registered in CP children before (black bars) and after (white bars) the treatment with GH and visual stimulation. * $p < 0.005$ vs. pretreatment.

Fig. 12. Latencies of Visual Evoked Potential waves in blind CP children.

The GH/IGF-I system appears to play a particularly critical role in myelin repair. IGF expression is induced in multiple models of demyelination and is increased during remyelination (Fushimi & Sharabe, 2004). Treatment with IGF-I or overexpression of IGF-I in transgenic mice inhibits oligodendrocyte death during demyelination and/or enhances remyelination following demyelinating lesions (McMorris & McKinnon, 1996; Mason et al., 2000; Kumar et al., 2007).

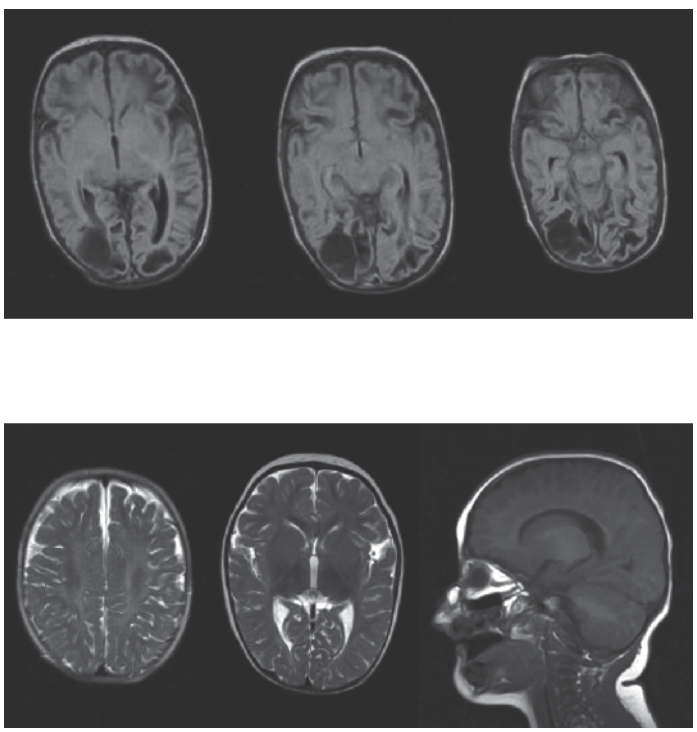
The possibility exists that remyelination observed in our study, as showed by the improvements in VEPs latencies, might be due to increased plasma IGF-I values. This does not exclude a direct effect of GH on myelination. As described before, GH induces EGF and EGF-R expression, factors recently involved on central nervous system remyelination (Gonzalez-Perez & Alvarez-Buylla, 2011).

Thus, it is feasible to assume that GH treatment played a significant role in the visual recovery elicited by specific visual stimulation.

Case report

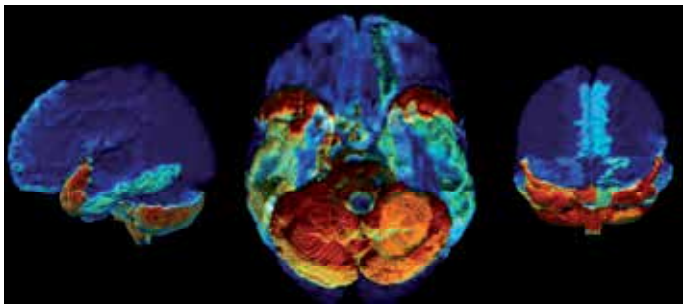
At age 3 weeks a CP baby was admitted for rehabilitation in our Center. He suffered a massive bleeding *in utero* that produced a 20-minute cardiac arrest. A MRI carried out at age

2 weeks showed a severe encephalopathy with cystic cavities in both occipital lobes. VEP and auditory evoked potentials were isoelectric without any signs of conduction. Prognosis could not be worst: blind, deaf, dumb and spastic tetraparesia.
After obtaining signed informed consent from the parents we commenced an intensive rehabilitation with him. Visual stimulation, auditory stimulation, sensorial and physical stimulation and GH treatment (0.04 mg/kg/day). Three months later the child was showing signs of positive evolution. There was electrical conduction from the retina to the cortex, he responded to auditory stimuli and spasticity was decreased. GH treatment lasted for 4 months, but physical and sensorial stimulation continued for 12 months. At this time the child was absolutely normal as functional physical and cognitive tests revealed. Currently, at age 17 months, he walks, he speaks, he laughs, he plays, he is like any other child of his age. No sequelae have been observed and the treatment did not produce any kind of adverse effects.
A new MRI taken when he was 1 year old showed that the brain was completely regenerated. Cystic cavities disappeared and occipital lobes were fully functional. Voxels based morphometry comparing both MRI indicated that a number of brain areas experienced a significant regeneration; this was particularly significant in thalamus.
Figures 13 and 14 respectively show MRI and voxels based morphometry studies in this child.



Upper images were taken at age 2-weeks. Notice the cavities affecting occipital lobes. Lower images were taken at age 1-year; no occipital cysts exist.

Fig. 13. Some images of MRI studies in the child described as a Case report.



These images were obtained after comparing MRI studies carried out at 1-year interval in the child described as a Case report. The intensity of red colours is associated to greater regeneration.

Fig. 14. Voxels based morphometry.

To our knowledge this is the first description about complete human brain regeneration demonstrated with brain images. Brain plasticity may be responsible for achieving important functional recoveries in patients after a brain injury. In our case these recoveries were found not only by clinical and specific physical and cognitive tests, but they have been demonstrated with brain images. It is clear that the age at which we commenced the treatment of this child had a significant influence on the results obtained; brain development continues for 1 year after birth, approximately, but it also clear that physical and cognitive stimulation alone are not able for achieving a complete and functional recovery of the brain. Since the system GH/IGF-I has been involved in playing a very important role during embryonic brain development it seems that the treatment with the hormone was the main factor responsible for results achieved in this study. This supports our previous postulate about commencing GH treatments as soon as possible after a brain injury (Devesa et al., 2010a).

3.2 Traumatic brain injury

Traumatic brain injury (TBI) is an important health problem and a leading cause of death and disability worldwide, mainly in people under 40. Specifically, TBI is an important identified risk factor for cognitive deficits, such as attention, concentration, learning, memory, conceptual thinking, problem-solving or language (Vogenthaler, 1987). Recent studies have demonstrated that hypopituitarism, and particularly growth hormone deficiency (GHD), is common among survivors of TBI tested several months or years following head trauma (Popovic, 2005a; Popovic et al., 2005b). The subjects at risk are those who have suffered moderate-to-severe head trauma, although mild intensity trauma or even minor head injuries may precede hypopituitarism (Popovic et al., 2005b). Complex pituitary deficits are found in 35–40% of TBI patients; severe GHD occurs in 10–15% and partial GHD in 15% of TBI patients (Popovic et al., 2005b).

Neuropsychological assessments have demonstrated that GHD is associated with reduced cognitive performance; specifically, most of the studies indicate that GHD can lead to clinically relevant changes in memory, processing speed, attention, vocabulary, perceptual speed, spatial learning and in reaction time tests as well (van Dam, 2006; Falletti et al., 2006; Nieves-Martinez et al., 2010).

Cognitive dysfunction appears to be specifically related to GH deficiency; this hypothesis is supported by the positive correlations between serum IGF-I concentration and IQ. Thus, it

has been shown that cognitive disorders secondary to GHD may be reversed by GH replacement (Maruff & Falletti, 2005).

We studied (Reimunde et al., 2010) 20 male adult patients with TBI suffered in the previous 10 years. Main brain injuries occurred because of frontal contusions affecting brain frontal lobes. Eleven of them presented GHD (mean age: 53.36 ± 17.35 years), while GH secretion seemed to be normal in the other nine patients (mean age: 47.12 ± 14.55 years).

All patients received daily cognitive rehabilitation, 1 hour per day, 5 days per week during 3 months. The contents of cognitive rehabilitation were aimed to improve the spatial, temporal and personal orientation, visual, auditory and tactile discrimination and perception, body recognition, planning and execution of motor acts, all memory types and processes, oral and writing language, calculation and executive function tasks. GHD patients were given human GH (0.5 mg/day during 20 days and then 1 mg/day, 5 days per week, for 3 months). Control patients were given placebo.

To assess different cognitive abilities we used a widely recognized neuropsychological test battery, the Wechsler Adults Intelligence Scale (WAIS) (Wechsler, 1990). This assessment battery involves several sections that specifically measure multiple cognitive functions. WAIS test was performed before commencing the treatment and after 3 months of treatment in all patients.

Results from our study demonstrate that individuals treated only with cognitive rehabilitation improved, specifically, their attentional functioning. However, the group treated with GH and cognitive rehabilitation improved their attentional functioning too, but moreover showed specific improvements in memory, understanding, associative thinking skills, information management and storing, learning, visual-motor dexterity and speed of execution.

In addition, the GHD group reached greater improvements in aspects related with memory, understanding, associative thinking skills and information management and storing information than the control patients.

These data correlated with previous results showing that GH-deficient subjects treated with GH experience significant improvements in concentration and attention, different memory modalities and learning.

It has been described that memory performance is weakly related to the mean daily GH dose, but there was a stronger positive correlation between the increase in mental performance and the GH-induced rise in serum IGF-I; this phenomenon can be explained by the large inter-individual differences in GH sensitivity (Deijen et al., 1998). However, in our study, no significant differences between plasma IGF-I levels in both groups of patients were observed at the end of the treatment period. Thus, it is unlikely that the IGF-I rise could be responsible for the results here obtained.

In conclusion, our study demonstrated, for the first time, the utility of a combination therapy involving GH replacement and cognitive rehabilitation in the recovery of cognitive impairments in patients who had suffered a TBI and a subsequent GHD. Our data show that the combination therapy has significantly greater effects than those achieved by carrying out only cognitive rehabilitation in patients with cognitive impairments secondary to a TBI without GHD. GH administration seems to be responsible for the results obtained. On these bases, we concluded that it woud be interesting to test whether GH administration to no GHD patients would improve cognitive functions in a number of neurological disorders (Reimunde et al., 2010).

4. Conclusions

Throughout this chapter we have reviewed the evidence showing that adult neurogenesis is a physiological mechanism to repair brain damage. We analyzed a number of factors directly involved in this process.

We demonstrated that both GH and its receptor are expressed in neural precursors in mice, and we showed that after a brain injury, in rats, the treatment with the hormone increases the number of newly born stem cells produced in response to the damage. We demonstrated that GH is present in the cytoplasm of these newly born cells, most likely for facilitating their migration from neurogenic niches. Our data in cell cultures obtained from mice stem cells demonstrate that the hormone plays a key role for neural cells survival.

In human patients with brain injury and GH-deficiency, we demonstrated that GH treatment together with physical and cognitive stimulation is responsible for the recovery of motor and cognitive abilities. GH treatment also appears to play a key role for remyelination of the visual pathways in children with cerebral palsy.

For the first time we showed here that an early treatment with the hormone can recover a brain injury in the first months of the life.

Although not presented here, GH treatment may repair central neurogenic dysphagias (Devesa et al., 2009; Reimunde et al., 2011, submitted for publication).

By analyzing the role of many factors positively involved on adult neurogenesis, we postulate that some of the positive effects of GH at the central level may be due to GH-induced expression of a number of neurotrophic factors and/or to enhanced release of some of them.

We conclude that GH treatment is a powerful tool for being used in a number of brain injuries, including neurodegenerative non-genetical diseases (for example, multiple sclerosis, Alzheimer, etc). It seems that there is no need that GH-deficiency exists for the hormone being useful.

GH treatments have to be well scheduled and short in time. The positive effects of the hormone remain in the time.

5. Acknowledgements

These studies were supported by Foundation Foltra (Teo, Spain). Studies in human patients were carried out in Medical Center Proyecto Foltra (Teo, Spain). Animal studies were carried out in the Department of Physiology, School of Medicine, Santiago de Compostela, Spain, under the direction of Professor Jesús Devesa and Professor Víctor Arce. Studies in NSCs were performed in the Institute of Neurosciences of the School of Medicine of Coimbra, Portugal, under the direction of Professor Joao Malva; his collaboration is greatly appreciated. Voxel based morphometries were performed by Brain Dynamics (Málaga, Spain).

6. References

Abdipranoto A, Wu A, Stayte S, et al. (2008). The role of neurogenesis in neurodegenerative diseases and its implications for therapeutic development. *CNS Neurol Disord Drug Targets*. 7:187-210.

Abdipranoto-Cowley A, Park JS, Croucher D, et al. (2009). Activin A Is Essential for Neurogenesis Following Neurodegeneration. *Stem Cells*. 27: 1330-46.

Aberg MA, Aberg ND, Hedbacker H, Oscarsson J & Eriksson PS. (2000). Peripheral infusion of IGF-I selectively induces neurogenesis in the adult rat hippocampus. *J Neurosci.* 20: 2896–2903.

Aberg ND, Johansson UE, Aberg MA, et al. (2007). Peripheral infusion of insulin-like growth factor-I increases the number of newborn oligodendrocytes in the cerebral cortex of adult hypophysectomized rats. *Endocrinology*. 148: 3765–3772.

Aberg ND, Johansson I, Aberg MA, et al. (2009). Peripheral administration of GH induces cell proliferation in the brain of adult hypophysectomized rats. *J Endocrinol*. 201:141-50.

Aberg D, Jood K, Blomstrand C, et al. (2011). Serum IGF-I levels correlate to improvement of functional outcome after ischemic stroke. *J Clin Endocrinol Metab.* 96:E1055-64.

Aimone JB, Deng W & Gage FH. (2010). Adult neurogenesis: integrating theories and separating functions. *Trends Cogn Sci.* 14: 325-37.

Ajo R, Cacicedo L, Navarro C & Sanchez-Franco F. (2003). Growth hormone action on proliferation and differentiation of cerebral cortical cells from fetal rat. *Endocrinology.* 144:1086–1097.

Ali O, Shim M, Fowler E, Cohen P & Oppenheim W. (2007a). Spinal bone mineral density, IGF-1 and IGFBP-3 in children with cerebral palsy. *Horm Res*. 68:316–320.

Ali O, Shim M, Fowler E, et al. (2007b). Growth hormone therapy improves bone mineral density in children with cerebral palsy: a preliminary pilot study. *J Clin Endocrinol Metab*. 92:932–937.

Altman J & Das GD. (1965). Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. *J Comp Neurol.* 124:319-35.

Anacker C, Zunszain PA, Cattaneo A., et al. (2011). Antidepressants increase human hippocampal neurogenesis by activating the glucocorticoid receptor. *Mol Psychiatry.* 16: 738-750.

Azcoitia I, Pérez Martín M, Salazar V., et al. (2005) Growth hormone prevents neuronal loss in the aged rat hippocampus. *Neurobiol Aging*. 5:697-703.

Barclay JL, Kerr LM, Arthur L, et al. (2010). In vivo targeting of the growth hormone receptor (GHR) Box1 sequence demonstrates that the GHR does not signal exclusively through JAK2. *Mol Endocrinol.* 24:204-17.

Beckinschtein P, Oomen CA, Saksida LM & Bussey TJ. (2011). Effects of environmental enrichment and voluntary exercise on neurogenesis, learning and memory, and pattern separation: BDNF as a critical variable? *Semin Cell Dev Biol*. Jul 7. [Epub ahead of print].

Bennett BL, Sasaki DT, Murray BW, et al. (2001). SP600125, an anthrapyrazolone inhibitor of Jun N-terminal kinase. *Proc Natl Acad Sci U S A*. 98:13681-6.

Betarbet R, Zigova T, Bakay RA & Luskin MB. Dopaminergic and GABAergic interneurons of the olfactory bulb are derived from the neonatal subventricular zone. *Int J Dev Neurosci.* 14: 921-30.

Bohannon RW & Smith MB. (1987) Interrater reliability of a modified Ashworth scale of muscle spasticity. *Phys Ther*. 67:206–207.

Boldrini M, Underwood MD, Hen R, et al. (2009). Antidepressants increase neural progenitor cells in the human hippocampus. *Neuropsychopharmacology*. 34:2376–2389.

Bowers JM, Waddell J & McCarthy MM. (2010). A developmental sex difference in hippocampal neurogenesis is mediated by endogenous oestradiol. *Biol Sex Differ*. 1:8-15.

Bruel-Jungerman E, Veyrac A, Dufour F, Horwood J, Laroche S & Davis S. (2009). Inhibition of PI3-Akt signaling blocks exercise-mediated enhancement of adult neurogenesis and synaptic plasticity in the dentate gyrus. *PloS One*. 4:e7901.

Byts N, Samoylenko A, Fasshauer T, et al. (2008). Essential role for Stat5 in the neurotrophic but not in the neuroprotective effect of erythropoietin. *Cell Death Differ*. 15:783–792.

Byts N & Sirén AL. (2009). Erythropoietin: a multimodal neuroprotective agent. *Exp Transl Stroke Med*. 1:4-11.

Cameron HA & McKay RD. (1999). Restoring production of hippocampal neurons in old age. *Nat Neurosci*. 2:894–897.

Campbell NR, Fernandes CC, Halff AW & Berg DK. (2010). Endogenous signaling through alpha7-containing nicotinic receptors promotes maturation and integration of adult-born neurons in the hippocampus. *J Neurosci*. 30: 8734– 8744.

Carracedo A, Ma L, Teruya-Feldstein J, et al. (2008). Inhibition of mTORC1 leads to MAPK pathway activation through a PI3K-dependent feedback loop in human cancer. *J. Clin. Invest*. 118:3065–3074.

Chen ZY, Asavaritikrai P, Prchal JT & Noguchi CT. (2007). Endogenous erythropoietin signaling is required for normal neural progenitor cell proliferation. *J Biol Chem*. 282:25875–25883.

Christophidis LJ, Gorba T, Gustavsson M, et al. (2009). Growth hormone receptor immunoreactivity is increased in the subventricular zone of juvenile rat brain after focal ischemia: a potential role for growth hormone in injury- induced neurogenesis. *Growth Horm IGF Res*. 19:497-506.

Coniglio SJ & Stevenson RD. (1995). Growth hormone deficiency in two children with cerebral palsy. *Dev Med Child Neurol*. 37:1013–1015.

Costoya JA, Finidori J, Moutoussamy S, Señaris R, Devesa J & Arce VM. (1999). Activation of growth hormone receptor delivers an antiapoptotic signal: evidence for a role of Akt in this pathway. *Endocrinology*. 140:5937-43.

Darnaudery M, Perez-Martin M, Belizaire G, Maccari S & Garcia-Segura LM. (2006). Insulin-like growth factor 1 reduces age-related disorders induced by prenatal stress in female rats. *Neurobiol Aging*. 27:119–127.

David DJ, Samuels BA, Rainer Q, et al. (2009). Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/depression. *Neuron*. 62:479–493.

Devesa J, Lima L & Tresguerres JA. (1992). Neuroendocrine control of growth hormone secretion in humans. *Trends Endocrinol Metab*. 3:175-83.

Devesa J, Reimunde P, Devesa A, et al. (2009). Recovery from neurological sequelae secondary to oncological brain surgery in an adult growth hormone-deficient patient after growth hormone treatment. *J Rehabil Med*. 41:775-7.

Devesa J, Casteleiro N, Rodicio C, López N & Reimunde P. (2010a). Growth hormone deficiency and cerebral palsy. *Ther Clin Risk Manag*. 6:413-8.

Devesa J, Devesa P & Reimunde P. (2010b). [Growth hormone revisited]. *Med Clin (Barc)* 6:413-18.

Devesa J, Alonso B, Casteleiro N, et al (2011). Effects of recombinant growth hormone (GH) replacement and psychomotor and cognitive stimulation in the neurodevelopment of GH-deficient (GHD) children with cerebral palsy: a pilot study. *Ther Clin Risk Manag*. 7:199-206.

Devesa P, Reimunde P, Gallego R, Devesa J & Arce VM. (2011). Growth hormone (GH) treatment may cooperate with locally-produced GH in increasing the proliferative response of hippocampal progenitors to kainate-induced injury. *Brain Inj*. 25: 503-10.

Devesa P. (2011). Effects of growth hormone on the central and peripheral neural repair. *Doctoral thesis*. University of Santiago de Compostela, Spain.

Deijen JB, de Boer H & van der Veen EA. Cognitive changes during growth hormone replacement in adult men. *Psychoneuroendocrinology*. 23:45–55.

Doetsch F, Petreanu L, Caille I, Garcia-Verdugo JM & Alvarez-Buylla A. (2002). EGF converts transit-amplifying neurogenic precursors in the adult brain into multipotent stem cells. *Neuron*. 36:1021–1034.

Donahue CP, Jensen RV, Ochiishi T, et al. (2002). Transcriptional profiling reveals regulated genes in the hippocampus during memory formation. *Hippocampus*. 12:821–833.

Donahue CP, Kosik KS, & Shors TJ. (2006). Growth hormone is produced within the hippocampus where it responds to age, sex, and stress. *Proc. Natl. Acad. Sci.* 103:6031–6036.

Dutheil S, Lacour M & Tighilry B. (2011). Discovering a new functional neurogenic zone-The vestibular nuclei of the brainstem. *Med Sci (Paris)*. 27: 605-613.

Eriksson PS, Perfilieva E, Björk-Eriksson T, et al. (1998) Neurogenesis in the adult human hippocampus. *Nat Med*. 4:1313- 7.

Falleti MG, Maruff P, Burman P & Harris A. (2996). The effects of growth hormone (GH) deficiency and GH replacement on cognitive performance in adults: A meta-analysis of the current literature. *Psychoneuroendocrinology*. 31: 681– 691.

Franklin RJ, Zhao C & Sim FJ. (2002). Ageing and CNS remyelination. *Neuroreport*. 13: 923–928.

Fukuda S, Kato F, Tozuka Y, Yamaguchi M, Miyamoto Y & Hisatsune T. (2003). Two distinct subpopulations of nestin- positive cells in adult mouse dentate gyrus. *J Neurosci* .23:9357-66.

Fushimi S & Shirabe T. (2004). Expression of insulin-like growth factors in remyelination following ethidium bromide- induced demyelination in the mouse spinal cord. *Neuropathology*. 24:208–218.

Fukul H & Toyoshima K. (2008). Music facilitates the neurogenesis, regeneration and repair of neurons. *Med Hypotheses* 271 : 765-9.

Galea LA, Kavaliers M & Ossenkopp KP. (1996). Sexually dimorphic spatial learning in meadow voles Microtus pennsylvanicus and deer mice Peromyscus maniculatus. *J Exp Biol*. 199: 195–200

Galea LA, Spritzer MD, Barker JM & Pawluski JL. (2006). Gonadal hormone modulation of hippocampal neurogenesis in the adult. *Hippocampus*. 16: 225-32.

Gampe K, Brill MS, Momma S, Götz M & Zimmermann H. (2011). EGF induces CREB and ERK activation at the wall of the mouse lateral ventricles. *Brain Res*. 1376:31-41.

García-Aragón J, Lobie PE, Muscat GE, Gobius KS, Norstedt G & Waters MJ. (1992). Prenatal expression of the growth hormone (GH) receptor/binding protein in the rat: a role for GH in embryonic and fetal development?. *Development.* 114: 869–876.

Gheusi G, Cremer H, McLean H, Chazal G, Vincent JD & Lledo PM. (2000). Importance of newly generated neurons in the adult olfactory bulb for odor discrimination. *Proc Natl Acad Sci U S A*. 97: 1823-8.

Gilson J & Blakemore WF. (1993). Failure of remyelination in areas of demyelination produced in the spinal cord of old rats. *Neuropathol Appl Neurobiol.* 19:173–181.

Goldman SA & Nottebohm F. (1983). Neuronal production, migration, and differentiation in a vocal control nucleus of the adult female canary brain. *Proc Natl Acad Sci.* 80:2390-4.

Gonzalez-Perez O & Alvarez-Buylla A. (2011). Oligodendrogenesis in the subventricular zone and the role of epidermal growth factor. *Brain Res Rev*. 67: 147-56.

Gould E, Reeves AJ, Graziano MS & Gross CG. (1999). Neurogenesis in the neocortex of adult primates. *Science.* 286: 548- 52.

Gritti A, Frolichsthal-Schoeller P, Galli R, et al. (1999). Epidermal and fibroblast growth factors behave as mitogenic regulators for a single multipotent stem cell-like population from the subventricular region of the adult mouse forebrain. *J Neurosci.* 19: 3287–3297.

Hansen JA, Lindberg K, Hilton DJ, Nielsen JH & Billestrup N. (1999). Mechanism of inhibition of growth hormone receptor signaling by suppressor of cytokine signaling proteins. *Molecular Endocrinology.* 13:1832–1843.

Hidalgo A, Barami K, Iversen K & Goldman SA. (1995). Estrogens and non-estrogenic ovarian influences combine to promote the recruitment and decrease the turnover of new neurons in the adult female canary brain. *J Neurobiol*. 27:470–487.

High WM, Briones-Galang M, Clark JA, et al. (2010). Effect of Growth Hormone replacement therapy on cognition after traumatic brain injury. *J Neurotrauma.* 27:1565-75.

Hua K, Forbes EM, Lichtenwalner RJ, Sonntag WE & Riddle DR. (2009). Adult-onset deficiency in growth hormone and insulin-like growth factor-I alters oligodendrocyte turnover in the corpus callosum. *Glia.* 57:1062-71.

Hoyt, CS. (2003). Visual function in the brain-damaged child. *Eye.* 17:369–384.

Huang GJ & Herbert J. (2006). Stimulation of neurogenesis in the hippocampus of the adult rat by fluoxetine requires rhythmic change in corticosterone. *Biol Psychiatry.* 59:619–624.

Iguchi H, Mitsui T, Ishida M, Kaba S & Arita J. (2011). cAMP response element-binding protein (CREB) is required for epidermal growth factor (EGF)-induced cell proliferation and serum response element ativation in neural stem cells isolated from the forebrain subventricular zone of adult mice. *Endocr J.* Jun 24. [Epub ahead of print].

Johansson BB. (2011). Current trends in stroke rehabilitation. A review with focus on brain plasticity. *Acta Neurol Scand.* 123: 147-59

Johnson F & Bottjer SW. (1995). Differential estrogen accumulation among populations of projection neurons in the higher vocal center of male canaries. *J Neurobiol.* 26:87–108.

Kaplan MS & Hinds JW. (1977). Neurogenesis in the adult rat: electron microscopic analysis of light radioautographs. *Science.* 197:1092-4.

Katakowski M, Zhang ZG, Chen J., et al. (2003). Phosphoinositide 3-kinase promotes adult subventricular neuroblast migration after stroke. *J Neurosci Res.* 74:494-501.

Kempermann G, Kuhn HG & Gage FH. (1997). More hippocampal neurons in adult mice living in an enriched environment. *Nature.* 386: 493-5.

Kempermann G, Wiskott L & Gage FH. (2004). Functional significance of adult neurogenesis. *Curr Opin Neurobiol.* 14:186-91.

Konkle AT & McCarthy MM. (2010). Developmental time course of Estradiol, Testosterone, and Dihydrotestosterone levels in discrete regions of male and female rat brain. *Endocrinology.* 152: 223-35.

Kronenberg G, Reuter K, Steiner B, et al. (2003). Subpopulations of proliferating cells of the adult hippocampus respond differently to physiologic neurogenic stimuli. *J Comp Neurol.* 467:455- 63.

Kuhn HG, Dickinson-Anson H & Gage FH. (1996). Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. *J Neurosci.* 16:2027–2033.

Kuhn HG, Winkler J, Dempermann G, Thal LJ & Gage FH. (1997). Epidermal growth factor and fibroblast growth factor- 2 have different effects on neural progenitors in the adult rat brain. *J Neurosci.* 17:5820–5829.

Kumar S, Biancotti JC, Yamaguchi M & de Vellis J. (2007). Combination of growth factors enhances remyelination in a cuprizone-induced demyelination mouse model. *Neurochem Res.* 32:783–797.

Lam TT & Leranth C. (2003). Role of the medial septum diagonal band of Broca cholinergic neurons in oestrogen- induced spine synapse formation on hippocampal CA1 pyramidal cells of female rats. *Eur J Neurosci.* 17:1997– 2005.

Lecca D, Trincavelli ML, Gelosa P, et al. (2008). The recently identified P2Y-like receptor GPR17 is a sensor of brain damage and a new target for brain repair. *PLos One.* 3:e3579.

Lennington JB, Yang Z & Conover JC. (2003). Neural stem cells and the regulation of adult neurogenesis. *Reprod Biol Endocrinol*. 1:99-105.

Lichtenwalner RJ, Forbes ME, Bennett SA, Lynch CD, Sonntag WE & Riddle DR. (2001). Intracerebroventricular infusion of insulin-like growth factor-I ameliorates the age-related decline in hippocampal neurogenesis. *Neuroscience.* 107:603–613.

Lichtenwalner RJ, Forbes ME, Sonntag WE & Riddle DR. (2006). Adult-onset deficiency in growth hormone and insulin- like growth factor-I decreases survival of dentate granule neurons: insights into the regulation of adult hippocampal neurogenesis. *J Neurosci Res.* 83:199–210.

Leuner B, Caponiti JM & Gould E. (2011). Oxytocin stimulates adult neurogenesis even under conditions of stress and elevated glucocorticoids. *Hippocampus.* Jun 20. doi: 10.1002/hipo.20947. [Epub ahead of print].

Lledo PM, Alonso M & Grubb MS. (2006). Adult neurogenesis and functional plasticity in neuronal circuits. *Nat Rev Neurosci*. 7: 179-93.

Lobie PE, Garcia-Aragon J, Lincoln DT, Barnard R, Wilcox JN & Waters MJ. (1993). Localization and ontogeny of growth hormone receptor gene expression in the central nervous system. *Brain Res Dev Brain Res*. 74:225-33.

Lois C, & Alvarez-Buylla A. (1993). Proliferating subventricular zone cells in the adult mammalian forebrain can differentiate into neurons and glia. *Proc Natl Acad Sci.* 90:2074-7.

Lyuh E, Kim HJ, Kim M, et al. (2007). Dose-specific or dose-dependent effect of growth hormone treatment on the proliferation and differentiation of cultured neuronal cells. *Growth Horm. IGF Res.* 17: 315–322.

Mason JL, Ye P, Suzuki K, D'Ercole AJ & Matsushima GK. (2000). Insulin-like growth factor-1 inhibits mature oligodendrocyte apoptosis during primary demyelination. *J Neurosci.* 20: 5703–5708.

McLenachan S, Lum MG, Waters MJ & Turnley AM. (2009). Growth hormone promotes proliferation of adult neurosphere cultures. *Growth Horm IGF Res.* 19:212-8.

McMorris FA & McKinnon RD. (1996). Regulation of oligodendrocyte development and CNS myelination by growth factors: prospects for therapy of demyelinating disease. *Brain Pathol*. 6: 313–329.

Mak GK & Weiss S. (2010). Paternal recognition of adult offspring mediated by newly generated CNS neurons. *Nat Neurosci.* 13: 652-3.

Maren S, De Oca B & Fanselow MS. (1994). Sex differences in hippocampal long-term potentiation (LTP) and Pavlovian fear conditioning in rats: positive correlation between LTP and contextual learning. *Brain Res.* 661:25–34.

Maric D, Maric I, Chang YH & Barker JL. (2003). Prospective cell sorting of embryonic rat neural stem cells and neuronal and glial progenitors reveals selective effects of basic fibroblast growth factor and epidermal growth factor on self-renewal and differentiation. *J Neurosci*. 23:240–251.

Marrone DF, Ramirez-Amaya V & Barnes CA. (2011). Neurons generated in senescence maintain capacity for functional integration. *Hippocampus.* Jun 21. doi: 10.1002/hipo.20959. [Epub ahead of print].

Maruff P & Falleti M. (2005). Cognitive function in growth hormone deficiency and growth hormone replacement. *Hormone Research.* 64(Suppl 3):100–108.

Mason JL, Ye P, Suzuki K, D'Ercole AJ & Matsushima GK. (2000). Insulin-like growth factor-1 inhibits mature oligodendrocyte apoptosis during primary demyelination. *J Neurosci.* 20 :5703–5708.

McLenachan S, Lum MG, Waters MJ & Turnley AM. (2009). Growth hormone promotes proliferation of adult neurosphere cultures. *Growth Horm IGF Res.* 19:212-8.

Mitsushima D, Takase K, Takahashi T & Kimura F. (2009a). Activational and organisational effects of gonadal steroids on sex-specific acetylcholine release in the dorsal hippocampus. *J Neuroendocrinol*. 21:400–405.

Mitsushima D, Takase K, Funabashi T & Kimura F. (2009b). Gonadal steroids maintain 24 h acetylcholine release in the hippocampus: organizational and activational effects in behaving rats. *J Neurosci*. 29:3808–3815.

Möderscheim TA, Christophidis LJ, Williams CE & Scheepens A. (2007). Distinct neuronal growth hormone receptor ligand specificity in the rat brain. *Brain Res.* 1137:29-34.

Morshead CM & van der Kooy D. (1992). Postmitotic death is the fate of constitutively proliferating cells in the subependymal layer of the adult mouse brain. *J Neurosci.*12:249–256.

Morshead CM, Reynolds BA, Craig CG, et al. (1994). Neural stem cells in the adult mammalian forebrain: a relatively quiescent subpopulation of subependymal cells. *Neuron.* 13:1071–1082.

Moutoussamy S, Renaudie F, Lago F, Kelly PA & Finidori J. (1998). Grb10 identified as a potential regulator of growth hormone (GH) signaling by cloning of GH receptor target proteins. *Journal of Biological Chemistry.* 273:15906– 15912.

Newborg J, Stock JR, Wnek L, et al. (1988).*Battelle Developmental Inventory with Recalibrated Technical Data and Norms: Screening Test Examiner's Manual*. 2nd ed. Allen, TX: DLM, Inc.

Nieves-Martinez E, Sonntag WE, Wilson A, et al. (2010). Early-onset GH deficiency results in spatial memory impairment in midlife and is prevented by GH supplementation. *Journal of Endocrinology*. 204:31–36.

Nilsson M, Perfilieva E, Johansson U, Orwar O & Eriksson PS. (1999). Enriched environment increases neurogenesis in the adult rat dentate gyrus and improves spatial memory. *J Neurobiol.* 39:569-78.

Nordeen EJ & Nordeen KW. (1989). Estrogen stimulates the incorporation of new neurons into avian song nuclei during adolescence. *Brain Res Dev Brain Res.* 49:27–32.

Palisano RJ, Hanna SE, Rosenbaum PL, et al. (2000). Validation of a model of gross motor function for children with cerebral palsy. *Phys Ther.* 80:974–985.

Pan SN, Ma HM, Su Z, Zhang CX, Zhu SY & Du ML. (2011). Epidermal growth factor receptor signaling mediates growth hormone-induced growth of chondrocytes from sex hormone-inhibited adolescent rats. *Clin Exp Pharmacol Physiol.* Jun 1. doi: 10.1111/j.1440-1681.2011.05547.x. [Epub ahead of print].

Pang Y, Zheng B, Fan LW, Rhodes PG & Cai Z. (2007). IGF-1 protects oligodendrocyte progenitors against TNF alpha- induced damage by activation of PI3K/Akt and interruption of the mitochondrial apoptotic pathway. Glia. 55:1099–1107.

Parent JM. (2002). The role of seizure-induced neurogenesis in epileptogenesis and brain repair. *Epilepsy Res.* 50: 179-89.

Parent JM. (2003). Injury-induced neurogenesis in the adult mammalian brain. *Neuroscientist*. 9: 261–272.

Paton JA & Nottebohm FN. (1984). Neurons generated in the adult brain are recruited into functional circuits. *Science.* 225:1046-8.

Perrini S, Natalicchio A, Laviola L, et al. (2008). Abnormalities of insulin-like growth factor-I signaling and impaired cell proliferation in osteoblasts from subjects with osteoporosis. *Endocrinology.* 149:1302–1313.

Popovic V. (2005a). GH deficiency as the most common pituitary defect after TBI: Clinical implications. *Pituitary* 8: 239– 243.

Popovic V, Aimaretti G, Casanueva FF & Ghigo E. (2005b). Hypopituitarism following traumatic brain injury. *Growth Hormone & IGF Research.* 15:177–184.

Rasika S, Nottebohm F & Alvarez-Buylla A. (1994). Testosterone increases the recruitment and/or survival of new high vocal center neurons in adult female canaries. *Proc Natl Acad Sci U S A.* 91:7854–7858.

Reimunde P, Rodicio C, López N, Alonso A, Devesa P & Devesa J. (2010). Effects of recombinant growth hormone replacement and physical rehabilitation in recovery of gross motor function in children with cerebral palsy. *Ther Clin Risk Manag.* 6:585-92.

Reimunde P, Quintana A, Castañón B. et al. (2011). Effects of growth hormone (GH) replacement and cognitive rehabilitation in patients with cognitive disorders after traumatic brain injury. *Brain Inj*. 25:65-73.

Reynolds BA & Weiss S. (1992). Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science.* 255:1707–1710.

Robel S, Berninger B & Götz M. (2011). The stem cell potential of glia: lessons from reactive gliosis. *Nat Rev Reurosci.* 12: 88-104.

Romanko MJ, Rola R, Fike JR, et al. (2004). Roles of the mammalian subventricular zone in cell replacement after brain injury. *Prog Neurobiol.* 74: 77-99.

Roof RL & Havens MD. (1992). Testosterone improves maze performance and induces development of a male hippocampus in females. *Brain Res.* 572:310–313.

Roof RL, Zhang Q, Glasier MM & Stein DG. (1993). Gender-specific impairment on Morris water maze task after entorhinal cortex lesion. *Behav Brain Res.* 57:47–51.

Sanders EJ, Parker E & Harvey S. (2006). Retinal ganglion cell survival in development: mechanisms of retinal growth hormone action. *Experimental Eye Research.* 83:1205–1214.

Sanders EJ, Baudet ML, Parker E, & Harvey S. (2009). Signaling mechanisms mediating local GH action in the neural retina of the chick embryo. *Gen Comp Endocrinol.* 163:63-9.

Sapolsky RM. (1992). Do glucocorticoid concentrations rise with age in the rat? *Neurobiol Aging.* 13:171–174.

Schaevitz LR & Berger-Sweeney J. (2005). Neurogenesis of the cholinergic medial septum in female and male C57BL/6J mice. *J Neurobiol.* 65:294–303.

Scheepens A, Williams CE, Breier BH, Guan J & Gluckman PD. (2000). A role for the somatotropic axis in neural development, injury and disease. *J Pediatr Endocrinol Metab.* 13 Suppl 6:1483-91.

Scheepens A, Sirimanne ES, Breier BH, Clark RG, Gluckman PD & Williams CE. (2001).Growth hormone as a neuronal rescue factor during recovery from CNS injury. *Neuroscience*. 104:677-87.

Scott HJ, Stebbing MJ, Walters CE. et al. (2006). Differential effects of SOCS2 on neuronal differentiation and morphology. *Brain Res.* 1067:138–145.

Shields SA, Gilson JM, Blakemore WF & Franklin RJ. (1999). Remyelination occurs as extensively but more slowly in old rats compared to young rats following gliotoxin-induced CNS demyelination. *Glia.* 28: 77–83.

Shim ML, Moshang T Jr, Oppenheim WL & Cohen P. (2004). Is treatment with growth hormone effective in children with cerebral palsy? *Dev Med Child Neurol*. 46:569–571.

Shingo T, Gregg C, Enwere E, et al. (2003). Pregnancy-stimulated neurogenesis in the adult female forebrain mediated by prolactin. *Science.* 299:117–120.

Silva C, Zhang K, Tsutsui S, Holden JK, Gill MJ & Power C. (2003). Growth hormone prevents human immunodeficiency virus-induced neuronal p53 expression. *Ann. Neurol.* 54:605–614.

Sim FJ, Zhao C, Penderis J & Franklin RJ. (2002). The age-related decrease in CNS remyelination efficiency is attributable to an impairment of both oligodendrocyte progenitor recruitment and differentiation. *J Neurosci.* 22: 2451–2459.

Sun LY, Al-Regaiey K, Masternak MM, Wang J & Bartke A. (2005a). Local expression of GH and IGF-1 in the hippocampus of GH-deficient long-lived mice. *Neurobiol. Aging.* 26:929–937.

Sun LY, Evans MS, Hsieh J, Panici J & Bartke A. (2005b). Increased neurogenesis in dentate gyrus of long-lived Ames dwarf mice. *Endocrinology.* 146:1138–1144.

Sun LY & Bartke A. (2007). Adult neurogenesis in the hippocampus of long-lived mice during aging. *J. Gerontol. A – Biol. Sci. Med. Sci.* 62:117–125.

Svensson AL, Bucht N, Hallberg M & Nyberg F. (2008). Reversal of opiate-induced apoptosis by human recombinant growth hormone in murine foetus primary hippocampal neuronal cell cultures. *Proc Natl Acad Sci U S A.* 105:7304-8.

Tanapat P, Hastings NB, Reeves AJ & Gould E. (1999). Estrogen stimulates a transient increase in the number of new neurons in the dentate gyrus of the adult female rat. *J Neurosci.* 19:5792–5801.

Tonchev AB. (2011). The nerve growth factor in health and unhealth neurons. *Arch Ital Biol.* 149: 225-31.

Torner L, Karg S, Blume A, et al. (2009). Prolactin prevents chronic stress-induced decrease of adult hippocampal neurogenesis and promotes neuronal fate. *J Neurosci.* 29:1826-33.

Trejo JL, Carro E & Torres-Aleman I. (2001). Circulating insulin-like growth factor I mediates exercise-induced increases in the number of new neurons in the adult hippocampus. *J Neurosci.* 21: 1628-34.

Tropepe V, Sibilia M, Ciruna BG, Rossant J, Wagner EF & van der Kooy D. (1999). Distinct neural stem cells proliferate in response to EGF and FGF in the developing mouse telencephalon. *Dev Biol.* 208:166–188.

Tsai PT, Ohab JJ, Kertesz N, et al. (2006). A critical role of erythropoietin receptor in neurogenesis and post-stroke recovery. *J Neurosci.* 26:1269–1274.

Turnley AM, Faux CH, Rietze RL, Coonan JR & Bartlett PF. (2002). Suppressor of cytokine signaling 2 regulates neuronal differentiation by inhibiting growth hormone signaling. *Nat. Neurosci.* 5: 1155–1162.

Vanderkuur JA, Butch ER, Waters SB, Pessin JE, Guan KL & Carter-Su C. (1997). Signaling molecules involved in coupling growth hormone receptor to mitogen-activated protein kinase activation. *Endocrinology.* 138:4301–4307.

van Dam PS. (2006). Somatotropin therapy and cognitive function in adults with growth hormone deficiency: A critical review. *Treatment in Endocrinology.* 5:159-170.

van Marle G, Antony JM, Silva C, Sullivan A & Power C. (2005). Aberrant cortical neurogenesis in a pediatric neuroAIDS model: neurotrophic effects of growth hormone. *Aids.*19:1781–1791.

van Praag H, Christie BR, Sejnowski TJ & Gage FH. (1999). Running enhances neurogenesis, learning, and long-term potentiation in mice. *Proc Natl Acad Sci USA.* 96: 13427-31.

Vogenthaler DR. (1987). An overview of head injury: Its consequences and rehabilitation. *Brain Injury.* 1:113–127.

Wagner K, Couillard-Despres S, Lehner B., et al. (2009). Prolactin induces MAPK signaling in neural progenitors without alleviating glucocorticoid-induced inhibition of *in vitro* neurogenesis. *Cell Physiol Biochem.* 24:397-406.

Wang X, Yang N, Deng L, Li X, Jiang J, Gan Y & Frank SJ. (2009). Interruption of growth hormone signaling via SHC and ERK in 3T3-F442A preadipocytes upon knockdown of insulin receptor substrate-1. *Mol Endocrinol.* 23:486-96.

Wechsler D. (1990). *Manual for the Wechsler adult intelligence scale. 8th ed. Madrid: TEA*.

Yamauchi T, Ueki K, Tobe K et al. (1997). Tyrosine phosphorylation of the EGF receptor by the kinase Jak2 is induced by growth hormone. *Nature.* 390:91–96.

Xu J & Messina JL. (2009). Crosstalk between growth hormone and insulin signaling. *Vitam Horm.* 80:125-53.

Yamauchi T, Ueki K, Tobe K, et al. (1998). Growth hormone-induced tyrosine phosphorylation of EGF receptor as an essential element leading to MAP kinase activation and gene expression. *Endocr J.* Apr; 45 Suppl: S27-31.

Yu T-S, Zhang G, Liebl DJ & Kernie SG. (2008). Traumatic brain injury-induced hippocampal neurogenesis requires activation of early nestin-expressing progenitors. *J Neurosci.* 28: 12901-12.

Zhang R, Zhang Z, Wang L, et al. (2004). Activated neural stem cells contribute to stroke-induced neurogenesis and neuroblast migration toward the infarct boundary in adult rats. *J Cereb Blood Flow Metab.* 24: 441-8.

Zhang L, Chopp M, Zhang RL, et al. (2010) Erythropoietin amplifies stroke-induced oligodendrogenesis in the rat. *PloS One*. 5:e106.

Zhang Z, Yang R, Zhou R, Li L, Sokabe M & Chen L. (2010). Progesterone promotes the survival of newborn neurons in the dentate gyrus of adult male mice. *Hippocampus.* 20: 402-12.

Zhao C, Deng W & Gage FH. (2008). Mechanisms and functional implications of adult neurogenesis. *Cell*. 132:645-60.

Zhu T, Goh EL, LeRoith D & Lobie PE. (1998). Growth hormone stimulates the formation of a multiprotein signaling complex involving p130(Cas) and CrkII. Resultant activation of c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK). *J Biol Chem*. 273:33864-75.

Zhu T, Goh EL, Graichen R, Ling & Lobie PE. (2001). Signal transduction via the growth hormone receptor. *Cellular Signalling*. 13:599–616.

Zhu T, Ling L & Lobie PE. (2002). Identification of a JAK2-independent pathway regulating growth hormone (GH)- stimulated p44/42 mitogen-activated protein kinase activity. GH activation of Ral and phospholipase D is Src- dependent. *Journal of Biological Chemistry*. 277:45592–45603.

22

Novel Strategies for Discovery, Validation and FDA Approval of Biomarkers for Acute and Chronic Brain Injury

S. Mondello, F. H. Kobeissy, A. Jeromin, J. D. Guingab-Cagmat, Z. Zhiqun, J. Streeter, R. L. Hayes and K. K. Wang
University of Florida and Banyan Biomarkers, Inc.
USA

1. Introduction

The proposed chapter outlines novel approaches that provide an infrastructure for discovery and validation of new biomarkers of acute brain injury. Approaches to validation can be also applied to existing biomarkers of brain injury in order to provide more rigorous assessment of their clinical utility. Importantly, there are currently no biomarkers of brain injury approved by Food and Drug Administration (FDA). The chapter reviews approaches critical for securing FDA approval of biomarkers of brain injury and disease, focusing on traumatic brain injury (TBI).

The chapter reviews proteomics techniques applied for the first time to discovery of biomarkers of central nervous system (CNS). These techniques include refined mass spectrometry technology and high throughput immunoblot techniques. Output from these approaches can identify potential candidate biomarkers employing systems biology and data mining methods that will also be described.

Once potential biomarkers have been identified, it is important to provide information on their clinical utility for diagnosis, management and prognosis of patients exposed to brain injuries. This section of the chapter will review both preclinical and clinical methods for biomarker validation. Preclinical models discussed include rodent models of closed head injury such as the controlled cortical impact (CCI) device. Consideration will also be given to the design and results from human clinical trials validating biomarkers of mild, moderate and severe traumatic brain injury (TBI). Human studies will include detailed analyses of the biokinetics of different biomarkers in order to understand their utility in acute, subacute and chronic phases of TBI. Consideration will also be given to relationships between levels of biomarkers and magnitude of acute injury, CT imaging profiles, occurrence of secondary insults and long term outcome.

To achieve practical clinical utility, it is important to develop highly sensitive and specific assays for individual biomarkers. Enzyme-linked immunosorbent assays (ELISAs) are the current choice for clinical use, since this assay technology provides reliable, quantitative and accurate data. ELISA technologies relevant to biomarker measurement will be discussed. Once ELISA assays for individual biomarkers have been developed, these assays need to be transferred to devices that are appropriate for the clinical application and medical

environment in which biomarker analyses will be conducted. We will review devices for use in clinical laboratories, emergency rooms (ERs), intensive care units (ICUs) and austere medical environment. In addition, this section will include review developing technologies allowing concurrent assessment of multiple biomarkers (multiplex).
To date, studies of biomarkers for brain injury have been restricted to research applications only. Although there is broad recognition of clinical utility of biomarkers, the FDA has yet to approve any biomarkers of CNS injury of disease. This section of the chapter provides a detailed outline of the regulatory consideration necessary for a biomarker to file for approval by FDA. Importantly, many of these considerations need to be integrated into relatively early stages of biomarker validation, assay development and device selection.

2. Biomarker discovery: Methods and results

2.1 Proteomics/systems biology in the area of neurotrauma

The application of neuroproteomics/neurogenomics has revolutionized the characterization of protein/gene dynamics, leading to a greater understanding of post-injury biochemistry. Neuroproteomics and neurogenomics fields have undertaken major advances in the area of neurotrauma research focusing on biomarker identification. Several candidate markers have been identified and are being evaluated for their efficacy as biological biomarkers utilizing these "omics approaches". The identification of these differentially expressed candidate markers using these techniques is proving to be only the first step in the biomarker development process. However, to translate these findings into the clinic, data-driven development cycle incorporating data-mining steps for discovery, qualification, verification, and clinical validation is needed. Data mining steps extend beyond the collected data level into an integrated scheme of animal modeling, instrumentation, and functional data analysis.
Proteomics is the identification and quantification of all expressed proteins of a cell type, tissue or organism. The advancement in the field of proteomics has coincided with the completion of the human genome sequencing project (Fenn et al. 1989, Tanaka 1988, Karas & Hillenkamp 1988). In recent years, the term proteomics is often mentioned together with biomarker discovery, as proteomic studies have the capability of identifying sensitive and unique signature protein biomarkers from tissues or biofluids derived from animal models or human clinical samples inflicted with various diseases. Neuroproteomics and neurogenomics, the application of proteomics and genomics in the field of neuronal injury, have been identified as a potential means for biomarker discovery, with the ability to identify proteome dynamics in response to brain injury (Guingab-Cagmat 2009, Haskins et al. 2005, Davidsson & Sjogren 2005, Shin et al. 2002, Celis et al. 2004).
In the area of brain injury, several studies have demonstrated the role of proteomics (Denslow et al. 2003, Katano et al. 2006) and genomics (Redell et al. 2009, Ding et al. 2006) in providing significant insight into understanding changes, modifications and functions in certain proteins post TBI. In addition, genomics and proteomics are powerful, complementary tools that play an important role in the area of biomarker identification. Over the past few years, advances in the fields of neuroproteomics and neurogenomics have led to the discovery of many candidate biomarkers and are becoming the primary methods for initial candidate marker selection (Kobeissy et al. 2008, Wang et al. 2006, Ottens et al. 2007, Nogoy 2007, Ottens et al. 2006). The identification of differentially expressed candidate markers using these techniques is proving to be only the first step in the biomarker

development process. However, to translate these into the clinic, these novel assays require a data-driven development cycle that incorporates data-mining steps for discovery, qualification, verification, and clinical validation (Rifai et al. 2006).

TBI neuroproteomics studies have utilized biofluids such as blood/serum in addition to injured tissue to identify clinical markers that may correlate with injury severity. One of the studies by Burgess et al, evaluated altered differential proteins in normal human post mortem cerebrospinal fluid (CSF) (Burgess et al. 2006). The rationale of using post-mortem CSF is that it resembles a model of massive brain injury and cell death which post-mortem, thus comparing protein profile of post-mortem CSF with brain injury CSF would be ideal for identifying protein markers of injury. Of the 229 proteins identified, a total of 172 were novel and not previously described. The findings showed that the use of post mortem CSF (non-TBI samples) to evaluate altered protein levels mimicked the changes occurring in the brain following a traumatic insult. Furthermore, the identification of differential proteins of intracellular origin in the CSF corroborates the suggestion that there is protein leakage into the CSF following brain injury (Dumont et al. 2004, Hammack et al. 2004). This is a key step in identifying protein markers since, neuronal specific proteins leak from injured brain directly to the CSF.

In one of the TBI studies conducted in our laboratory, 1D-differential gel electrophoresis (DIGE) protein separation in series with mass spectrometry analysis was used to discover putative TBI biomarkers in brain tissues from a rat model (Haskins et al. 2005). These included 57 downregulated and 74 upregulated proteins; however data were not so informative due to limited separation capability. In an advanced study, we utilized a multidimensional separation platform called (CAX-PAGE/RPLC-MSMS) which consisted of different levels of separation including ion chromatography, 1-D gel electrophoresis and mass spectrometry as a novel approach for identifying biomarkers and protein breakdown products (degradomes); for detailed reviews, refer to (Svetlov et al. 2006, Kobeissy et al. 2006). As an application, the CAX/neuroproteomic analysis was employed on cortical samples of rat subjected to controlled cortical impact (CCI) model of experimental TBI (48 hrs post injury). Of interest, our neuroproteomic analysis identified 59 differential protein components of which 21 decreased and 38 increased in levels after TBI. One main advantage of this technique is its ability to elucidate degradomic substrates of different protease systems; thus our data identified the elevated levels of the breakdown products of several proteins (Kobeissy et al. 2006). Several of these are now being investigated as potential biomarkers specific for TBI to assess severity and recovery by evaluating their levels at different time points post TBI.

2.1.1 Data mining coupled neurosystems biology analysis in brain Injury

Coupled to data-mining steps, systems biology (SB) represents a mathematical model capable of predicting the altered processes or functions of a complex system under normal and perturbed conditions. It combines experimental, basic science data sets, proteomic and genetic data sets, literature and text mining, integration with computational modeling, bioinformatics and pathway/interaction mapping methods. When constructed properly, SB databases can provided a context or framework for understanding biological responses within physiological networks at the organism level, rather than in isolation (Chen et al. 2007).

In this regard, "omics" output constitutes one key component of neurosystems biology. It discusses the global changes involved in neurological perturbations integrating the final outcomes into a global functional network map which incorporates potential biomarkers

identified (Grant 2003, Grant & Blackstock 2001). In the area of brain injury, neurosystems biology platform harnesses data sets that, by themselves, would be overwhelming, into an organized, interlinked database that can be queried to identify non-redundant brain injury pathways or convert hot spots. These can be exploited to determine their utilities as diagnostic biomarkers and/or therapeutic targets. The ultimate goals of system biology are: first by exploring the systems component (gene, protein, small molecule, metabolite etc.), help biologist, pharmaceutical companies and doctors to better understand the mechanisms underlying the disease components. Thus, it allows for suitable targets for treatment. Secondly, the systems biology approach enables one to be able to predict the functions and behavior of various components of the system upon varying any on the interconnected component since the whole system will be viewed globally rather than on micro, individual component level (Beltrao et al. 2007).

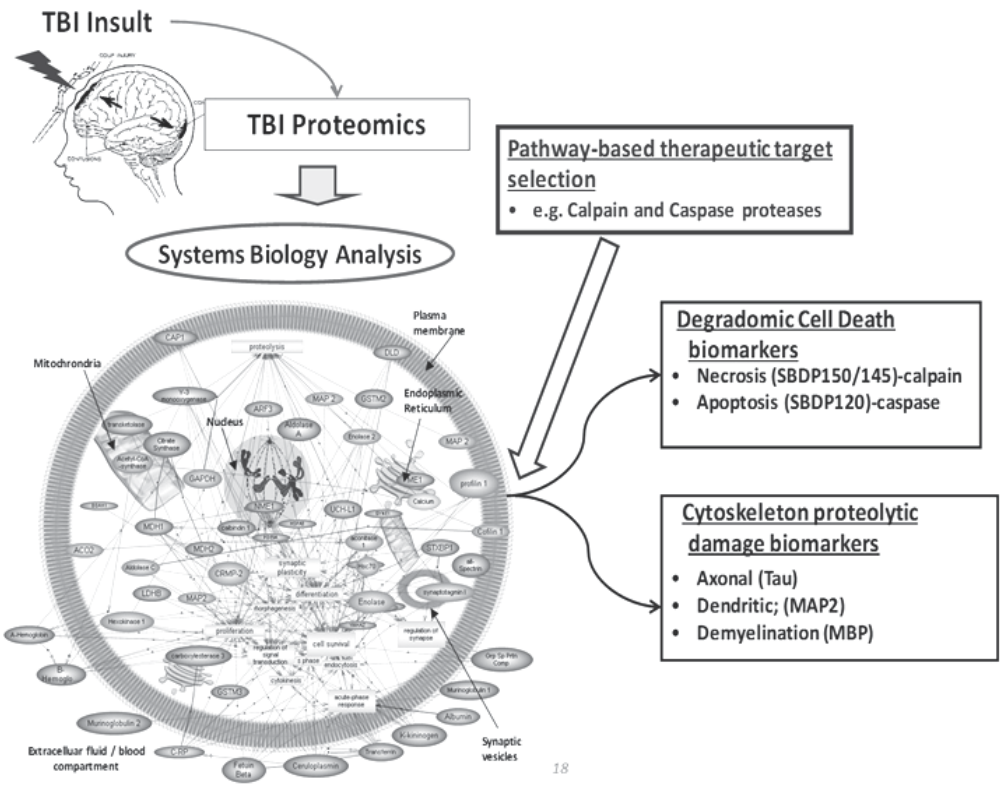


Fig. 1. Systems biology–based therapeutic target identification and target-specific biomarker selection.

In the field of neurotrauma, identifying and analyzing brain injury-related networks plays important and practical clues relating to biological pathways relevant to disease processes. However, the more important underlying goal in this analysis is to provide important clues that may suggest radically new approaches to therapeutics. Systems modeling and simulation is now considered fundamental to the future development of effective therapies. In the brain injury for example, it has been shown that calpain and caspase proteases are major components in cell death pathways taking part in two destructive proteolytic

pathways that not only contribute to key forms of cell death (necrosis and apoptosis), but also in the destruction of important structural components of the axons (alphaII-spectrin breakdown products (SBDPs) and tau), dendrites (MAP2) and myelin (MBP) (Figure. 1). Interestingly, two different forms of SBDPs reflect either neuronal necrosis (SBDP150 and SBDP145 cleaved by calpain) or neuronal apoptosis (SBDP120 cleaved by caspase-3) (Wang et al. 2005). These SBDPs and other similar neural protein breakdown products can serve as target pathway specific biomarkers as illustrated in Figure 1.

Calpain and caspase proteases are used here as examples of therapeutic targets with proteolytic brain biomarkers representing non-redundant pathways relevant to the pathobiology of these therapeutic targets and the disease itself. TBI (traumatic brain injury); MAP2 (microtubule-associated protein 2); MBP (myelin basic protein); SBDP (spectrin breakdown product).

3. Biomarker validation: Methods and results

Traumatic injury to the brain results in a cellular activation and disintegration, leading to release of cell-type–specific proteins. Measurable amounts of these damage markers are present in cerebrospinal fluid (CSF) and blood. These markers not only indicate the pathoanatomic injury type and the severity of injury but also might provide specific

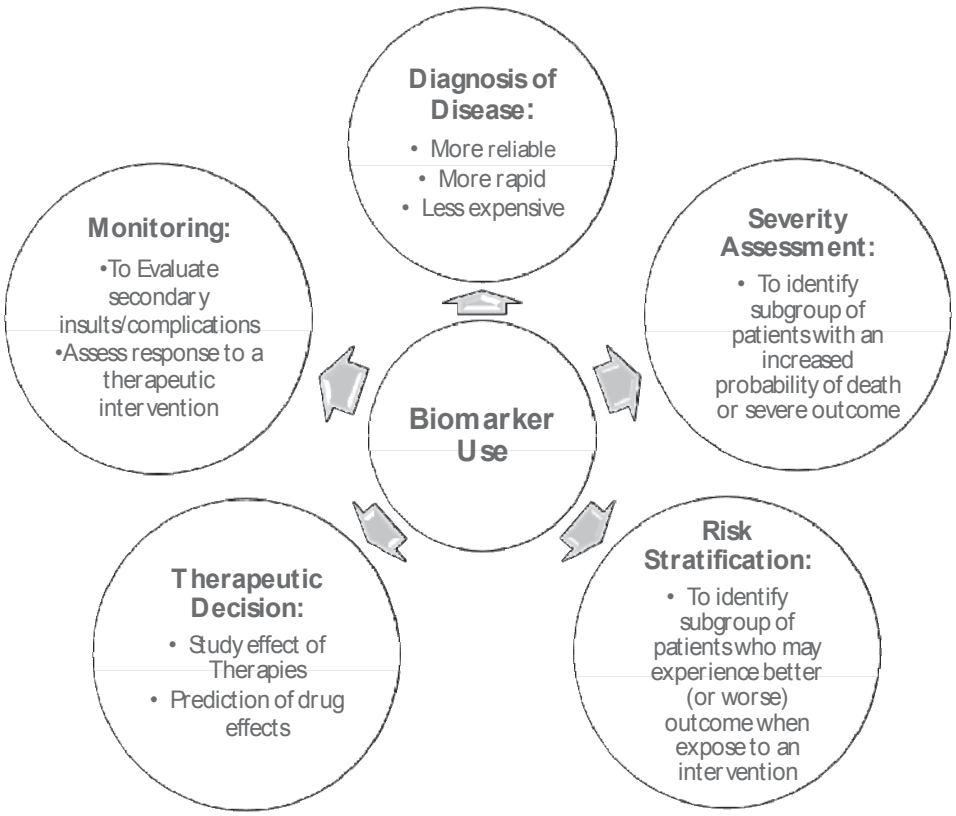


Fig. 2. Potential use of biomarkers in TBI

information about the pathophysiologic mechanisms which can be targeted by therapeutic interventions. Therefore, during the last decade, neurobiochemical markers for TBI have attracted increased attention, they can be used to screen for, diagnose or monitor the patients and to guide targeted therapy or assess therapeutic response (Etzioni et al. 2003, Vitzthum et al. 2005). (Figure. 2) Furthermore, biomarkers might be valuable tool in drug development (Blennow 2010).

3.1 Clinical evaluation

However, several studies have measured a variety of neurochemical substances in the CSF or blood, and a number of proteins synthesized in astroglial cells or neurons have been proposed as markers of cell damage in the CNS and after TBI, to date, none has been approved for clinical use. Critical criteria of the diagnostic performance of a clinically valid biomarker for TBI include its diagnostic accuracy and predictability.

The diagnostic accuracy of a test is the proportion of correctly classified patients (the sum of true positive and true negative tests) and is determined by calculating the test's sensitivity, specificity, likelihood ratio and receiver operating characteristic (ROC) curve (Bossuyt et al. 2003). Sensitivity is the ability to detect a disease in subjects in whom the disease is truly present (i.e., a true positive), and specificity is the ability to rule out the disease in subjects in whom the disease is truly absent (i.e., a true negative). Likelihood ratio (LHR), used to assess diagnostic value of a test, is the likelihood that a given test result would be expected in a patient with the specific disease compared to the likelihood that that same result would be expected in a patient without the target disorder. Two dimensions of accuracy might be considered, the LHR for a positive test (positive LHR) and the LHR for a negative test (negative LHR) (Albert 1982, Altman 1991). ROC curve is the graphical way of presenting the sensitivity (true positive rate) versus false positive rate (1 − specificity). A ROC curve enables the determination of appropriate cut-points, depending on the intended clinical utility of the test (Zweig& Campbell 1993). The area under the curve (AUC) is a measure of predictive discrimination: 50% is equivalent to random guessing and 100% is perfect prediction.

Although sensitivity and specificity are the most commonly provided variables in diagnostic studies, they do not directly apply to many clinical situations because the physician would rather know the probability that the disease is truly present or absent if the diagnostic test is positive or negative rather than probability of a positive test given the presence of the disease (sensitivity). This information is provided by the diagnostic predictability. Diagnostic predictability establishes the ability of the test to predict the presence or absence of disease for a given test result and is determined by calculating the positive and negative predictive values. The positive predictive value denotes the proportion of patients with positive test results who have the disease, and the negative predictive value defines the proportion of patients with a negative test who do not have the disease. The predictive values of a test vary with the prevalence of the disease in the population examined. Bayes' theorem allows calculation of the predictive values for any prevalence of disease using the prevalence and the sensitivity and specificity measures derived from previous studies (Altman 1991).

To demonstrate its clinical utility, a novel biomarker needs to be evaluated in a series of human studies (phase 1–4 trials) in order to establish the performance characteristics. The phase 1 examines whether the biomarker is significantly different for diseased patients as compared to those known not to have the disease. If a satisfactory discrimination between patients and controls is proven, the following step is to determine diagnostic accuracy

(phase 2). Phase 3 evaluates the performance of the test in the target population (Sackett & Haynes 2002). Phase 3 assesses whether the measurement of the biomarkers modifies outcome and influence therapeutic interventions and the subsequent effect on health outcome (intervention studies) (Sackett & Haynes 2002). Phase 4 trial is also known as post-marketing surveillance trial because this phase can be done after the marker has been made commercially available.

3.2 Novel candidate biomarkers

Many publications can be found for candidate biomarkers, although the initially promising results have often not been confirmed. Here, we review novel biomarkers that have shown high sensitivity and specificity in at least two independent studies. Biomarkers reflect damage and release from each of the major cell types/structures in brain parenchyma, therefore they can be divided into neuronal/synaptic and glial biomarkers (Table 1). We also discuss selected candidate biomarkers related to cellular and subcellular origins.

Biomarker	**Location**	**Function/ Pathogenic process**	**Comment**
Neuronal and Axonal Markers			
NSE	Prominently in the cytoplasm of neurons	Upregulated NSE is released from damaged axons to maintain cellular homeostasis.	Also present in erythrocytes and platelets
UCH-L1	Cell Body (perikarya)	Protein de-ubiquitinization	Implicated in familial Parkinsonism
SBPDs	Axons	Cortical cytoskeleton matrix support	Pathology--dependent generation of αII-spectrin breakdown products (SBDPs): Calpain generates SBDP145 as a signature event in acute neuronal necrosis Caspase-3 generates SBDP120 as a signature event in delayed neuronal apoptosis.
Phosphorylated neurofilament	Predominantly in axons	Main component of the axonal cytoskeleton	Increased serum concentrations of this protein are expected to provide a specific measure of axonal injury
Glial Markers			
S100b			
GFAP	Major protein constituent of glial filaments in astrocytes	Cytoskeleton support	Induced during " reactive astrogliosis " after TBI

Table 1. Biomarkers for TBI

3.2.2 Neuronal and axonal markers

Neuronal and axonal proteins could prove to be valuable biomarkers as these molecules might correlate with cognitive function and long term outcome.

3.2.2.1 Neuron-specific enolase

Neuron-specific enolase (NSE) is a glycolytic pathway enzyme, localized predominantly in neuronal cytoplasm. Several studies demonstrated that NSE is a sensitive and specific indicator of neuronal cell death (Selakovic et al. 2005, Oertel et al. 2006). In addition, studies have been conducted examining CSF and serum NSE levels from adults with severe TBI, and their relationship with severity of injury and clinical outcome. Increased CSF and serum levels of NSE have been reported after TBI. NSE concentrations were also associated with severity of injury, CT scan findings and outcome (Selakovic et al. 2005, Herrmann et al. 2000, Ross et al. 1996). Although, NSE was originally identified in neurons, further studies have shown the protein in erythrocytes and platelets. False positive values have been reported in the setting of combined CNS injury plus shock and in the setting of hemolysis (Piazza et al. 2005).

3.2.2.2 Ubiquitin C-terminal hydrolase

Ubiquitin C-terminal hydrolase (UCH-L1), a neuron-specific cytoplasmic enzyme, is highly enriched in neurons representing between 1 and 5% of total soluble brain protein (Lincoln et al. 1999). This protein is involved in either the addition or removal of ubiquitin from proteins that are destined for metabolism (via the ATP-dependent proteosome pathway) (Laser et al. 2003), thus playing an important role in the removal of excessive, oxidized or misfolded proteins during both normal and neuropathological conditions, such as neurodegenerative disorders (Kobeissy et al. 2006). Because of the important function and its high brain specificity, UCH-L1 has been proposed as a novel biomarker for TBI. CSF levels of UCH-L1 in CSF have been found significantly increased in severe TBI patients compared with uninjured patients, with significant associations observed between levels of UCH-L1 in CSF, injury severity and clinical outcome (Papa et al. 2010). A study has been conducted investigating the exposure and kinetic metrics of UCH-L1 in adults with severe TBI, and their relationship with severity of injury and clinical outcome (Brophy et al. 2010). A strong correlation between CSF and serum median concentrations and biokinetics, especially during the acute period, and relationships with clinical outcome were observed (Brophy et al. 2011). Furthermore, a recent study reported that serum concentrations of UCHL1 were associated with abnormal blood brain barrier (BBB) permeability, suggesting that UCH-L1 might be used to monitor BBB disruption in patients with TBI (Blyth et al. 2011).

3.2.2.3 Spectrin Breakdown Products (SBDPs)

Alpha II-spectrin is primarily found in neurons and is abundant in axons and presynaptic terminals (Riederer et al. 1986). The protein is processed to breakdown products (SBDPs) of molecular weights 150 kDa (SBDP150) and 145 kDa (SBDP145) by calpain and is also cleaved to a 120-kDa product (SBDP120) by caspase-3. Calpain and caspase-3 are major executioners of necrotic and apoptotic cell death, respectively, during ischemia or TBI (Ringger et al. 2004, Pineda et al. 2007, Mondello et al. 2010). Thus, a unique feature of this technique is the ability to concurrently detect calpain and caspase-3 proteolysis of aII-spectrin, providing crucial information on the underlying cell death mechanisms. In addition, distinct temporal release patterns of CSF SBDP145 and SBDP120 were reported to reflect different temporal characteristics of protease activation (Mondello et al. 2010). Pineda

et al., employing Western blot analyses, reported elevated levels of SBDPs in CSF from adults with severe TBI and their significant relationships with severity of injury and outcome (Pineda et al. 2007). Increased CSF SBDP levels were found to be significantly associated with mortality in patients with severe TBI. In addition, the temporal profile of SBDPs in nonsurvivors also differed from survivors (Mondello et al. 2010).These data suggest that SBDPs may provide crucial information not only on severity of brain injury, but also on underlying pathophysiological mechanisms associated with necrotic and apoptotic cell death.

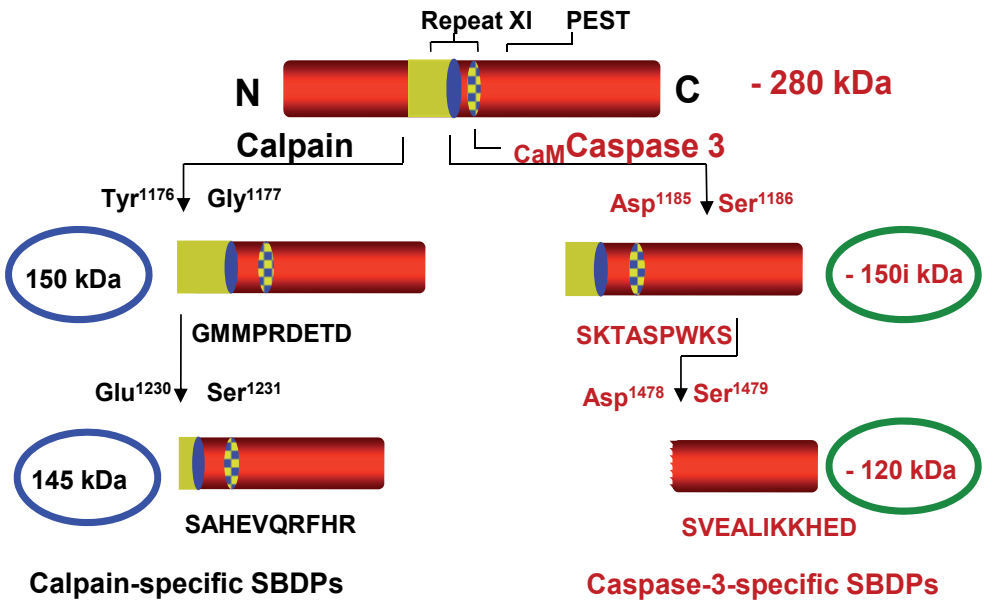


Fig. 3. Brain Injury-Dependent Generation of SBDPs

3.2.2.4 Phosphorylated neurofilament

A recent addition to the growing number of markers of brain injury is the heavily phosphorylated form of the major neurofilament subunit NF-H (pNF-H). Because pNF-H is specific for axons, increased serum concentrations of this protein are expected to provide a specific measure of ongoing axonal damage or degeneration. Increased pNF-H concentrations in serum and CSF have been observed following a variety of CNS damage and disease states, both in animal models and in human patients (Anderson et al. 2008, Boylan et al. 2009, Ganesalingam et al. 2011, Douglas-Escobar et al. 2010).

3.2.3 Glial markers

Several candidate glial markers have been proposed. In the section below, we focus on s100 and GFAP, which are the most extensively examined glial markers in TBI.

3.2.3.1 S100B

S-100b is a low-affinity calcium-binding protein that is primarily expressed and secreted astrocytes (Xiong et al., 2000). The biological function of S100B is still somewhat unclear.

Intracellularly, this protein is involved in in the regulation of a large variety of cell activities and in the regulation of cell morphology (Goncalves et al., 2008, Kleindienst & Bullock 2006). S100B is also released into the extracellular space where seems to have both toxic/degenerative and trophic/reparative roles depending on the concentration (Goncalves et al., 2008). S100B found in low levels in healthy individuals, rises rapidly after head injury. The serum half-life of s100 is about 30 to 90 minutes and can be detected soon after injury (Jonsson et al., 2000). Several studies have demonstrated effectiveness of S100B as a diagnostic marker in the setting of TBI (Romner et al. 2000, Raabe et al. 1999). Increased levels of this protein marker have been shown to correlate with injury severity, mortality, and poor neurologic outcome following severe TBI (Vos et al. 2004). In addition, since 1995 several studies have investigated the clinical utility of this biomarker to predict normal CT findings after minor head injury in adults (Ingebrigtsen et al. 1995, Biberthaler et al. 2006). However, several observations concerning S100B have tempered the original enthusiasm regarding the usefulness of this protein as brain damage biomarker. One of the limitations to the use of S100B as a potential screening agent for brain injury is the lack of specificity. Indeed, S-100b, originally considered specific to astroglia in the central nervous system, is present in other extracerebral tissues such as adipocytes, chondrocytes and bone marrow cells (Donato 2001). High serum S-100b levels have been observed after extracranial trauma and burns (Anderson et al. 2001, Romner &Ingebritsen 2001). In addition, increased S100B may reflect either glial damage or astrocytic reactions to neural injury, referred to as reactive astrogliosis, which might have beneficial or detrimental physiological purposes (Herrmann et al. 2003, Kleindienst et al. 2007). This has raised concerns as to whether the serum levels of this protein actually correlate with the degree of brain damage or are more reflective of other processes. Finally, because of the high normative values in the pediatric population, S100B is not a useful marker in children (Piazza et al. 2007, Berger et al. 2006).

3.2.3.2 Glial fibrillary acidic protein

Glial fibrillary acidic protein (GFAP), a monomeric intermediate filament protein found in the astroglial skeleton, is not found outside the central nervous system and is thus considered a marker worth focusing upon in the search for brain specificity (Vos et al. 2004, Missler et al. 1999). In addition GFAP can be measured in peripheral blood (van Geel et al. 2002, Missler et al. 1999).

Recent evidence indicates elevates serum GFAP levels in various types of brain damage, ranging from neurodegerative disorders (Baydas et al. 2003) and stroke (Herrmann et al. 2000) to traumatic brain injury (TBI) (Vos et al. 2004, Nylen et al. 2006, Pelinka et al. 2004). Clinical studies have proposed that the serum GFAP level is a reliable marker of primary brain damage after TBI with the further advantage that it is not released in situations of multiple traumas without brain injury (Vos et al. 2004, Pelinka et al. 2004). GFAP has also been demonstrated to be a potential useful biomarker to predict clinical outcome. Recently, Vos and colleagues showed a high specificity of GFAP in predicting death or unfavorable outcome at 6 months (0.93– 0.95), with a falsepositive rate for unfavorable outcome below 5% (Vos et al. 2010).

4. Assay development and hand-held devices

A variety of different analytical assay formats have been developed to measure biomarkers in biofluids such as cerebrospinal fluid (CSF) and plasma and serum (Mondello et al. 2011).

The most commonly used format is a two-sided enzyme-linked sandwich immunoassay (ELISA). This format is based on the coating of a solid surface in a plate to capture the biomarker/analyte of interest, followed by incubation with a directly or indirectly-labeled labeled detection antibody. In the ELISA, this label is of enzyme-based, but there are a variety of different surface structures, labels and detection methods available. These include fluorescence, chemiluminescence, optical (absorbance) and electro-chemiluminescence.

Key elements in the consideration of the assay format are the analytical performance of the assay in terms of sensitivity and specificity, which assessed as specific assay parameters. The importance of these assay parameters and their assessment can be found in a recent paper by Mondello et al. (Mondello et al. 2011). Over the past few years, a practical iterative approach "fit-for-purpose" approach to biomarker assay development and validation has evolved, which centers around the "intended use" of the assay and the regulatory requirements associated with it (Lee et al. 2006). Assays "evolve" through different stages from the original assay development phase through an analytical qualification and validation phase. The underlying concept here is to fully understand the analytical performance of these assays for their intended use, before testing on clinical samples. Laboratories performing testing on human specimen for diagnosis, prevention or treatment of any disease are certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988 or have similar accreditation outside the US. These standard practices frequently required for CLIA certification were published by the Clinical and Laboratory Standard Institute (CLSI). In May 2001, the Food and Drug Administration (FDA) issued guidance for industry for bioanalytical method validation of assay to support pharmacokinetic (PK) assessment of small molecule drugs (Guidance for Industry on Bioanalytical Method Validation: availability. Fed. Regist. 66, 28526-28527, 2001).

In view of the diverse of biomarker assays for either PK , drug development or diagnostic purposes, neither the FDA bioanalytical validation nor the CLSI guidelines fully meet the needs of drug development and diagnostic applications of biomarker assays, which has resulted in additional recommendations for example by the American Association of Pharmaceutical Scientists (AAPS). A more comprehensive overview can be found in Lee et al, 2006. While it is important to develop "fit-for purpose" biomarker assays and analytically define and understand their performance, this procedure should be mirrored in the standardized collection of biofluids to be analyzed. For example, the Alzheimer Disease Neuroimaging initiative (ADNI) has identified the crucial parameters in the collection and storage primarily of CSF, which has resulted in the formulation of standard operating procedures (SOPs) and the quality-control assessment of collected samples. This has also led to the formation of a global CSF consortium, which based on the availability of biofluids such as CSF collected under standardized conditions allowed the identification of the variability when performing biomarker assessment at multiple sites (Mattson et al. 2011).

The translation of a "fit-for-purpose assay into a clinically useful and commercially viable diagnostic product requires the transfer of such an assay onto an appropriate platform, suitable for its intended use. While the laboratory assays described above are typically performed in a clinical reference laboratory setting over a course of hours, it is desirable to perform the measurement of acute biomarkers of brain injury in point-of-care (POC) type setting, outside the clinical laboratory. This includes the accident site, emergency room, sidelines of a sports field and military field. Rather than performing the biomarker assay in a benchtop multiwell plate format, in these setting a smaller and portable POC system is desirable, which requires little to no sample preparation and can performed as a rapid

diagnostic test in 30 min or less. In the battle field, these requirements are even more stringent in that the assay system should be carried around as handheld device, simple and lightweight and withstand extreme environments.

Over the last couple of years a variety of different POC platforms have been developed (Price & Kricka 2007). These POC assay platform comprise different design concepts and detection technologies based on their intended use. Glucose testing in diabetic patients is one of successful examples of POC test. The first blood glucose measurements were carried out with small paper strips invented and introduced by Ames in 1965. A measurement if performed by adding a drop of blood to a strip wherein the reaction of glucose in the blood results in the development of blue color and is compared to a color chart for analysis.

For tests, which do not require quantification, but simply detect the presence of a biomarker such as pregnancy test, a lateral flow-strip design might be sufficient. These are membrane strips, through which a small volume of sample liquid is transported by capillary action. These lateral flow strips are based on the binding of labels in the presence of biomarker through an immunochemical reaction, typically, small gold particles.

The assessment of biomarkers in the POC or handheld device format might require a more semi-quantitative assessment and here solutions have been developed, which mimic several of the assay steps a central laboratory test in a miniaturized format. One of the earliest examples is the i-STATR system (Abbott Laboratories, Abbott Park, IL) for measuring electrolyte, coagulation, glucose, cardiac markers such as troponin, and other tests. Wet assay reagents are stored in a cartridge and are actively pumped by the device through mechanical displacement to perform the various steps of the assays. Other examples such as AtolyzerR, originally developed by Atonomics, and the Roche cobas h232R exist and many more new technologies are being developed. A modification is the Philips MagnotechR system, which replace the liquid manipulation steps by magnetically controlled movement. The implementation of molecular-diagnostic-based POCs also shows promise for the future. These assays typically include a polymerase-chain reaction-based amplification step.

All these implementations of the POC tests in a miniaturized cartridge-type format are designed to allow a high level of assay control. The overall assay performance is influenced by minor variation for example in timing of the individual assay steps and can results in large overall assay performance variability. The successful adaptation of any biomarker assay to a POC/handheld device is dependent on the requirement for sensitivity and precision in its intended use. The development of new POC technologies and solutions shows great promise for the adaptation of CNS biomarker assays in unmet medical needs.

5. Strategy for regulatory approval by FDA

Biomarkers, whether proteomic based or based on more traditional technologies, can be used for a variety of purposes. Generally speaking, the term 'biomarker' describes any measurable diagnostic indicator used to assess the presence or risk of disease. For the US Food and Drug Administration (FDA), the term 'diagnostic' includes the diagnosis, screening and prediction of disease. But it also encompass other uses such as staging or the prognosis of disease, monitoring patients and the effectiveness of a treatment and/or optimizing treatment outcomes by aiding health-care providers in medical and therapeutic decision making.

The FDA has been involved in the regulation of medical devices, including in vitro diagnostic devices (IVDs) since 1976 when the US Congress established laws for the

regulation of medical devices under the Medical Device Amendments act (REF. 8). According to the Medical Device Amendments act, for an in vitro diagnostic (IVD) device to enter the US market it must comply with a set of rules and regulations in order to prove safety and effectiveness for its intended use. In 21 CFR 860.7(d)(1) device safety requires "the probable benefit to health from use of device for its intended use and conditions of use … outweigh any probable risk." In 21 CFR 860.7(e)(1) device effectiveness requires "that in a significant portion of the target population, the use of the device for its intended use and conditions for use … will provide clinically significant results." In addition, a new assay is required to demonstrate an adequate analytical performance (appropriate accuracy and precision) and clinical performance (sensitivity, specificity and some indication of clinical utility) (21 CFR 807; 21 CFR 814).

In order to be commercialized in a kit, newly discovered biomarkers device must follow specific pathways. Investigational studies of diagnostic devices can be performed with various design configurations, but they must conform to FDA requirements. The researchers are required to apply for an investigational device exemption (IDE) before initiating the study, as described in Title 21 of the Code of Federal Regulations 812 (21 CFR 812)(US Food and Drugs Administration [FDA], 2006). The IDE submission should describe the nature of the proposed study, include details of informed consent and ensure patient protection and that the risks associated with participation in the study will be clearly communicated to individuals.

After the appropriate investigational studies have been completed, the FDA requires premarket submissions before a test can be approved for clinical use in the United States. Depending on the nature of the test and its classification, the product could be reviewed as a 510(k) premarketing clearance with a 90-day timeline or Premarket Approval (PMA) with a 180-day timeline (21 CFR 807; 21 CFR 814). FDA considers three classes of devices (class I, II, or III) (FDA, 2009, 2010).

The 510(k) process is used when the new test measures an existing FDA classified analyte (class I or II) where there exists a commercially available predicate test method that has been cleared by the FDA or that was in commercial distribution before May 28, 1976 (21 CFR 860.84). Premarket clearance requires the sponsor to provide information for the new product including its intended use and classification and 'substantially equivalence' to the predicate device. This ensures that a high level of safety and effectiveness is maintained. In addition the sponsor must show characterization of analytical capability of the test (e.g., specificity and accuracy, precision and linearity by correlating patient studies against the predicate device) (http://www. fda.gov/ oc/mdufma/coversheet.html).

The PMA process is used when the test is classified as class III; that is, either it is associated with high risk (e.g., when the outcome determines cancer treatment or diagnosis) or the clinical utility of the marker or the technology of the measurement are novel and no predicate device can be identified (21 CFR 814). FDA requires the sponsor to submit the same data required for 510(k) as well as clinical outcomes data, where the level of the marker is related to disease status defined by clinical criteria (http://www.fda.gov/ oc/mdufma/coversheet.html).

In 1997, since some new biomarkers have no obvious predicate devices and do not have safety concerns, FDA created a new hybrid 'de novo' or 'risk-based' classification (21 CFR 814). This process allows a new biomarker to be regulated as in a 510(k), but requires the demonstration of clinical effectiveness.

Therefore, in the absence of a predicate device and depending on the intended use and clinical utility, either a PMA or a 'de novo' process will be required for a new protein of interest before it achieves commercial availability in a kit or device.

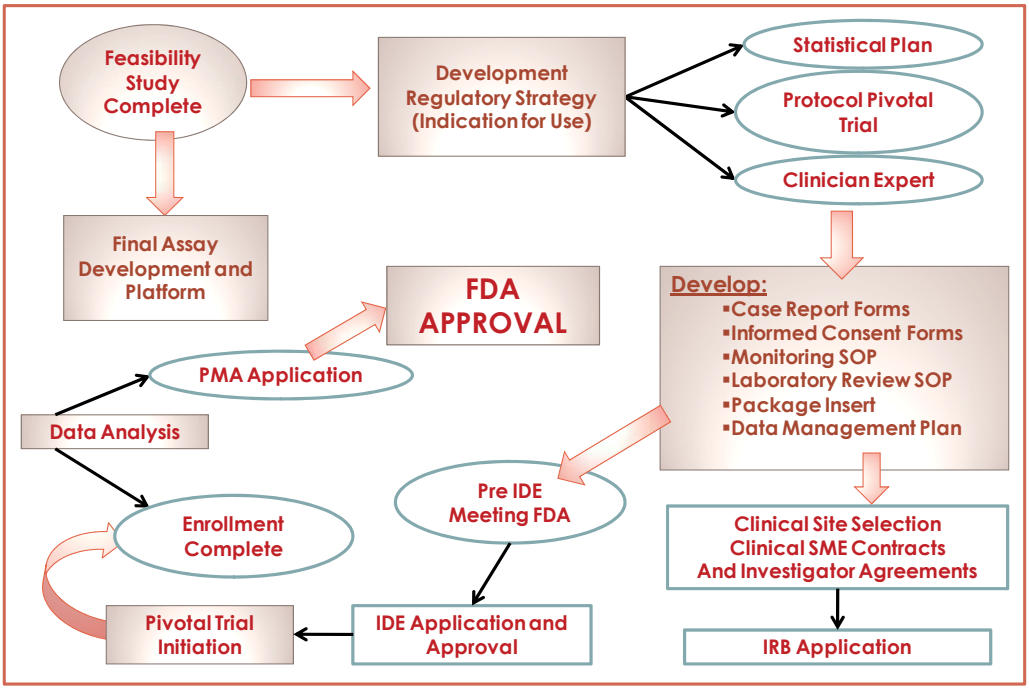


Fig. 4. Regulatory Pathway for PMA Application

6. Conclusion

This chapter provides an introductory review of techniques that have been applied to biomarker identification and clinical validation. Demonstration of clinical utility and compliance with regulatory requirements is critical for the commercialization of novel biochemical markers but also formidable, uncertain and costly.

At present, there are still many unanswered questions in this area of research, however, such as the best statistical methods to analyze the large volume of data generated, the role of potential demographic and clinical confounders, such as age, sex, and on the other hand the broad and complex spectrum of types and severities of brain injuries. In addition, issues such as sample integrity and preservation, normalization, and appropriate control data also must be given careful consideration. A multimarker strategy will probably be needed to provide a greatly expanded approach to the detection of brain injury, elucidating its pathogenesis and making it possible to guide and monitor the therapy in new ways and ultimately to improve outcome.

7. References

Albert, A. (1982). On the use and computation of likelihood ratios in clinical chemistry. *Clin Chem*, Vol.28, pp.1113–1119

Altman, D.G. Practical Statistics for Medical Research (Chapman & Hall, London, UK, 1991)

Anderson, R.E.H.L.; Nilsson, O.; Dijai-Merzoug, R. & Settergen, G. (2001). High serum S100B levels for trauma patients without head injuries. *Neurosurgery,* Vol.49, pp.1272–1273

Anderson, K.J.; Scheff, S.W.; Miller, K.M. et al. (2008). The phosphorylated axonal form of the neurofilament subunit NF-H (pNF-H). as a blood biomarker of traumatic brain injury. *J Neurotrauma,* Vol.25, pp.1079–1085

Beltrao, P.; Kiel, C. & Serrano, L. (2007). Structures in systems biology. *Curr Opin Struct Biol*, Vol.17, pp.378-384

Baydas, G.; Nedzvetskii, V.S.; Tuzcu, M. et al. (2003). Increase of glial fibrillary acidic protein and S-100B in hippocampus and cortex of diabetic rats: effects of vitamin E. *Eur J Pharmacol*, Vol.462, pp.67–71

Berger, R.P.; Dulani, T.; Adelson, P.D. et al. (2006). Identification of brain injury in well appearing infants using serum and cerebrospinal markers: A possible screening tool. *Pediatrics,* Vol.117, pp.325–332

Biberthaler, P.; Linsenmeier, U.; Pfeifer, K.J. et al. (2006). Serum S-100B concentration provides additional information for the indication of computed tomography in patients after minor head injury: a prospective multicenter study. *Shock,* Vol.25, No.5, pp.446–453

Blyth, B.J.; Farahvar, A.; He H.; et al. (2011). Elevated Serum Ubiquitin Carboxy-Terminal Hydrolase L1 Is Associated with Abnormal Blood-Brain Barrier Function after Traumatic Brain Injury. *J Neurotrauma*, [Epub ahead of print]

Bossuyt, P.M.; Reitsma, J.B.; Bruns, D.E. et al. (2003). The STARD statement for reporting studies of diagnostic accuracy: explanation and elaboration. *Clin Chem,* Vol.49, pp.7-18

Boylan, K.; Yang, C.; Crook, J. et al. (2009). Immunoreactivity of the phosphorylated axonal neurofilament H subunit (pNF-H). in blood of ALS model rodents and ALS patients: evaluation of blood pNF-H as a potential ALS biomarker. *J Neurochem*, Vol.111, pp.1182–1191

Brophy, G.M. ; Mondello, S.; Papa, L. et al. (2011). Biokinetic analysis of ubiquitin C-terminal hydrolase-L1 (UCH-L1). in severe traumatic brain injury patient biofluids. *J Neurotrauma*, Vol.28, No.6, pp.861-70

Blennow, K. (2010). Biomarkers in Alzheimer's disease drug development. *Nat Med.* Vol.16, No.11, pp. 1218-22

Burgess, J. A.; Lescuyer, P.; Hainard, A. et al. (2006). Identification of brain cell death associated proteins in human post-mortem cerebrospinal fluid. *J Proteome Res*, Vol.5, pp. 1674-1681

Celis, J. E.; Gromov, P.; Cabezon, T. et al. (2004). Proteomic characterization of the interstitial fluid perfusing the breast tumor microenvironment: a novel resource for biomarker and therapeutic target discovery. *Mol Cell Proteomics*, Vol.3, pp.327-344

Chen, S. S. ; Haskins, W. E. ; Ottens, A. K. et al. (2007). Bioinformatics for traumatic brain injury: Proteimic Data Mining. *Data Mining in Biomedicine.* Ed. Panos Pardalos, Boginski, V.L., Vazacopoulos, A. Springer, pp. 1-26

Code Federal Regulations, vol. 21 CFR807, Available from http://frwebgate.access.gpo.gov/cgi-bin/getcfr.cgi?YEAR=current&TITLE=21&PART=807&SECTION=81&SUBPART= &TYPE=TEXT

Code Federal Regulations, vol. 21 CFR814, Available from http://frwebgate.access.gpo.gov/cgi-bin/getcfr.cgi?YEAR=current&TITLE=21&PART=814&SECTION=1&SUBPART= &TYPE=TEXT)

Davidsson, P. & Sjogren, M. (2005) The use of proteomics in biomarker discovery in neurodegenerative diseases. *Dis Markers*, Vol.21, pp.81-92

Denslow, N.; Michel, M.E.; Temple, M.D. et al. (2003) Application of proteomics technology to the field of neurotrauma. *J Neurotrauma*, Vol.20, pp.401-407

Ding, Q.; Wu, Z.; Guo, Y. et al. (2006) Proteome analysis of up-regulated proteins in the rat spinal cord induced by transection injury. *Proteomics*, Vol.6, pp.505-518

Donato, R. (2001). S100: a multigenic family of calcium-modulated proteins of the EF-hand type with intracellular and extracellular functional roles. *Int J Biochem Cell Biol*, Vol.33, No.7, pp.637– 668

Douglas-Escobar, M.; Yang, C.; Bennett, J. et al. (2010). A pilot study of novel biomarkers in neonates with hypoxic-ischemic encephalopathy. *Pediatr Res*, Vol.68, No. 6, pp.531-6

Dumont, D. ; Noben, J. P.; Raus, J. et al. (2004) Proteomic analysis of cerebrospinal fluid from multiple sclerosis patients. *Proteomics*, Vol.4, pp.2117-2124

Etzioni, R; Urban, N; Ramsey, S. et al. (2003) The case for early detection. Nat Rev Cancer. Vol.3, No.4, pp. 243-52

Fenn, J. B.; Mann, M.; Meng, C. K.; Wong, S. F. and Whitehouse, C. M. (1989) Electrospray ionization for mass spectrometry of large biomolecules. *Science*, Vol. 246, pp. 64-71

Food and Drugs Administration. Electronic code of federal regulations. US Food and Drugs Administration [online], Available from http://ecfr.gpoaccess.gov/cgi/t/text/textidx?c=ecfr&tpl=/ecfrbrowse/Title21/21cfr812_main_ 02.tpl (2006)

Food and Drug Administration. 21 USC 360 c (a)(1). Classifi cation of devices intended for human use; device classes, Available from http://www.fda.gov/RegulatoryInformation/Legislation/FederalFoodDrugandCosmeticActFDCAct/FDCActChapterVDrugsandDevices/ucm110188.htm (accessed 2010-04-27) (2009).

Food and Drug Administration. Overview of IVD regulation, Available from http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/IVDRegulatoryAssistance/ucm123682.htm#4b: (accessed 2010-04-27). Within section "Medical devices" (2010)

Ganesalingam, J.; An, J.; Shaw, C.E. et al. (2011). Combination of neurofilament heavy chain and complement C3 as CSF biomarkers for ALS. *J Neurochem*, Vol.117, No.3, pp.:528-37

Goncalves, C.A.; Leite, M.C. & Nardin, P. (2008). Biological and methodological features of the measurement of S100B, a putative marker of brain injury [published online ahead of print April 18, 2008]. *Clin Biochem*, Vol.41, No.10/11, pp.755–763

Grant, S. G. (2003). Systems biology in neuroscience: bridging genes to cognition. *Curr Opin Neurobiol*, Vol.13, pp.577-582

Grant, S. G. & Blackstock, W. P. (2001). Proteomics in neuroscience: from protein to network. *J Neurosci*, Vol.21, pp.8315-8318

Guingab-Cagmat, J.D.; Kobeissy, F.; Ratliff, M.V. et al. (2009). Neurogenomics and Neuroproteomics Approaches of Studying Neural Injury. In: Essentials of Spinal Cord Injury, (M. Fehlings, et.al ed.).. Thieme, Toronto

Hammack, B. N.; Fung, K. Y.; Hunsucker, S. W. et al. (2004). Proteomic analysis of multiple sclerosis cerebrospinal fluid. *Mult Scler*, Vol.10, pp.245-260

Haskins, W. E.; Kobeissy, F. H.; Wolper, R.A. et al. (2005). Rapid discovery of putative protein biomarkers of traumatic brain injury by SDS-PAGE-capillary liquid chromatography-tandem mass spectrometry. *J Neurotrauma*, Vol.22, pp.629-644.

Herrmann, M.; Vos, P.E.; Wunderlich, M.T. et al. (2000). Release of glial tissue-specific proteins after acute stroke: a comparative analysis of serum concentrations of protein S-100B and glial fibrillary acidic protein. *Stroke,* Vol.31, pp.2670–2677

Herrmann, M.; Jost, S.; Kutz, S. et al. (2000). Temporal profile of release of neurobiochemical markers of brain damage after traumatic brain injury is associated with intracranial pathology as demonstrated in cranial computerized tomography. *J Neurotrauma*, Vol.17, pp.113–122

Herrmann, M. & Ehrenreich, H. (2003). Brain derived proteins as markers of acute stroke: their relation to pathophysiology, outcome prediction and neuroprotective drug monitoring. *Restor Neurol Neurosci,* Vol.21, No.3-4, pp.177-190

Ingebrigtsen, T.; Romner, B.; Kongstad, P. & Langbakk, B. (1995). Increased serum concentrations of S-100 after minor head injury. A biochemical marker with prognostic value? *J Neurol Neurosurg Psychiatry*, Vol.59, pp.103–104

Jonsson, H.; Johnsson, P.; Hoglund, P. et al. (2000). Elimination of S100B and renal function after cardiac surgery. *J Cardiothorac Vasc Anesth,* Vol.14, No.6, pp.698–701

Karas, M. & Hillenkamp, F. (1988). Laser desorption ionization of proteins with molecular masses exceeding 10,000 daltons. *Anal Chem*, Vol.60, pp.2299-2301

Katano, T.; Mabuchi, T.; Okuda-Ashitaka, E. et al. (2006). Proteomic identification of a novel isoform of collapsin response mediator protein-2 in spinal nerves peripheral to dorsal root ganglia. *Proteomics*, Vol.6, pp.6085-6094

Kleindienst, A. & Bullock R.M. (2006). A critical analysis of the role of the neurotrophic protein S100B in acute brain injury. *J Neurotrauma*, Vol.23, No.8, pp.1185-200

Kleindienst, A.; Hesse, F.; Bullock, M.R. & Buchfelder, M. (2007). The neurotrophic protein S100B: value as a marker of brain damage and possible therapeutic implications. *Prog Brain Res*. Vol.161, pp.317-325

Kobeissy, F. H.; Ottens, A. K.; Zhang, Z. et al. (2006). Novel differential neuroproteomics analysis of traumatic brain injury in rats. *Mol Cell Proteomics*, Vol.5, pp.1887-1898

Kobeissy, F. H.; Sadasivan, S.; Oli, M.W. et al. (2008). Neuroproteomics and systems biology-based discovery of protein biomarkers for traumatic brain injury and clinical validation. *Proteomics Clin Appl*, Vol.2, pp.1467-1483

Laser, H.; Mack, T.G.; Wagner, D. & Coleman, M.P. (2003). Proteasome inhibition arrests neurite outgrowth and causes "dying-back" degeneration in primary culture. *J Neurosci Res,* Vol.74, pp.906-916

Lee, J.W.; Devanarayan, V.; Barrett, Y.C. et al. (2006). Fit-for-purpose method development and validation for successful biomarker measurement. *Pharm Res,* Vol.23, No.2, pp.312-28

Lincoln, S.; Vaughan, J.; Wood, N. et al. (1999). Low frequency of pathogenic mutations in the ubiquitin carboxy-terminal hydrolase gene in familial Parkinson's disease. *Neuroreport,* Vol.10, pp.427-429

Mattsson, N.; Andreasson, U. ; Persson, S.; Arai H et al. (2011). The Alzheimer's Association external quality control program for cerebrospinal fluid biomarkers. *Alzheimers Dement*, Vol.7, No.4, pp.386-395

Missler, U.; Wiesmann, M.; Wittmann, G. et al. (1999). Measurement of glial fibrillary acidic protein in human blood: analytical method and preliminary clinical results. *Clin Chem,* Vol.45, pp.138 –141

Mondello, S.; Robicsek, S.; Gabrielli, A. et al. (2010). aII-spectrin breakdown products (SBDPs). diagnosis and outcome in severe traumatic brain injury patients. *J Neurotrauma,* Vol.27, pp.1203–1213

Mondello, S.; Muller, U.; Jeromin, A. et al. (2011). Blood-based diagnostics of traumatic brain injuries. *Expert Rev Mol Diagn,* Vol.11, No.1, pp.65-78

Nylen, K.; Ost, M.; Csajbok, L.Z. et al. (2006). Increased serum-GFAP in patients with severe traumatic brain injury is related to outcome. *J Neurol Sci,* Vol.240, pp.85–91

Nogoy, N. (2007). Neuroproteomics: the hunt for biomarkers of neurotrauma. Andrew Ottens talks to Nicole Nogoy. *Expert Rev Proteomics*, Vol.4, pp.343-345

Oertel, M.; Schumacher, U.; McArthur, D.L et al. (2005). S-100B and NSE: markers of initial impact of subarachnoid haemorrhage and their relation to vasospasm and outcome. *J Clin Neurosci*, Vol.13, pp.834–840

Ottens, A. K.; Kobeissy, F. H.; Fuller, B. F. et al. (2007). Novel neuroproteomic approaches to studying traumatic brain injury. *Prog Brain Res*, Vol.161, pp.401-418

Ottens, A. K.; Kobeissy, F. H.; Golden, E. C. et al. (2006). Neuroproteomics in neurotrauma. *Mass Spectrom Rev*, Vol.25, pp.380-408

Papa, L.; Akinyi, L.; Liu, M.C. et al. (2010). Ubiquitin C terminal hydrolase is a novel biomarker in humans for severe traumatic brain injury. *Crit Care Med*, Vol.38, pp.138-144

Pelinka, L.E.; Kroepfl, A.; Schmidhammer, R. et al. (2004). Glial fibrillary acidic protein in serum after traumatic brain injury and multiple trauma. *J Trauma,* Vol.57, pp.1006 – 1012

Piazza, O.; Cotena, S.; Esposito, G. et al. (2005). S100B is a sensitive but not specific prognostic index in comatose patients after cardiac arrest. *Minerva Chir*, Vol.60, pp.477–480

Piazza, O.; Storti, M.P.; Cotena, S. et al. (2007). S100B is not a reliable prognostic index in paediatric TBI. *Pediatr Neurosurg*, Vol.43, pp.258–264

Pineda, J.A.; Lewis, S.B.; Valadka, A.B. et al. (2007). Clinical significance of aII-spectrin breakdown products in cerebrospinal fluid after severe traumatic brain injury. *J Neurotrauma,* Vol.24, pp.354–366

Price, C.P. & Kricka, L.J. (2007). Improving healthcare accessibility through point-of-care technologies. *Clin Chem,* Vol.53, No.9, pp.1665-75

Raabe, A.; Grolms, C.; Sorge, O.; Zimmermann, M. & Seifert, V. (1999). Serum S-100B protein in severe head injury. *J Neurosurg,* Vol.45, pp.477–483

Redell, J. B.; Liu, Y. & Dash, P. K. (2009). Traumatic brain injury alters expression of hippocampal microRNAs: potential regulators of multiple pathophysiological processes. *J Neurosci Res*, Vol.87, pp.1435-1448

Rifai, N.; Gillette, M. A. & Carr, S. A. (2006). Protein biomarker discovery and validation: the long and uncertain path to clinical utility. *Nat Biotechnol*, Vol.24, pp.971-983

Riederer, B.M.; Zagon, I.S. & Goodman, S.R. (1986). Brain spectrin (240/235). and brain spectrin (240/235E): two distinct spectrin subtypes with different locations within mammalian neural cells. *J Cell Biol,* Vol.102, pp.2088–2097

Ringger, N.C.; O'Steen, B.E.; Brabham, J.G. et al. (2004). A novel marker for traumatic brain injury, CSF aII-spectrin breakdown product levels. *J Neurotrauma,* Vol.21, pp.1443–1456

Romner, B.; Ingebrigtsen, T.; Kongstad, P. & Borgesen, S.E. (2000). Traumatic brain injury: serum S-100 measurements related to neuroradiological findings. *J Neurotrauma,* Vol.17, pp.641– 647

Romner, B. & Ingebrigtsen, T. (2001). High serum S100B levels for traumapatients without head injuries. *J. Neurosurg*, Vol.49, pp.1490

Ross, S.A.; Cunningham, R.T.; Johnston, C.F & Rowlands, B.J. (1996). Neuronspecific enolase as an aid to outcome prediction in head injury. *Br J Neurosurg*, Vol.10, pp.471–476

Sackett, D.L. & Haynes, R.B. (2002). The architecture of diagnostic research. *Br Med J,* Vol.324, pp.539–541

Savola, O.; Pyhtinen, J.; Leino, T.K. et al. (2004). Effects of head and extracranial injuries on serum protein S100B levels in trauma patients. *J Trauma*, Vol.56, pp.1229 –1234

Selakovic, V.; Faicevic, R. & Radenovic, L. (2005). The increase of neuron-specific enolase in cerebrospinal fluid and plasma as a marker of neuronal damage in patients with acute brain infarction. *J Clin Neurosci*, Vol.12, pp.542– 547

Shin, B. K. ; Wang, H. & Hanash, S. (2002). Proteomics approaches to uncover the repertoire of circulating biomarkers for breast cancer. *J Mammary Gland Biol Neoplasia*, Vol.7, pp.407-413

Svetlov, S. I.; Xiang, Y.; Oli, M. W. et al. (2006). Identification and preliminary validation of novel biomarkers of acute hepatic ischaemia/reperfusion injury using dual-platform proteomic/degradomic approaches. *Biomarkers*, Vol.11, pp.355-369

Tanaka, K.; Waki, H.; Ido, Y. et al. (1988). Protein and polymer analysis analyses up to m/z 10000 by laser ionization time-of-flight mass spectrometry. *Rapid Commun Mass Spectrom*, Vol.2, pp.3

Vitzthum, F.; Behrens, F.; Anderson, N.L. & Shaw, J.H. (2006). Proteomics: from basic research to diagnostic application. A review of requirements & needs. *J Proteome Res,* Vol.4, No.4, pp.1086-97

van Geel, W.J.A.; de Reus, H.P.M.; Nijzing, H. et al. (2002). Measurement of glial fibrillary acidic protein in blood: an analytical method. *Clin Chim Acta,* Vol.326, pp.151–154

Vos, P.E.; Lamers, K.J.; Hendriks, J.C. et al. (2004). Glial and neuronal proteins in serum predict outcome after severe traumatic brain injury. *Neurology,* Vol.62, pp.1303–1310

Wang, K. K.; Larner, S. F.; Robinson, G. & Hayes, R. L. Neuroprotection targets after traumatic brain injury. *Curr Opin Neurol*, Vol.19, pp.514-519

Wang, K. K. ; Ottens, A. K.; Liu, M. C. et al. (2005). Proteomic identification of biomarkers of traumatic brain injury. *Expert Rev Proteomics*, Vol.2, pp.603-614

Zweig, M.H. & Campbell, G. (1993). Receiver-operating characteristic (ROC). plots: a fundamental evaluation tool in clinical medicine. *Clin Chem*, Vol.39, pp.561–577

23

Decompressive Craniectomy: Surgical Indications, Clinical Considerations and Rationale

Dare Adewumi and Austin Colohan
Loma Linda University Medical Center Department of Neurosurgery
Loma Linda
USA

"If there's no CSF pressure, but brain pressure exists, then pressure relief must be achieved by opening the skull" -Kocher 1901

1. Introduction

The management of increased intracranial pressure is a common clinical scenario encountered in a large portion of medicine. It is encountered more often in the practices of trauma, neurology and neurological surgery and as such is very common in intensive care settings. Treatment approaches remain one of the more controversial fields in medicine. In this chapter we will address the treatment approaches for elevated intracranial pressure. Indications for assessment, clinical considerations and approaches to proper management of elevated pressures focusing mainly on the decompressive craniectomy. We will address the role of the decompressive craniectomy in trauma stroke and address the option of lumbar drainage.

2. Intracranial pressure

Intracranial pressure is defined as the pressure within the cerebral vault that the systemic perfusion must overcome in order to adequately perfuse the brain. A mathematical expression of this relationship is that of CPP (Cerebral Perfusion Pressure) = MAP (Mean Arterial Pressure) - ICP (Intracranial Pressure). This relationship predicts that under settings where intracranial pressure approaches or surpasses mean arterial pressure the perfusion pressure declines resulting in hypoxic brain injury. The Monro-Kellie doctrine is often highlighted in illustrations of intracranial pressure. It holds that the adult cranial vault is a rigid structure composed of brain parenchyma, cerebrospinal fluid, and blood. The cranial vault being a rigid bony structure offers very little compliance and is not easily subject to shifts in space composition. As such, expansion in any of these compartments results in an increase in the amount of pressure contained within the rigid vault. There are inherent safeguards that allow for an element of expansion without excessive effects on the intracranial pressure. However once a certain a threshold is surpassed further expansion

results in marked elevation in intracranial pressure. As intracranial pressure rises beyond adequate perfusion, the resultant effect is reduced perfusion and oxygen availability to the cerebrum resulting in hypoxic injury and cell death. The autolytic cascade leads to an increase in cell volume as the cell membrane is unable to maintain the electrochemical gradient due to a break down of the ATP dependent Na/K ATPase pump. Water and calcium influx leads to the expansion in the cell volume and a feed-forward positive cycle where cell death causes more edema and swelling, which causes increased intracranial pressure, which in turn causes less brain perfusion and results in further cell death. CSF flow through normal pathways can also be obstructed resulting in further elevation in intracranial pressure. The end result is often herniation as pressure becomes greater than the resistance of surrounding structures and brain structures actively transgress the boundaries established by dura mater and bone. There exist several ways to manage the complication of elevated intracranial pressure. Most of these are subdivided into a step wise systematic approach. One of such divides ICP management into two categories. Category 1 involves volume reduction. This utilizes the principles of the Monroe-Kellie doctrine. Removal of volume either in the form of CSF drainage or hyperosmotic diuresis will allow for more expansion of the edematous parenchyma. Category 2 describes the removal of mechanical constraints. This category primarily relies on the decompressive craniectomy. Removal of the rigid limitations preventing brain expansion would allow for more swelling thereby limiting and or preventing herniation (Scmidek H 2006). The AANS guidelines approach the management of elevated ICP similarly. The treatment paradigm is divided into 2 Tiers. Tier 1 involves the use of sedatives, paralytics, ventricular drainage, hyperosmolar therapy and mild hyperventilation. Tier 2 involves hypothermia, longer acting sedatives such as barbiturates, lumbar drainage via an intrathecal catheter and surgical decompression of the cranial vault. The decompressive craniectomy as we will discuss later allows for an outward herniation of brain structures as the limitations of the cranial vault are expanded theoretically minimizing any further injury the brain tissue would suffer by being forced into other compartments.

3. Management of elevated intracranial pressure

In this chapter we will focus primarily on the management of intracranial pressure in patients suffering from either stroke or traumatic brain injury. There remain other clinical scenarios where patients could suffer from elevated intracranial pressure. They include but are not limited to: infection, either meningeal or encephalitic, spontaneous intracerebral hemorrhage, and aneurysmal subarachnoid hemorrhage. Although decompressive craniectomy may play a role in these settings, it is beyond the scope of this chapter. The application of decompressive craniectomy in the posterior fossa will also not be addressed in this chapter.

Various cutoff values are used at different centers above which treatment of elevated intracranial pressure is indicated. Levels of 15, 20 and 25 are quoted but most centers use ICP > 20-25mmHg as the upper limit (Bullock 1995). There is a high mortality and even higher morbidity in patients with ICP persistently above 20mmHg. The physician's clinical discretion remains paramount in these settings. A 25 year-old trauma victim with an ICP of 30 but is awake, not intubated and following commands briskly is not as concerning as a 45 year-old with an ICP of 20 but has become acutely unresponsive. Several measurements can

be employed in the management of elevated intracranial pressure. 1. Ensure adequate patient head positioning. The position of the patient's head can either optimize or hinder venous drainage. Venous congestion can lead to increased intracranial pressure. 2. Optimize the patient's vitals, specifically avoiding hypercarbia and hyperthermia. 3. Osmotic diuretics such as mannitol and hypertonic saline can also be utilized. In settings where adequate control of elevated ICP remains difficult, another option that can be employed is lumbar drainage. Lumbar drainage plays a role after ventriculostomy drainage has proven insufficient. At our institution we ensure patency of the ventriculostomy catheter with adequate drainage. If ICP remains elevated at this point a lumbar drain is placed. Initially the lumbar drain is left clamped and in most cases ICP control improves. However in situations where ICP elevation persists the lumbar drain is unclamped and placed at the same level as the ventriculostomy drain to minimize risk of brainstem herniation. The placement of lumbar drain plays a very valuable role in the reduction of elevated intracranial pressure (Munch E 2001; Murad A 2008).

4. Assessment of the trauma patient

The initial assessment of the trauma patient often begins in the emergency department. The basics must not be forgotten. Obtain a thorough history paying particular attention to mechanism of injury. Enough emphasis cannot be placed on obtaining a good overview of the situation. Even in the presence of an experienced trauma team the alert neurosurgeon can still play an additive role in helping to ensure that all bases are covered. A trauma team focused on placing a chest tube may not initially notice a lack of movement in a patient's lower extremities. The alert neurosurgeon would function well to inform the trauma team of this finding and help facilitate the appropriate imaging modality needed. Pay attention to the patient as a whole without focusing on the brain alone. In every trauma setting ensure that the ABC's of trauma (airway, breathing and circulation) are addressed before moving on to secondary surveys. A thorough physical and neurologic exam is always paramount. Initial head CT is indicated in settings where there are any moderate or high risk factors including but not limited to unresponsiveness, amnesia, altered mental status, deteriorating mental status (including intoxication), signs of calvarial fracture, focal neurologic deficits, penetrating skull injury, progressive headache, posttraumatic seizure, unreliable or inadequate history, multi-system trauma, severe facial injury, and significant subgaleal swelling (Stein S C 1992). Follow up Head CT remains at the clinical discretion of the physician and in the context of the nature of the presenting injury. A non-surgical epidural hematoma may require earlier follow up head CT than a small focal contusion. There exist practice guidelines and indications for ICP monitoring in the trauma setting. For salvageable patients with severe traumatic brain injury (GCS<8 after cardiopulmonary resuscitation), level II evidence dictates a need for ICP monitoring in patients with an abnormal Head CT. Level III evidence states a need for ICP monitoring in patients with a normal Head CT but with risk factors of elevated intracranial pressure, such as: age > 40 years, SBP < 90 mmHg, flexor or extensor posturing(Bullock 1995). However the final decision remains at the clinical discretion of the physician. Radiographic information can be used as an adjunct in determining a need for ICP monitoring but should not be used as a sole determinant. In one study 13% of patients with a normal CT scan will have elevated ICP, however patients with a normal CT and risk factors for elevated ICP have a 60% chance of elevated ICP (Narayan R

K 1082) . Decompressive craniectomy in trauma unlike in stroke remains controversial. In animal studies with artificially induced intracranial lesions, craniectomy has been linked to increased cerebral edema hemorrhagic infarcts and cortical necrosis (Moody R 1968; Cooper P 1979; Forsting M 1995). However decreased intracranial pressure, improved cerebral perfusion pressure and increased oxygen tension are also reported following craniectomy in trauma (Moody R 1968; Burkert W 1988). A review of more than 30 articles failed to demonstrate a clear benefit for craniectomy in the setting of trauma (Munch E 2000). A definitive answer to benefit of decompressive craniectomy still requires definitive randomized trials. At this point there does appear to be benefit especially in young patients with GCS >4.

Until the recently published DECRA trial (Cooper D 2011) no class I evidence supporting or disputing decompressive craniectomy in trauma existed. All previous evidence is class III at best. One of the more cited articles supporting decompressive craniectomy is a study designed at the University of Virginia (Polin 1997). In this study 35 bifrontal decompressive craniectomies were performed on patients with post-traumatic edema but no mass lesions between 1984 and 1993. The results were matched to control patients from the Traumatic Coma Data Bank. Their results showed good recovery and moderate disability in 37% of patients with mortality in 23% of patients as opposed to 16% moderate disability and 34% mortality in the Traumatic Coma Data Bank control. Post operative ICP control was better than the preoperative group and the control group. The study also found that patients with ICP>40 who underwent surgery >48hrs after time of injury did poorly.

A separate study published by the University of Maryland in Baltimore(Aarabi B 2006) discussed the findings of decompressive craniectomies performed between 2000-2004. 50 decompressive craniectomies were performed to control elevated ICP. 10 of these were performed before ICP elevation and 40 performed after ICP elevation. They found that decompressive craniectomies lowered the ICP to lower than 20 in 85% of patients. 14 of 50 patients died, 16 remained in a vegetative or severely disabled state, 20 had what was defined as a good outcome. Decompressive craniectomy was associated with a better than expected functional outcome in patients with medically uncontrollable ICP and or brain herniation compared with outcomes in other control cohorts reported in the literature.

A study out of Italy (Chibbaro S 2007) retrospectively reviewed 48 decompressive craniectomies and compared their outcomes to the Traumatic Coma Data Bank. They found that decompressive craniectomy reduced the midline shift and ameliorated basal cistern effacement. Younger patients (mean age of 31 years) had a better outcome. They also found that patients with early surgery, less than 16hrs of injury had, a better outcome than late intervention.

The DECRA trial as described earlier remains the only Class I evidence to date. The trial ran from December 2002 to April 2010. Eligibility criteria for inclusion in the study were patients between 15 and 59 years, patients with severe non-penetrating traumatic brain and patients with a GCS of 3 to 8. They excluded patients with dilated un-reactive pupils and those with mass lesions, including, but not limited to subdural and epidural hematomas. All patients had ICP monitoring and were treated medically if ICP was greater than 20. They defined early refractory ICP as a spontaneous elevation for more than 15 minutes continuously or intermittently within a 1 hour period despite first tier intervention. Patients were randomized within 72 hours to either surgery or standard care. The bifrontoparietal craniectomy technique used was based on the bifrontal craniectomy technique described by

Polin (Polin 1997). They randomly assigned 155 adults with severe traumatic brain injury and ICP refractory to first tier therapy to undergo either bifrontotemporoparietal decompressive craniectomy or standard medical care. 73 patients were enrolled in the surgical arm and 82 patients were enrolled in the medical arm. Patients in the the surgical group were found to have less time with elevated intracranial pressure, required fewer interventions for increased ICP post op and spent fewer days in the ICU. However patients undergoing craniectomy were found to have a worse outcome on the Glasgow outcome scale than those receiving standard care. The rates of death at 6 months were similar, 19% in the surgical arm and 18% in the medical arm.

The DECRA trial came under a large amount of criticism primarily related to the study design. The greatest word of caution arises in defining refractory ICP elevation has having reached levels greater than 20 for 15 minutes continuously or intermittently. Most neurosurgeons, neurologists and neurointensivists would address this as being too low a threshold for aggressive management of raised ICP. Some centers advocate the use of hyperosmotics such as Mannitol as a drip over 30 minutes instead of a bolus. Although "time is brain", operating on a patient based on an ICP elevation of 15 minutes can be seen as being very rapid and somewhat rash. Another criticism lies in the screening of 3,478 patients over a 7 year period to only enroll 155 patients. Finally the exclusion of patients with mass lesions is also a concern. Several of the patients involved in the DECRA trial with no mass lesion might fall into a category of diffuse axonal injury that did not require and would not be expected to benefit from a surgical decompression.

The RESCUEicp trial is a currently ongoing international prospective multi-center randomized controlled trial comparing the efficacy of decompressive craniectomy versus optimal medical management for the treatment of refractory intracranial hypertension following brain trauma. This study differs from the DECRA trial in terms of ICP threshold (25 vs 20 mmHg), timing of surgery (any time after injury vs within 72 hours post injury) and the acceptance of mass lesions. The RESCUEicp also holds a longer follow up period, 2 years as opposed to 6 months with the DECRA trial and a larger patient population with a goal of 400 (300 enrolled as of April 2011).

5. Assessment of the stroke patient

Decompressive craniectomy plays a vital role in patients diagnosed with malignant cerebral infarction. Malignant cerebral infarction refers to large territorial parenchymal strokes with ischemic edema and associated herniation. They typically involve occlusion of the MCA or ICA distribution causing infarction of the supplied territory. They account for approximately 15% of all strokes but mortality ranges from 50-80%. Patients typically present with rapid neurologic deterioration , gaze preference towards the infarcted hemisphere, contralateral hemiplegia and progressive decline in level of consciousness. Patients can rapidly progress from a state of being awake, alert, oriented and following commands to an obtunded state. The validity of decompressive craniectomy in stroke patients has been better studied and thereby better accepted in stroke patients than in trauma patients. Decompressive craniectomy in stroke patients as been show to reduce mortality from 50% to as as low as 32% in non-dominant hemisphere strokes with reduction of hemiplegia and in dominant hemisphere strokes only a mild-moderate aphasia. Better results occur with earlier surgery (Carter BS 1997). In 2007, three key landmark articles were published that proposed

class I evidence supporting the use of decompressive craniectomy as valid treatment in the management of patients with malignant MCA infarction. The DECIMAL trial (Vahedi K 2007) was a study conducted in France after a poll of 47 neurology departments showed that only 2 departments were convinced of the efficacy of the decompressive craniectomy. The decompressive craniectomy in malignant MCA infarction trial was a multi center prospective randomized open but with blind evaluation of the primary end point study comparing early decompressive craniectomy versus standard medical therapy. It was conducted in 13 selected stroke centers including a stroke unit and a neurosurgery department from 2001 to 2005. Patients selected were between the ages of 18 and 55.

They included strokes as defined by an NIHSS > 16 within 24 hours of initial symptoms. The imaging criteria included head CTs that showed greater than a 50% MCA distribution involvement and MRI-DWI showing > 145 cm infarct volume. Exclusion criteria included patients with significant contralateral infarction, secondary hemorrhage of more than 50% of MCA territory pre-infarct significant disability, coagulopathy or use of tPA. For patients in the surgical arm of the study, decompressive craniectomy was performed less than 6hrs after randomization and up to 30hrs after initial onset of symptoms. 38 pts from 7 centers had been end enrolled when the study was prematurely ended (18 medical therapy, 20 surgical therapy). The study was ended due to slow recruitment of patients, a high difference in mortality between the 2 groups and to organize pooled data from the other ongoing trials, the DESTINY(Juttler E 2007) and HAMLET(Hotmeijer J 2009) trials. With the results gathered it was noted that the early decompression increased by more than half the number of patients with moderate disability and reduced by more than half the mortality rate at 1 year (mRS <3 of 50% in surgical group and 22% in medical group). The DECIMAL trial was able to conclude that in young patients (55yrs or younger) with malignant MCA infarction, early decompressive craniectomy had a great benefit on survival and led to a better functional outcome. No patients remained bedridden or had severe residual disability. Young patients had a significantly better outcome after surgery. However no patient had a complete recovery.

The DESTINY trial (Juttler E 2007)was a prospective, multi center, randomized, controlled clinical trial also based on a sequential design that used mortality after 30 days as the first end point. Although this trial was also ended prematurely it was able to show a statistically significant reduction in mortality after 32 patients had been enrolled. 15 of 17 patients randomized to the surgical group as opposed to 7 of 15 in the medical group survived after 30 days. DESTINY showed that hemicraniectomy reduces mortality in large hemispheric stroke patients. With only 32 patients enrolled the study was unable to demonstrate statistical superiority in functional outcome however the trial was terminated in light of the results of its joint analysis of the 3 European hemicraniectomy trials.

The HAMLET trial (Hotmeijer J 2009) was the third European trial and its aim was to assess the effect of decompressive surgery within 4 days of symptoms in patients with space-occupying hemispheric infarction. It showed that surgical decompression reduces fatality and poor outcome in patients with space occupying infarctions who are treated within 48hrs of stroke onset. The results however, showed no evidence that functional outcome is improved when decompression is delayed for up to 96hrs after stroke onset. They stress that the decision to operate should depend on the emphasis patients and relatives attribute to survival and dependency. Patient's lives can be saved but functional independence will either be severely impaired or completely lost.

A Meta-Analysis of the 3 randomized controlled trials found that hemicraniectomy within 48hrs after stroke onset resulted in reduced mortality and more favorable functional outcome. The trials were ongoing when the pooled analysis was planned. DESTINY and DECIMAL were stopped due to a clear reduction in mortality. HAMLET was still ongoing. The goal was to obtain results as soon as possible to avoid ongoing randomization in unnecessary and unethical situations. Indications include age <60 years. Stronger consideration in right hemisphere strokes and radiographic evidence of acute ICA or MCA infarcts with signs of impending severe brain swelling (Vahedi K 2007). 93 patients were included in the pooled analysis, (DESTINY 32pts, DECIMAL 38pts and HAMLET 23pts). More patients in the surgery group than in the medical group had mRS <4 (78% vs 24%). Also more patients in the surgical group survived than in the medical group (78% vs 29%). Interestingly certain rehab centers describe better improvement in patients with dominant hemisphere infarctions than in non-dominant hemisphere infarctions.

Perhaps this can be attributed to a retained capacity to learn activities of daily living. The most compelling argument for craniectomy in stroke patients regards the timing of surgery. This is supported by the 3 European trials as well as a retrospective study of 52 decompressions stratified by time of surgery into groups with intervention in under 6 hours from injury, intervention 6 hours after injury and no intervention. These showed mortality rates of 8%, 36% and 80% respectively. The average length of ICU stay was 12 days, 18 days and 7 days (shortened due to fatality)(Cho D 2003). A separate but similar study retrospectively stratified 63 interventions into decompression within 24 hours (early, 31 patients) and after 24 hours (late, 32 patients) of initial injury. Mortality was 16% for early decompression and 34% for late decompression. The average length of intensive care stay was 7 days for early decompression and 13 days for late decompression (Schwab S 1998). However early detection is only capable and confirmatory with adequate radiographic evidence.

MRI with DWI offers the best correlation to stroke severity and clinical outcome. The 90% sensitivity and 96% specificity of predictive value of malignant cerebral infarction with MRI-DWI are superior to the 60% and 70 to 90% specificity reported with Head CT (Arenillas J 2002; Manno E 2003). Given the limited availability of MRI either due to hospital constraints or patient co-morbidities, as well as the time restraints imposed by timely use of tPA in the setting of hyperacute stroke, the Head CT remains the most available and widely used radiographic reference tool.

At our institution we have developed a standard protocol applied to patients with malignant MCA stroke to assess their eligibility for decompressive craniectomy:

- LLUMC Protocol for Decompressive Craniectomy in Stroke
- Patients < 60 years old
- Large MCA stroke (greater than 145ml volume on DWI or greater than 50% MCA territory)
- Presentation within 48hrs of initial symptoms
- Exclude dilated un-reactive pupils
- Exclude bleeding diathesis
- Ventriculostomy placement ipsilateral to infarcted brain
- Lumbar drain if ICP remains elevated
- Cranioplasty between 6 weeks and 6 months

These parameters although not standardized nationally take into consideration the findings of other institutions and several published works. The ubiquity of factors such as age and

timing of intervention are far reaching, as exhibited by the findings of Eghwrudjakpor and Allison (Eghwrudjakpor P 2010). They describe a glasgow coma core of 8 and above, age less than 50 years and early intervention as being the most significant determinants of prognosis.

6. Surgical considerations

A wide variety of surgical techniques have been reported for decompressive craniectomy. All approaches can be performed either unilaterally or bilaterally. The bone flap can be stored in various locations, the patients abdominal subcutaneous fat, cryopreservation and in situ using the hinge craniectomy method (Ko 2007). A standard trauma flap skin incision is made with the goal of exposing the following margins: anteriorly to the superior border of the orbital roof, avoiding entry into the frontal sinus, posteriorly to at least 2cm posterior to the external meatus, medially to a point 2cm lateral to the midline to avoid the sagittal sinus and inferiorly to the floor of the middle cranial fossa (Scmidek H 2006). The temporalis muscle is reflected anteriorly. Burrholes are placed at the keyhole, the root of the zygoma and as preferred along the planned craniotomy route. A high speed drill is used for the craniotomy. The lesser wing of the sphenoid is fractured and removed to the superior orbital fissure. The dural edges can be tacked up to the skull to minimize formation of epidural hematoma. Dura can be opened in several manners but typically is done in a stellate fashion. Dura closure is not mandatory at this point and can either be left open, with mild approximation of dural leaflets or replaced with dural substitute. The decompressive craniectomy alone without durotomy reduces the intracranial pressure by 15%. A duraplasty further reduces the intracranial pressure by an additional 55% (Scmidek H 2006). At our institution we rely more on the rapid closure technique as described by Guresir et al. This technique has been found to significantly shorten operation time without increased complication rates or additional complications (Guresir E 2011). There have been incidences of CSF leak attributed to open dural leaflets but this has not been our experience. In the setting of trauma, evacuation of hematoma or contusion can proceed as indicated by the nature of the injury.

The size of the craniectomy directly correlates with degree of expansion (Gaa M 1990; Yoo D 1999). Small craniectomies are associated with further infarction and hemorrhage at the sites of the craniectomy margin. Mortality rates have also been reported as elevated in small diameter craniectomies (Wagner S 2001). This is due to the venous congestion that occurs in the herniated brain tissue as it is restricted and compressed by the bony boundary of the skull defect. Brain parenchyma herniates through the bony defect which in essence is the desired effect but compression of parenchyma adjacent to the bony boundary in a small craniectomy leads to venous congestion, venous infarction and further damage to brain tissue. This is more common in craniectomies smaller than 8cm in diameter.

Doubling the diameter of a craniectomy from 6cm to 12cm increases the decompressed brain volume from 9ml to 86ml. A lower margin of craniectomy relative to the floor of the middle fossa has also been described with improved outcomes. This can be related to the state of decompression of the mesencephalic cisterns. Compression of the basal cisterns is known to impair clinical outcome, a larger craniectomy to the base of the brainstem could minimize brain stem compression (Toutant S 1984; Munch E 2000). The state of the mesencephalic cisterns correlates greatly with the distance of the craniectomy to the temporal cranial floor. As such decompression or out-fracturing of the temporal floor after removal of the bone flap remains exceedingly important. Compression of the cisterns impairs clinical outcome and a

large craniectomy to the base of the cranium could minimize brain stem compression. A mortality rate of 77%, 39% and 22% as been described in those with absent, compressed and normal cisterns respectively. Patients presenting with a GCS of 7-8 who were expected to fair well did not recover as expected if the basal cisterns were compressed or absent in studies evaluated within the first 48hrs of admission(Toutant S 1984). As such recommendations for craniectomy size are typically in the range of 10x15cm with the lower margin extending to less than 1cm from the floor of the middle cranial fossa.

The frontal craniotomy is typically used in cases of frontal contusions or infarction. Bifrontal craniectomy is the most widely used. The surgical technique involves placing the patient supine. A bicoronal skin incision is planned posterior to the coronal suture. After incision the temporalis muscle is reflected inferiorly. Burr holes are made at the keyhole and at the root of the zygoma. Burr holes are also placed on either side of the sagittal sinus and along the planned craniectomy. Bilateral craniectomies are performed leaving a strip of bone covering the sagittal sinus.This strip of bone is then removed after freeing the sagittal sinus. A separate technique involves placing burrholes directly on the superior sagittal sinus and a burr hole at each key hole. The dura is then stripped from the bone, taking particular caution at the sinus. A bone flap is created connecting the keyhole burrholes with the most posterior burrhole at the sinus. Bilateral U shaped durotomies are created.

If the sinus is to be ligated and sacrificed, tributary veins are coagulated as they drain into the sinus. The sinus is then ligated at the most anterior margin as dictated by the craniotomy margin and posteriorly to a maximum of 1/3 of the length of the entire superior sagittal sinus. The ligated sinus can then be separated from the falx as it dives into the intercerebral hemisphere. Further consideration and care must be taken in settings of trauma with frontal skull fractures and frontal contusions. Bony structures may serve a tamponade effect on a lacerated sinus and may need to be left in place or anterior portions of the sinus may need to be sacrificed for adequate decompression. Bilateral dural openings are made into U-shaped flaps extending to the anterior portion of the sagittal sinus followed by ligation of the sinus. Some authors advocate preservation of the strip of bone overlying the sagittal sinus, others argue that sacrificing the anterior 1/3 of the sinus could lead to increased venous pressure and worsening cerebral edema (Polin 1997).

The removal of large areas of contused, infarcted or hemorrhagic theoretically further enhances the decompression. It also removes regions of disrupted blood brain barrier that could lead to further edema. Temporal lobectomy can be performed with removal of no more than 4-5cm of brain from the temporal tip on the dominant side and 6-7cm on the non-dominant side. Frontal lobectomy has also been described but neither has shown great therapeutic promise (Nussbaum E S 1991).

7. Recovery after hemicraniectomy

Decompressive hemicraniectomy, although life saving typically leaves survivors with severe disability. The alternative to this however being death. Most patients fail to ever re-attain functional independence. Patients typically have their bone flap replaced 6 weeks to 6 months after the initial injury to ensure resolution of the initial insult. Patients suffering from the post craniectomy syndrome may have their bone flap replaced sooner rather than latter.

Complications include but are not limited to intracerebral hematoma formation, extra-axial collections, cerebrospinal fluid leakage and cranioplasty failure. Post craniectomy hematoma formation primarily occurs due to inadequate surgical hemostasis or rupture of

friable vessels as herniating brain tissue is compressed along the craniotomy margin. This is seen more often in small hemicraniectomies (Wagner S 2001).. The vast majority of these are clinically silent. Extra axial fluid accumulations occur as a result of CSF leakage through the dural leaflets or secondary to post traumatic extra axial hydrocephalus. Some patients may require a ventricular or sub-dural shunt.

CSF leak occurs in conjunction with open dura and inadequate closure of galea and skin. The incidence of this is in the range of 3% to 5% (Polin 1997; Wagner S 2001). Cranioplasty failure occurs as a result of bone flap resorption or infection. This occurs in 2% to 6% of cases. Poor graft fixation and approximation, excessive use of bone wax and a poorly vascularized or infected scalp can lead to this (Polin 1997). Patients with a compromised flap could subsequently require an acrylic bone flap. Postoperative seizure disorder are also reported in the range of 5% to 30%. The etiology of these remain unclear but can be attributed to the initial injury, resultant decompression, and a complicated hospital course.

8. Non-traumatic brain injury

In the setting of non-traumatic brain injury, the role of decompressive craniectomy remains unclear similar to traumatic brain injury. These include patients in categories including but not limited to subarachnoid hemorrhage secondary to ruptured aneurysm, intracerebral hemorrhage (ICH), and infectious processes. Kim et al (Ki-Tae Kim 2008) describe a series in which 75 patients underwent decompressive craniectomy and were analyzed retrospectively. In this group 28 patients were classified as traumatic brain injury, 24 as intracerebral hemorrhage and 23 with major infarction. Patients with a GCS score less than 8 and midline shift on head CT greater than 6mm were considered surgical candidates. Patients outcomes at 6 months revealed a mortality rate of 21.4% in TBI, 25% in ICH and 60.9% in major infarction.

Favorable outcomes defined by glasgow outcome score of 4-5 (moderate disability or better) were observed in 57.1% of patients with TBI, 50% with ICH and 30.4% with major infarction. They also describe changes in intracranial pressure that were further increased with dural opening regardless of the disease group. Although this study describes the decompressive craniectomy as being more effective in ICH and TBI patients, we would encourage the reader to bear in mind these findings relate to retrospective studies. As described earlier in the chapter there exists class I evidence supporting the use of decompressive craniectomy in major infarction or stroke patients provided certain parameters are met.

Hitchings and Delaney further describe a series of patients who underwent decompressive craniectomy for non trauma related conditions (Hitchings L 2010). They describe 54 patients who underwent 56 procedures. They noted that although intracranial pressure was reduced by the procedure. patients had long hospital stays and consumed a very significant amount of resources. Among survivors, two-thirds sustained a good outcome however most patients suffered residual deficits. They noted a mortality rate of 39%. These findings remain in concordance with the general concordance regarding decompressive craniectomies in that patients lives typically can be saved but functionality and quality of life suffer greatly.

9. Conclusion

Intracranial hypertension is a common sequelae of several illnesses and traumatic injury. Decompressive craniectomy provides an effective means of reducing and managing

intracranial pressure. Lumbar drainage is also a useful adjunct in addition. Selection criteria remains in evolution primarily in the trauma setting but at the present time it appears the best outcomes are in young otherwise healthy patients. The decision to proceed with decompressive craniectomy should take into consideration several factors including family wishes and reasonable expectations of level of recovery.

10. References

Aarabi B, H. D., Ahn E, Aresco C, Scalea T, Eisenberg H (2006). "Outcome following decompressive craniectomy for malignant swelling due to severe head injury." *Journal of Neurosurgery* 104: 469-479.

Arenillas J, R. A., Molina C, et al (2002). "Prediction of early neurological deterioration using diffusion and perfusion weighted imaging in hyperacute middle cerebral artery stroke." *Stroke* 33: 2120-2197.

Bullock, R. C. R. M., Clifton G, et al (1995). Guidelines for the management of severe head injury. *The Brain Trauma Foundation*
The American Association of Neurological Surgeons
The Joint Section of Neurotrauma and Critical Care. New York,
Park Ridge, Illinois.

Burkert W, P. H. (1988). "Decompressive trephination in therapy refractory brain edema." *Zentralbl Neurochir* 50: 318-323.

Carter BS, O. C., Candia GJ, et al (1997). "One year outcome after decompressive surgery for massive nondominant hemisphere infarction." *Neurosurgery* 40: 1168-1176.

Chibbaro S, T. L. (2007). "Role of decompressive craniectomy in the management of severe head injury with refractory cerebral edema and intractable intracranial pressure, Our experience with 48 cases." *Surgical Neurology* 68: 632-638.

Cho D, C. T., Lee H (2003). "Ultra-early decompressive craniectomy for malignant middle cerebral artery infarction." *Surgical Neurology* 60: 227-232.

Cooper D, R. J., Murray L, Arabi Y, Davies A, D'Urso P, Kossman T, Ponsford J, Seppelt I, Reilly P, Wolfe R (2011). "Decompressive Craniectomy in Diffuse Traumatic Brain Injury." *The New England Journal of Medicine* 364: 1493-1502.

Cooper P, H. H., Clark W, et al (1979). "Enhancement of experimental cerebral edema after decompressive craniectomy: Implications for the management of severe head injuries." *Neurosurgery* 4: 296-300.

Eghwrudjakpor P, A. A. (2010). "Decompressive Craniectomy Following Brain Injury: Factors Important to Patient Outcome." *Libyan Journal of Medicine* 5(4620).

Forsting M, R. W., Schabitz W, et al (1995). "Decompressive Craniectomy for cerebral infarction: An experimental study in rats." *Stroke* 26: 259-264.

Gaa M, R. M., Lorenz M, et al (1990). "Traumatic brain swelling and operative decompression: A prospective investigation." *Acta Neurochirugica Supplementum* 51: 326-328.

Guresir E, V. H., Schuss P, Oszvald A, Raabe A, Seifert V, Beck J (2011). "Rapid closure technique in decompressive craniectomy." *Journal of Neurosurgery* 114: 954-960.

Hitchings L, D. A. (2010). "Decompressive Craniectomy for patients with severe non-traumatic brain injury: a retrospective cohort study." *Critical Care Resuscitation* 12: 16-23.

Hotmeijer J, K. L., Algra A, Amelink G, Van Gijn J, Van Der Worp H (2009). "Surgical decompression for space-occupying cerebral infarction (the Hemicraniectomy After Middle Cerebral Artery infarctin with Life-threatening Edema Trial [HAMLET]): a multicentre, open, randomised trial." *The Lancet* 8(4): 326-333.

Juttler E, S. S., Schiedek P, Unterberg A, Hennerici M, Woitzk J, Witte S, Ekkehart J, Hacke W (2007). "Decompressive Surgery for the Treatment of Malignant Infarction of the Middle Cerebral Artery (DESTINY)." *Stroke* 38: 2518-2525.

Ki-Tae Kim, J.-K. P., Seok-Gu Kang, Kyung-Suck Cho, Do-Sung Yoo, Dong-Kyu Jang, Pil-Woo Huh, Dal-Soo Kim (2008). "Comparison of the effect of decompressive craniecomty on different neurosurgical diseases." *Acta Neurochirurgica* 151(1): 21-30.

Ko, S. (2007). "In situ Hinge Craniectomy." *Neurosurgery* 60: 255-259.

Manno E, N. D., Fulgham J, et al (2003). "Computed tomographic determinants of neurologic deterioration in patients with large middle cerebral artery infarctions." *Mayo Clinic Proc* 78: 156-160.

Moody R, R. S., Mullan S (1968). "An evaluation of decompression in experimental head injury." *Journal of Neurosurgery* 29: 586-590.

Munch E, B. C., Horn P, Roth H, Schmiedek P, Vajkoczy P (2001). "Therapy of malignant intracranial hypertension by controlled lumbar cerebrospinal fluid drainage." *Critical Care Medicine* 29: 976-981.

Munch E, H. P., Schurer, L et al (2000). "Management of severe traumatic brain injry by decompressive craniectomy." *Neurosurgery* 47: 315-322.

Murad A, G. S., Colohan AR (2008). "Controlled lumbar drainage in medically refractory increased intracranial pressure. A safe and effective treatment " *Acta Neurochirurgica Supplementum* 102: 89-91.

Narayan R K, K. P. R. S., Becker D P, et al (1082). "Intracranial Pressure: To monitor or not to monitor?" *Journal of Neurosurgery* 56: 650-659.

Nussbaum E S, W. A. L., Sebring L, et al (1991). "Complete temporal lobectomy for surgical resucitation of patients with transtentorial herniation secondary to unilateral hemispheric swelling." *Neurosurgery* 29: 62-66.

Polin, R., Shaffrey M, Bogaev Ce, et al (1997). "Decompressive bifrontal craniectomy in the treatment of severe refractory posttraumatic cerebral edema." *Neurosurgery* 41: 84-92.

Schwab S, S. T., Aschoff A, et al (1998). "Early hemicraniectomy in patients with complete middle cerebral artery infarction." *Stroke* 29: 1888-1893.

Scmidek H, R. D., Ed. (2006). *Operative Neurosurgical Techniques Indications, Methods and Results*. Decompressive Craniectomy: Physiologic Rationale, Clinical Indications and Surgical Considerations. Philadelphia PA, Elsevier.

Stein S C, R. S. E. (1992). "Moderate head injury: A guide to initial management." *Journal of Neurosurgery* 77: 652-654.

Toutant S, K. M., Marshall L, et al (1984). "Absent of compressed basal cisterns on first CT scan: Ominous Predictors of outcome in severe head injury." *Journal of Neurosurgery* 61: 84-92.

Vahedi K, e. a. (2007). "Sequential-Design, Multicenter, Randomized, Controlled Trial of Early Decompressive Craniectomy in Malignant Middle Cerebral Artery Infarction (DECIMAL Trial)." *Stroke* 38: 2506-2517.

Vahedi K, H. J., Juettler E; et al (2007). "Early decompressive surgery in malignant infarction of the middle cerebral artery: A pooled analysis of three randomized controlled trials." *Lancet Neurology* 6: 215-222.

Wagner S, S. H., Aschoff A, et al (2001). "Suboptimum hemicraniectomy as a cause of additional cerebral lesions in patients with malignant infarction of the middle cerebral artery." *Journal of Neurosurgery* 94: 693-696.

Yoo D, K. D., Cho K et al (1999). "Ventricular pressure monitoring during bilateral decompression with dural expansion." *Journal of Neurosurgery* 91: 953-959.

24

The Role of Decompressive Craniectomy in the Management of Patients Suffering Severe Closed Head Injuries

Haralampos Gatos[1], Eftychia Z. Kapsalaki[2], Apostolos Komnos[3]
Konstantinos N. Paterakis[1] and Kostas N. Fountas[1]
[1]*Departments of Neurosurgery, University Hospital of Larissa*
School of Medicine, University of Thessaly, Larissa
[2]*Diagnostic Radiology, University Hospital of Larissa*
School of Medicine, University of Thessaly, Larissa
[3]*Department of Intensive Care Unit, General Hospital of Larissa, Larissa*
Greece

1. Introduction

Severe Traumatic Brain Injury (S-TBI) is the major cause of mortality, morbidity, and disability among people younger than 45 years old. It constitutes a major problem in developed countries, not only for the affected patients and their families but also for the society, with serious socio-economic ramifications. It cannot be overemphasized the society's burden from S-TBIs, which may exhaust even the most developed health care systems. During the last 30 years, advances in pre-hospital treatment, novel imaging modalities, intensive care monitoring improvements, rehabilitation advances, as well as better understanding of the S-TBI pathophysiology have decreased the overall mortality rate from 70-80% in 1970's to 30 % nowadays. Severe TBI is still associated with unfavorable outcome (death or severe disability) in up to 60%. Continuous efforts of the neurosurgical community focus not only on decreasing the S-TBI associated mortality, but also on improving the quality of life and the functional outcome of patients suffering S-TBIs [Danish, et al., 2009; Honeybul, et al., 2011].

It has been demonstrated by Marmarou and his colleagues [Marmarou et al., 1991] that increased intracranial pressure (ICP) is strongly associated with poor outcome in patients suffering S-TBIs, making thus intracranial hypertension the most frequent cause of death and disability. Moreover, it has been postulated that this association between increased ICP and poor outcome is linear. Frequently, the greatest challenge for a neurosurgeon treating a patient suffering a S-TBI is the management of increased ICP, which in a large number of cases overwhelms brain's ability to regulate cerebral blood flow (CBF), resulting thus to cerebral ischemia and consequently to severe disability and/or death. Elevated ICP is usually defined as an ICP above a threshold of 20 mmHg, measured within any intracranial space (subdural, intraventricular, extradural or intraparenchymal compartments). The cause of increased ICP in patients with S-TBIs is the result of an increase in brain parenchyma volume at the expense of one or more of the other two intracranial components (cerebral

blood volume, cerebral spinal fluid). An increase in brain water content (edema), increased cerebral blood volume, and /or the presence of hematomas contribute significantly to increases of ICP in patients with S-TBI.
It has been proposed that the employment of DC may drastically lower the increased ICP by allowing expansion of the edematous cerebral hemispheres. Furthermore, it has been postulated that DC interrupts the vicious cycle of intracranial hypertension via the impairment of the cerebral perfusion pressure (CPP), which inevitably results into further ICP increasing and may eventually lead to cellular injury and death. In this chapter the historical evolution of the surgical technique of DC is presented along with a brief description of the various surgical types and techniques, which are currently utilized in clinical practice. Moreover, the current concepts and controversies, the ongoing clinical trials, and the procedure associated complications are presented and discussed.

2. Decompressive craniectomy: Historical landmarks

Decompressive craniectomy has been proposed for many years as a valid treatment option for severe medically refractory intracranial hypertension, caused by various pathological conditions such as, S-TBIs, extensive cerebral infraction, massive subarachnoid hemorrhage, large intraparenchymal hemorrhage, severe intracranial infections, and extensive venous sinus thrombosis.
Hippocrates was the first who clearly described the indications for trephination in severe head injuries. Indeed, trephination remained for centuries the major surgical intervention for managing patients with severe closed head injuries. The concept of decompression (removal of a variable amount of calvaria) was introduced in the late 1890 by Annandale. Kocher in 1901 proposed the opening of the skull for relieving increased intracranial pressure, while Harvey Cushing [Cushing, 1905] performed a subtemporal decompressive craniectomy for treating moribund edema caused by an intracranial neoplastic disorder.
The concept of large cranial and dural decompression along with the removal of any underlying masses was initially described by Miyazaki in 1966, while Kjellberr and Prieto refined this surgical technique in 1971. For decades, DC was known as an occasionally life saving procedure, associated however with numerous serious complications. Therefore, the vast majority of neurosurgeons were not very eager in incorporating DC in the trauma neurosurgical armamentarium. Characteristically, Clarke in 1968 stated that the only reason for reporting his experience from performing DCs in S-TBI patients was for warning other neurosurgeons to avoid performing similar surgery.
Decompressive craniectomy in its current form was recently re-introduced by Guerra and his coworkers [Guerra et al., 1999], who reported favorable outcome in more than 50% of their cases, undergoing DC after suffering S-TBIs. Since then, many non-randomized, usually retrospective, and small size clinical studies have suggested that DC may be a valuable treatment option, when maximal medical treatment has failed to control increased ICP. It has to be pointed out that the number of the published articles in the medical literature regarding the role of DC in the management of patients with S-TBI has been geometrically increased during the last decade (Fig. 1). However, the pertinent literature demonstrates a wide variation in clinical outcomes, and ill-defined indications for performing DC in patients with S-TBIs [Aarabi et al., 2006; Howard et al., 2008; Jagannathan, et al., 2007; Morgalla et al., 2008; Münch et al., 2000].

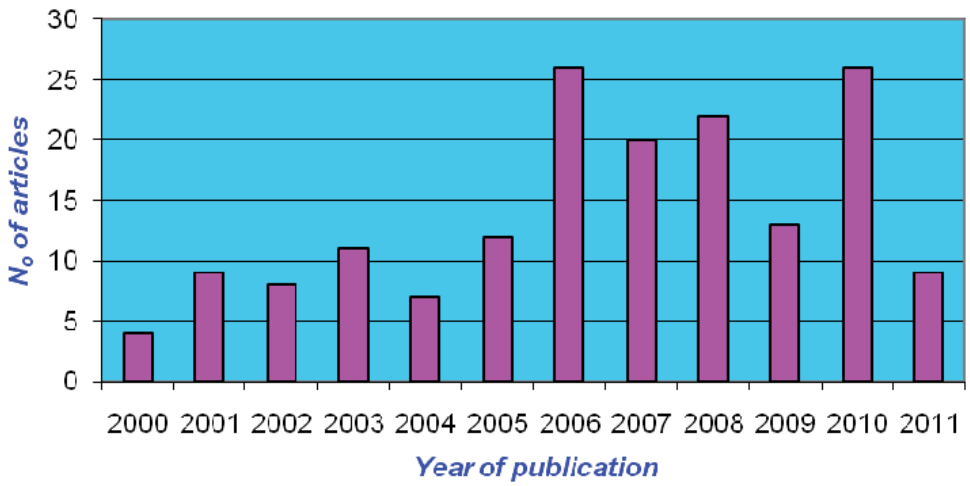


Fig. 1. Diagrammatic representation of the number of PubMed listed articles regarding the role of DC in S-TBI patients, during the decade 2000-2010.

2.1 Types of surgical decompression and surgical procedures

Historically, DC is defined as the removal of different parts and portions of the skull, with or without opening of the underlying dura, and augmentative duroplasty. A variety of operations have been proposed and different surgical techniques have been developed for brain decompression. These include: a) the classic and widely used fronto-temporo-parietal craniectomy (either unilateral or bilateral), b) the bifrontal (bicoronal) craniectomy, c) the subtemporal decompression or the recently modified temporal craniectomy, and d) the hinge (door-like) craniotomy.

The fronto-temporo-parietal craniectomy (hemicraniectomy), theoretically consists of extensive bone resection, exposing practically almost the whole underlying cerebral hemisphere. The patient is placed in supine position, with his/her head turned towards the opposite direction. An extended reverse questionmark skin incision is performed starting one cm in front of the tragus, extending above and behind the ipsilateral ear (approximately to the posterior mastoid line) and then curving forward one or two cm laterally from the midline, ending at or just behind the frontal hair line. Key points of the procedure are the extension of the decompression to the floor of the middle cranial fossa (all the way to the zygomatic arch with preservation of the superficial temporal artery and the branches of the facial nerve), (Fig. 2), and adequate size decompression with at least 12cm in its largest diameter (Fig. 3). Usually, the temporalis muscle is dissected in one plane (osteoplastic flap), by using monopolar cautery. According to another technique, the temporalis muscle may be mobilized separately, and its fascia may be dissected and harvested for the duraplasty. The pterion and the temporal bones have to be adequately exposed. Burr holes are placed to the pterion, temporal bone, posterior parietal and frontal regions, as close as possible to the scalp incision, taking advantage of the whole skin flap. Then, the underlying bulging dura is carefully stripped off the bone, in all the burr holes with the use of a fine dissector. The burr holes are connected by using a high-speed craniotome, and then the inferior rim of the temporal bone is carefully removed in pieces, by a large rongeur exposing thus the floor of the middle cranial fossa. At this point, the ipsilateral sphenoid wing can be

drilled off, by using a diamond burr, if the patient's condition allows such a time-consuming maneuver. The bone flap may be preserved by implanting it in an abdominal subcutaneous pocket, or it can be freezed and appropriately stored. The underlying dura is incised and opened in a cruciate fashion (Fig. 4). Augmentative duraplasty is performed then by using either gallea aponeurotica, or temporal fascia, or commercially available dural substitutes. The importance of performing meticulous hemostasis cannot be overemphasized. The wound is closed in anatomical layers, avoiding any tension at the skin margins. The surgeon judges according to brain edema and ICP measurements, whether the temporal fascia is sutured back or just a few approximating sutures are placed to the temporalis muscle [Apuzzo, 1993; Huang & Wen, 2010; Valadka & Robertson, 2007].

Fig. 2. Early postoperative CT scan showing adequate decompression of the middle cranial fossa in a patient undergoing decompressive craniectomy.

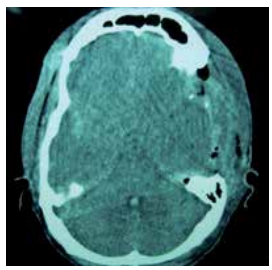


Fig. 3. Early postoperative CT scan demonstrating a large size decompressive craniectomy.

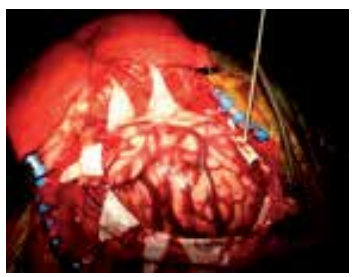


Fig. 4. Intraoperative picture showing cruciate opening of the dura. Please note the bulging, edematous brain, and the engorged cortical veins.

In bilateral hemicraniectomy, a bone ridge of approximately 3-4 cm in width is preserved over the superior sagittal sinus (SSS) (Fig 5). In bifrontal DC, a bicoronal skin flap is

performed and bilateral frontal bones including the bone over the SSS are removed. A key point of this procedure is the careful elevation of the bone flap, which requires careful dissection of the underlying SSS.

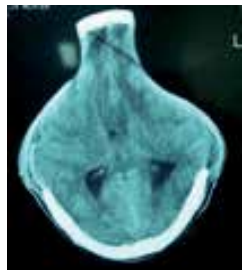


Fig. 5. Delayed CT scan of a patient undergoing bilateral hemicraniectomy. Please note the preservation of the bone over the superior sagittal sinus.

In the hinge craniotomy, the bone flap is repositioned in its place with the use of three titanium miniplates. A Y-shaped miniplate is placed just posterior to the coronal suture, a simple straight two-hole miniplate is placed at the sphenoid wing, and another miniplate at the posterior temporal region. It has to be emphasized, that only the Y-shaped miniplate is secured to the surrounding skull, while the other two miniplates are secured only at one side acting as buttress plates, preventing this way future bone flap settling, while allowing temporary expansion of the underlying edematous brain [Kenning, et al., 2009; Schmidt, et al., 2007].

In selected cases, DC can be combined with ipsilateral temporal lobectomy (anterior temporal lobectomy and uncusectomy), preventing thus the risk of transtentorial brain herniation. In this procedure the head of the patient should be turned 45^0 towards the contralateral side, and a fronto-temporo-parietal craniectomy is performed. After the dural incision and under microscopic magnification, an anterior temporal lobectomy is performed, sparing the superior temporal gyrus. A sub-pial aspiration/resection technique is used for removing the anterior 4-5 cm of the inferior and middle temporal gyri, as well as the fusiform gyrus, the uncus, the parahippocampal gyrus, and potentially the mesial temporal structures [Chibbaro, et al., 2008].

A decompressive craniectomy may be performed either for preventing or for treating severe brain swelling. Prophylactic or primary DC is defined as the surgical decompression performed primarily for evacuation of an underlying mass of any type, whenever the surgeon decides that removal of the bone flap along with the overlying bone flap will benefit the patient. According to the Congress of Neurological Surgeons' guidelines, a prophylactic DC may be performed in: a) comatose patients with epidural hematoma, b) in patients with acute subdural hematoma with thickness greater than 10 mm, or midline shift greater than 5 mm, c) in patients with admitting GCS score <8 and traumatic parenchymal lesions greater than 50 cm^3 in volume, or greater than 20 cm^3 with midline shift of at least 5 mm and/or cisternal compression, and d) in patients with open (compound) depressed cranial fractures, greater in thickness than that of the adjacent cranium, or with underlying hematoma, dural penetration, pneumocephalus, infection, or frontal sinus involvement [Bullock, et al., 2006; Sahuquillo & Arikan, 2006]. Secondary DC or therapeutic decompression is defined as the surgical decompression performed in patients with massive unilateral or bilateral brain edema in order to control high ICP refractory to maximal medical therapy.

2.2 Current concepts and controversies

Decompressive craniectomy has recently become a valid, widely-performed treatment option for managing patients with medically refractory intracranial hypertension. The Brain Trauma Foundation (BTF) and the Brain Injury Consortium (BIC) consider DC as a second-tier therapy for medically intractable intracranial hypertension. The algorithm of treating increased ICP of traumatic origin is based on a set of therapeutic maneuvers and first-line measures as head elevation, maintenance of adequate oxygen tension, maintenance of normovolemia and normal osmosis, normothermia, appropriate sedation and analgesia, avoiding pyrexia and seizures, adequate CSF draining via an external ventriculostomy, mild to moderate hypocapnia, administration of mannitol and hypertonic solutions, and neuromuscular blockade. When these first-level measures fail, only a few therapeutic options are available. These second-tier therapies are the administration of high dose barbiturates, induction of hypothermia, and DC. The BIC state that DC may be considered in exceptional situations, while Bullock et al suggest that DC may be the procedure of choice in patients with post-traumatic edema, hemispheric swelling, or diffuse injury given the appropriate clinical context. This context however, remains to be defined [Brain Trauma Foundation [BTF], 2007; Bullock, et al., 2006; Maas, et al., 1997].

Numerous experimental models have demonstrated that DC reduces secondary brain injury. These effects are thought to be the result of an increase in collateral cerebral circulation, reduction in tissue edema, and improvement in oxygenation and energy metabolism [Stiver, 2009; Weiner et al., 2010]. Furthermore, postoperative radiological evaluation in cases of DC shows amelioration of midline shift, and improvement of the preoperative compression of the basal cisterns [Laalo, et al., 2009].

Despite the constantly increasing clinical employment of DC in the management of patients with S-TBIs, there are still several points of controversy, regarding its exact role in the treatment of these patients. The most important controversial points may be summarized to the following:

- Lack of clear indications and guidelines, regarding the selection of candidates for DC. Cochrane data base analysis in 2007 [Sahuquillo & Arikan, 2006] concluded that there was no evidence to support the routine use of secondary DC to reduce unfavorable outcome in adults suffering S-TBIs and refractory intracranial hypertension. Contrariwise, it seems that there is more solid evidence in pediatric trauma patients, in whom DC seems to reduce the risk of death and unfavorable outcome [Sahuquillo & Arikan, 2006].
- The exact role of ICP measurements and the ICP waveform type in selecting patients for DC. Many investigators suggest that a single episode of ICP> 20 mmHg lasting at least for 5 minutes is an indication for performing DC, while others suggest a higher ICP threshold of 25 to 30 mmHg.
- Patient's age. Most neurosurgeons are very reluctant to perform DC in patients over 60 years old. Indeed, outcome seems to be worse in elderly patients. However, this issue remains to be addressed.
- The presence of commorbitity. It appears that multi systemic trauma patients have worse outcome. The presence of cardiological and other systemic underlying pathology, as well as the preoperative use of anti-platelet or anticoagulant medication should be seriously assessed before deciding to perform a DC.
- Ideal timing for performing DC. The question of early versus late intervention remains still unanswered. It is apparent that surgical decompression needs to be performed

before irreversible brain stem compression and/or herniation occur [Ruf, et al., 2003; Timofeev, et al., 2008].

- Ideal size of decompression. It was previously mentioned that adequate decompression of the floor of the middle cranial fossa is essential for achieving optimal relaxation of the perimesencephalic cisterns. It has been also proven that small DCs and small dural openings may cause swollen brain tissue to herniate through the bony defect, causing strangulation, infarction and worsening of the brain swelling. The current trend is to perform large size DCs with a diameter larger than 12 cm. However, the issue of the appropriate size DC has to be addressed in a prospective, randomized study.
- The exact role of other parameters of neuromonitoring, such as brain tissue oxygen, markers of anaerobic metabolism (microdialysis), transcranial Doppler ultrasonography measurements, and electroencephalographic monitoring may provide further information, making the selection of ideal surgical candidate for DC more accurate [Bor-Seng-Shu, et al., 2006; Weiner, et al., 2010].
- The effect of DC in the functional outcome of patients with S-TBIs remains to be proven. Does the performance of DC provide better functional outcome? Several clinical studies have suggested that DC reduces ICP but the overall functional outcome remains essentially unchanged [Danish, et al., 2009; Howard, et al., 2008; Morgalla, et al., 2008].

During the last decade a systematic attempt was made to prospectively assess the role of DC in the management of patients suffering S-TBIs and/or presenting medically refractory intracranial hypertension [Ban, et al., 2010; Morgalla et al., 2008; Valadka & Robertson, 2007].Two independent, parallel, multi-centric, prospective clinical studies (the DECRA and the Rescue ICP trials) were designed and are underway for evaluating the exact role of DC in the management of patients with S-TBIs. These studies are supposed to address the issue of the efficacy of DC but also may clarify other DC-associated controversial points.

2.3 The DECRA clinical trial

The early DEcompressive CRAniectomy (DECRA) in patients with severe traumatic brain injury is a multi-centric, prospective, randomized trial, coordinated by The National Trauma Research Institute, the National Health and Medical Research Council of Australia, the Victorian Trauma Foundation, the ANZICS Foundation, and the Western Australian Institute for Medical Research. The primary objective of the trial is to determine whether early decompressive craniectomy compared to conventional management strategies in patients with severe diffuse traumatic brain injury and early refractory intracranial hypertension improves neurological outcomes, at six months post injury. The inclusion criteria are: severe diffuse traumatic brain injury defined as GCS<9 and CT-scan with evidence of brain swelling (Marshall score grade D2-4), or GCS score > 8 before intubation and Marshall score D3 or D4 on the obtained brain CT scan, age 15-60 years, ICP monitor insertion, decompression within 72 hours from the injury, and medically refractory ICP (defined as ICP> 20mmHg for more than 15 minutes continuously or cumulative during one hour) [Cooper, et al., 2008].

The exclusion criteria are: intracranial hemorrhage>3cm in diameter, intracranial mixed hemorrhagic contusion>5cm in long axis, previous craniectomy, presence of epi-dural and/or sub-dural hematoma > 0.5 cm in thickness, co-existent spinal cord injury, penetrating brain injury, arrest at the scene, unreactive pupils >4mm in diameter, GCS score =3, general contraindications for neurosurgical intervention, or no change of survival after careful consideration of the obtained brain CT and the patient's clinical examination.

The surgical technique used is the bifrontal decompressive craniectomy (as described by Polin and his coworkers) with a single fronto-temporal bone flap extending across the midline. The underlying dura could be opened either by making a bilateral cruciate incision or by employing a large L-shaped incision, with the lower corner of the L facing laterally. The dural openings are covered with dural or fascial patches [Polin, et al., 1997].
DECRA trial investigators presented their initial results in April 2011. They reported on 155 randomly assigned cases (73 patients underwent DC while 82 had standard care). According to their results the investigators concluded that in adults with severe diffuse traumatic brain injury and refractory intracranial hypertension, early bifronto-temporo-parietal decompressive craniectomy decreased intracranial pressure and the length of stay in the ICU. However, they found that patients undergoing craniectomy had worse scores on the Extended Glasgow Outcome Scale than those receiving standard care (odds ratio for a worse score in the craniectomy group: 1.84). Similarly, patients undergoing DC demonstrated a greater risk of an unfavorable outcome (odds ratio: 2.21). The observed death rates at six months were similar in the craniectomy group (19%) and the standard-care group (18%) [Cooper, et al., 2011].
It has to be emphasized however, that the DECRA study carries significant weaknesses and biases. It is of great interest that out of 3700 patients, who were potential candidates for participating in this study, only 155 were finally recruited. This may be a significant selection bias. In addition, the small number of study participants significantly decreases the statistical strength of the DECRA study. Furthermore, the utilized ICP threshold of 20 mmHg for assigning patients for DC may be considered too low for patients with S-TBIs.

2.4 The rescue ICP trial

The Randomized Evaluation of Surgery with Craniectomy for Uncontrollable Elevation of Intra-Cranial Pressure, is a multi-centric 48 (centers from 19 different countries) clinical study, organized as a collaborative research project between the university of Cambridge (Departments of Neurosurgery and Neurointensive Care), and the European Brain Injury Consortium. It is a randomized controlled trial comparing the efficacy of DC versus optimal medical management for the treatment of refractory intracranial hypertension following brain trauma. The inclusion criteria are: patients with S-TBI, age 10-65 y.o, with an abnormal CT-scan requiring ICP monitoring, and raised ICP > 25 mmHg for > 1 hour to 12 hours. Patients may have immediate operation for a mass lesion but not a decompressive craniectomy. Patients with immunological, hepatic, or renal compromise may be included in this study as long as there is a description of the type and the extent of their impairment. Contrariwise, patients with bilateral fixed and dilated pupils, bleeding diathesis, a devastating injury, inability to follow them, inability to monitor ICP, primary decompression, brainstem injury, or patients that were treated according to the Lund protocol, or have received barbiturates before their randomization, should be excluded from the study. The surgical treatment comprises of a large unilateral fronto-temporo-parietal craniectomy for unilateral brain edema or of large bilateral fronto-temporo-parietal craniectomies for diffuse bilateral hemisphere swelling. The craniectomy extends from the frontal sinus anteriorly to the coronal suture posteriorly, and to the pterion laterally. It is accompanied by a large dural opening (dural flap pedicles are based on the superior sagittal sinus medially, and also a division of the falx anteriorly is required).
The primary endpoint of the Rescue-ICP study will be the outcome assessment at discharge (GOS score), and then at six months after injury (Extended GOS score), while secondary

endpoints will be: a) the assessment of outcome using the SF-36 and the SF-10 questionnaires, b) assessment of ICP control, c) length of stay in the ICU, d) hospital length of stay, and e) a detailed health-economic analysis of the collected data [Hutchinson, et al., 2006]. The results of the Rescue-ICP study are expected with great interest, and may greatly influence the future of DC and the overall management of patients suffering S-TBIs.

3. Decompressive craniectomy complications

Decompressive craniectomy has been associated with high mortality rates, mainly due to the severity of the underlying trauma, and also with numerous, and occasionally severe complications. Several factors have been identified as predisposing to the development of DC-associated complications. These include low GCS score upon admission, patient's old age, the presence of commorbidity, and the systematic preoperative anti-coagulant administration. Yang et al [Yang, et al., 2008] reported that the frequency of DC associated complications was 62% for admitting GCS scores 3-5, 39% for GCS scores 6-9, and 36 % for GCS scores >9. In addition, older patients (> 60 y.o) tend to have higher complications rates and prolonged ICU stay. Likewise, preoperative administration of anti-coagulant or antiplatelet medication increases the risk of intraoperative and/or postoperative bleeding.
The alterations in cerebral compliance, CBF autoregulation, and altered CSF dynamics associated with S-TBIs, are the main reason for developing DC-associated complications. Several theories suggesting that the sudden change from prolonged severe compression to a state of maximal vasolidation and hyperemia following DC may cause loss of autoregulation, and may be responsible for the occurrence of DC associated complications [Aarabi, et al., 2009; Yang, et al., 2008]. The most commonly occurred complications may be divided to:

1. Perioperative complications

a. Blossoming of pre-existing cerebral contusions. Expansion of pre-existing hemorrhagic contusions, as demonstrated on serial CT scans, has been reported as frequently as 40% of the cases, and may be considered inherent to the injury evolving process.
b. Evolution and expansion of contralateral masses. The decompression provided by DC allows the expansion of contralateral masses (hematomas), which that were preoperatively tamponated by the swollen brain.
c. External cerebral herniation. Expansion of the edematous brain through the bony defect may occur, especially during the first two postoperative weeks. The compression of the cortical veins of the herniated cerebral tissue leads to infarction, and unfortunately to further swelling. In small size decompressions, a mushroom-like herniation of the swollen parenchyma may occur. Therefore, most surgeons propose large craniectomies and wide dural openings for protecting the underlying cortical veins and for minimizing the risk of external cerebral herniation.

2. Early postoperative complications.

a. Subdural effusions and hygromas are very often developed in cases of DC (Fig 6). Aarabi and his coworkers [Aarabi, et al., 2009] have reported subdural effusions and hygromas in 50% of their cases. Alteration of CSF circulation after a DC may incite the development of hygromas, which may progressively expand in volume. However, the hygromas very rarely demand surgical evacuation, and they usually resolve spontaneously.

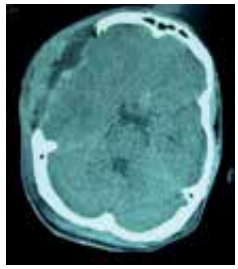


Fig. 6. Postoperative CT scan demonstrating the development of a subdural hygroma.

b. Impaired wound healing and infection may complicate a DC, and prolong patient's hospitalization. The performance of an extensive DC may compromise the skin flap blood supply (especially in the occipital area), and thus may increase the possibility of postoperative wound healing problems. Therefore, it is of paramount importance to maintain adequate skin flap blood supply by preserving the superficial temporal artery, and by avoiding tight skin suturing during closure. The observed high infection rate in patients undergoing DC may also be partially explained by the fact that the vast majority of these patients require prolonged ICU treatment and multiple interventions.
c. Post-DC hydrocephalus incidence has been reported to be as high as 15 %, and is associated with poorer clinical outcome (Fig 7). The treatment of hydrocephalus with CSF shunting remains controversial, and the timing of intervention before or after cranioplasty is still disputable.

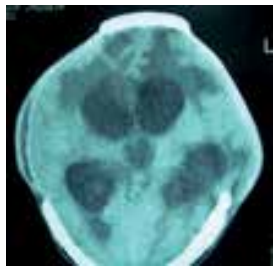


Fig. 7. Delayed postoperative CT scan demonstrating severe ventriculomegaly in a patient undergoing bilateral hemicraniectomy.

d. The syndrome of the trephined is a frequent delayed complication and its diagnosis is often overlooked. Occasionally it may be presented with delayed onset of focal neurological deficits.
e. Paradoxical herniation with brain stem compression and neurological deterioration is a very rare complication and may be precipitated by a lumbar puncture in a craniectomized patient.

3.1 Cranioplasty

One of the drawbacks of DC is the fact that a second surgical intervention is required to repair the bone defect. Autologous bone grafts may be used, which are preserved subcutaneously, or stored freezed under specific conditions in storage bone-banks. Tailor-made heterologous or synthetic bone grafts may also be used. The previous belief ofdelayed cranioplasty for minimizing the risk of infection, is seriously questioned nowadays, and an

increasing number of surgeons prefer early surgical skull reconstruction. Several surgeons support the idea of immediate cranioplasty even during the initial hospitalisation, as early as 2-4 weeks after trauma, when there is no suspicion of infection. The possibility of a new head injury with the exposed brain unprotected must be taken into consideration. Moreover, the observation of better functional outcome after cranioplasty, may further increase the number of early cranioplasty cases [Beauchamp, et al., 2010; Morina, et al., 2011].

4. Conclusions

The management of refractory post-traumatic intracranial hypertension remains a challenge for neurosurgeons, anesthesiologists, and neuro-intensivists. Cerebral ischemia leading to severe disability or death is unfortunately the only result expected. In order to deal with this dramatic situation only few treatment options exist. The ultimate measure to relieve uncontrollable ICP is an extensive decompressive craniectomy. It is proven that DC increases the volumetric compensatory capacity and reduces ICP. However, the well-documented risks and drastically complications of DC have to be seriously considered before performing a DC. In carefully selected cases these risks may be outweighted by the expected benefit. The results of the two ongoing prospective, randomized, controlled trials are expected to enlighten us on the exact role of DC in the management of patients with S-TBIs, and its effect on their long-term functional outcome.

5. References

Aarabi, B., Hesdorffer, DC., Ahn, ES., Aresco, C., Scalea, TM., & Eisenberg, HM. (2006). Outcome following decompressive craniectomy for malignant swelling due to severe head injury. *J Neurosurg* Vol.104, No.4, (April 2006), pp. 469-79, ISSN 0022-3085

Aarabi, B., Chesler, D., Maulucci, C., Blacklock, T., & Alexander, M. (2009). Dynamics of subdural hygroma following decompressive craniectomy: a comparative study. *Neurosurg Focus* Vol.26, No.6, (June 2009), pp.E8, ISSN 1092-0684

Apuzzo, MLJ. (1993). Complication Avoidance and Management, In: *Brain Surgery,* Volume 2, part 4, pp.1283-1296, Churchill Livingstone Inc., ISBN 0-443-08709-1, New York

Ban, SP., Son, YJ., Yang, HJ., Chung, YS., Lee, SH., & Han, DH. (2010*).* Analysis of complications following decompressive craniectomy for traumatic brain injury. *J Korean Neurosurg Soc* Vol.48, No.3, (September 2010), pp.244-50, ISSN 1225-8245

Beauchamp, KM., Kashuk, J., Moore, EE., Bolles, G., Rabb, C., Seinfeld, J., Szentirmai, O., & Sauaia, A. (2010). Cranioplasty after post injury decompressive craniectomy: is timing of the essence? *J Trauma* Vol. 69, No.2, (August 2010), pp.270-4, ISSN 0022-5282

Bor-Seng-Shu, E., Hirsch, R., Teixeira, MJ., De Andrade, AF., & Marino, R Jr. (2006). Cerebral hemodynamic changes gauged by transcranial Doppler ultrasonography in patients with posttraumatic brain swelling treated by surgical decompression. *J Neurosurg* Vol.104, No.1, (January 2006), pp.93-100, ISSN 0022-3085

Brain Trauma Foundation BTF. (2007). Guidelines for the management of severe traumatic brain injury, 3rd edition*. J Neurotrauma* Vol. 24, No. 1, (2007), ISSN 0897-7151

Bullock, MR., Chesnut, R., Ghajar, J., Gordon, D., Hartl, R., Newell, DW., Servadei, F., Walters, BC., & Wilberger, JE. (2006). Guidelines for the Surgical Management of

Traumatic Brain Injury. N*eurosurgery* Vol. 58, No. 3, (March 2006), pp. S2-vi, ISSN 0148-396X

Chibbaro, S., Marsella, M., Romano, A., Ippolito, S., & Benericetti, E. (2008). Combined internal uncusectomy and decompressive craniectomy for the treatment of severe closed head injury: experience with 80 cases. *J Neurosurg* Vol.108, No.1, (January 2008), pp. 74-9, ISSN 0022-3085

Cooper, DJ., Rosenfeld, JV., Murray, L., Wolfe, R., Ponsford, J., Davies, A., D'Urso, P., Pellegrino, V., Malham, G., & Kossmann, T. (2008). Early decompressive craniectomy for patients with severe traumatic brain injury and refractory intracranial hypertension--a pilot randomized trial. *J Crit Care* Vol.23, No.3, (September 2008), pp. 387-93, ISSN 1541-6933

Cooper, DJ., Rosenfeld, JV., Murray, L., Arabi, YM., Davies, AR., D'Urso, P., Kossmann, T., Ponsford, J., Seppelt, I., Reilly, P., & Wolfe, R.; DECRA Trial Investigators; Australian and New Zealand Intensive Care Society Clinical Trials Group. (2011). Decompressive craniectomy in diffuse traumatic brain injury. *N Engl J Med* Vol.364, No.16, (2011 April 21), pp.1493-502, ISSN 0028-4793

Guerra, WK., Gaab, MR., Dietz, H., Mueller, JU., Piek, J., & Fritsch, MJ. (1999). Surgical decompression for traumatic brain swelling: indications and results. *J Neurosurg* Vol.90, No.2, (February 1999), pp.187-96, ISSN 0022-3085

Cushing H. (1905). The establishment of cerebral hernia as a decompressive measure for inaccessible brain tumors; with the description of intramuscular methods of making the bone defect in temporal and occipital regions. *Surg Gynecol Obstet* Vol.1, pp.297–314, ISSN 0039-6087

Danish, SF., Barone, D., Lega, BC., & Stein, SC. (2009). Quality of life after hemicraniectomy for traumatic brain injury in adults. A review of the literature. *Neurosurg Focus* Vol.26, No.6, (June 2009), pp.E2, ISSN 1092-0684

Honeybul, S., Ho, KM., Lind, CR., & Gillett, GR. (2011). Surgical intervention for severe head injury: ethical considerations when performing life-saving but non-restorative surgery. *Acta Neurochir (Wien)* Vol. 153, No.5, (May 2011), pp. 1105-10, ISSN 0001-6268

Howard, JL., Cipolle, MD., Anderson, M., Sabella, V., Shollenberger, D., Li, PM., & Pasquale, MD. (2008). Outcome after decompressive craniectomy for the treatment of severe traumatic brain injury. J *Trauma* Vol.65, No.2, (August 2008), pp.380-6, ISSN 0022-5282

Huang, X., & Wen, L. (2010). Technical considerations in decompressive craniectomy in the treatment of traumatic brain injury. *Int J Med Sci* Vol.7, No.6, (November 8), pp. 385-90, ISSN 1449-1907

Hutchinson, PJ., Menon, DK., & Kirkpatrick, PJ. (2005). Decompressive craniectomy in traumatic brain injury--time for randomised trials? *Acta Neurochir (Wien)* Vol.147, No.1, (January 2005), pp. 1-3, ISSN 0001-6268

Hutchinson, PJ., Corteen, E., Czosnyka, M., Mendelow, AD., Menon, DK., Mitchell, P., Murray, G., Pickard, JD., Rickels, E., Sahuquillo, J., Servadei, F., Teasdale, GM., Timofeev, I., Unterberg, A., & Kirkpatrick, PJ. (2006). Decompressive craniectomy in traumatic brain injury: the randomized multicenter RESCUE icp study (www.RESCUEicp.com). *Acta Neurochir Suppl* Vol.96, pp.17-20, ISSN 0001-6268

Jagannathan, J., Okonkwo, DO., Dumont, AS., Ahmed, H., Bahari, A., Prevedello, DM., Jane, JA Sr., & Jane, JA Jr. (2007). Outcome following decompressive craniectomy in children with severe traumatic brain injury: a 10-year single-center experience with long-term follow up. *J Neurosurg* Vol.106, No.4, (April 2007), pp.268-75, ISSN 0022-3085

Kenning, TJ., Gandhi, RH., & German, JW. (2009). A comparison of hinge craniotomy and decompressive craniectomy for the treatment of malignant intracranial hypertension: early clinical and radiographic analysis. *Neurosurg Focus* Vol.26, No.6, (June 2009),pp. E6, ISSN 1092-0684

Laalo, JP., Kurki, TJ., Sonninen, PH., & Tenovuo, OS. (2009). Reliability of diagnosis of traumatic brain injury by computed tomography in the acute phase. *J Neurotrauma* Vol. 26, No.12, (December 2009), pp. 2169-78, ISSN 1557-9042

Maas, AI., Dearden, M., Teasdale, GM., Braakman, R., Cohadon, F., Iannotti, F., Karimi, A., Lapierre, F., Murray, G., Ohman, J., Persson, L., Servadei, F., Stocchetti, N., & Unterberg, A. (1997). EBIC-guidelines for management of severe head injury in adults. European Brain Injury Consortium. *Acta Neurochir (Wien)* Vol.139, No.4, pp. 286-94, ISSN 0001-6268

Marmarou, A., Anderson, RL., Ward, JD., Choi, SC., Young, HF., Eisenberg, HM., Foulkes, MA., Marshall, LF., & Jane, JA. (1991). Impact of ICP instability and hypotension on outcome in patients with severe head trauma. *J Neurosurg Vol.* 75, (November 1991),pp. S59–66, ISSN 0022-3085

Morgalla, MH., Will, BE., Roser, F., & Tatagiba, M. (2008). Do long-term results justify decompressive craniectomy after severe traumatic brain injury? *J Neurosurg* Vol.109, No.4, (October 2008), pp.685-90, ISSN 0022-3085

Morina, A., Kelmendi, F., Morina, Q., Dragusha, S., Ahmeti, F., Morina, D., & Gashi, K. (2011). Cranioplasty with subcutaneously preserved autologous bone grafts in abdominal wall-Experience with 75 cases in a post-war country Kosova. *Surg Neurol Int* Vol.2, (May 28), pp. 72. ISSN 2152-7806

Münch, E. Horn, P., Schürer, L., Piepgras, A., Paul, T.,& Schmiedek, P. (2000). Management of Severe Traumatic Brain Injury by Decompressive Craniectomy. *Neurosurgery Vol*. 47, No. 2, (August 2000), pp. 315-323, ISSN 0148-396X

Polin, RS., Shaffrey, ME., Bogaev, CA., Tisdale, N., Germanson, T,. Bocchicchio, B, & Jane, JA. Decompressive bifrontal craniectomy in the treatment of severe refractory posttraumatic cerebral edema. (1997). *Neurosurgery* Vol.41, No.1, (July 1997), pp.84-92, ISSN 0148-396X

Ruf, B., Heckmann, M., Schroth, I., Hügens-Penzel, M., Reiss, I., Borkhardt, A., Gortner, L., & Jödicke, A. (2003). Early decompressive craniectomy and duraplasty for refractory intracranial hypertension in children: results of a pilot study. *Crit Care* Vol. 7, No.6, (December 2003), pp. R133-8, ISSN 1364-8535

Sahuquillo, J., & Arikan, F. (2006). Decompressive craniectomy for the treatment of refractory high intracranial pressure in traumatic brain injury. *Cochrane Database Syst* Rev Vol, 25, No.1, pp.CD003983

Schmidt JH, 3rd., Reyes, BJ., Fischer, R., & Flaherty, SK. (2007). Use of hinge craniotomy for cerebral decompression. Technical note. *J Neurosurg* Vol.107, No.3, (September 2007), pp.678-82, ISSN0022-3085

Stiver, SI. (2009). Complications of decompressive craniectomy for traumatic brain injury. *Neurosurg Focus* Vol.26, No.6, (June 2009), pp. E7, ISSN 1092-0684

Timofeev, I., Czosnyka, M., Nortje, J., Smielewski, P., Kirkpatrick, P., Gupta, A., & Hutchinson, P. (2008). Effect of decompressive craniectomy on intracranial pressure and cerebrospinal compensation following traumatic brain injury. *J Neurosurg* Vol.108, No.1, (January 2008), pp.66-73, ISSN 0022-3085

Valadka, AB., & Robertson, CS. (2007). Surgery of cerebral trauma and associated critical care. *Neurosurgery* Vol.61, No.1, (July 2007), pp.203-20, ISSN 1220-8841

Weiner, GM., Lacey, MR., Mackenzie, L., Shah, DP., Frangos, SG., Grady, MS., Kofke, A., Levine, J., Schuster, J., & Le Roux, PD. (2010). Decompressive craniectomy for elevated intracranial pressure and its effect on the cumulative ischemic burden and therapeutic intensity levels after severe traumatic brain injury. *Neurosurgery* Vol. 66,No.6, (June 2010), pp. 1111-8, ISSN 0148-396X

Yang, XF., Wen, L., Shen, F., Li, G., Lou, R., Liu, WG., & Zhan, RY. (2008). Surgical complications secondary to decompressive craniectomy in patients with a head injury: a series of 108 consecutive cases. *Acta Neurochir (Wien)* Vol.150, No.12, (December 2008), pp, 1241-7, ISSN 0001-6268

25

The Importance of Restriction from Physical Activity in the Metabolic Recovery of Concussed Brain

Giuseppe Lazzarino et al.*
Department of Biology, Geology and Environmental Sciences
Division of Biochemistry and Molecular Biology, University of Catania, Catania
Italy

1. Introduction

Brain concussion is unquestionably the most common form of traumatic brain injury (TBI) worldwide (Bruns & Hauser, 2003; Tagliaferri et al., 2006). In European countries, approximately 235 individual/100,000 people are admitted annually to the hospital following TBI, 80% of which receive a diagnosis of mild TBI (mTBI). (van der Naalt, 2001; Vos et al., 2002). It has been calculated that the ratio in the occurrence of mTBI to severe TBI (sTBI) is approximately 22 to 1, with mTBI accounting for at least 75% of patients who survive after TBI each year (Tagliaferri et al., 2006). These percentages are very similar to those recorded in the United States where it is estimated that approximately 1.5 – 8 million people per year suffer from TBI and, among those requiring hospitalization, a proportion ranging from 75% to 90% are classified as "mildly" injured or "concussed" (Bruns & Hauser, 2003). These wide ranges of annual incidence are probably due to the fact that an unknown proportion of mTBI victims do not seek any medical attention (McCrea et al., 2004) (HEADS UP) but it might also be due to the fact that there is still confusion and inconsistency among researchers and organizations in defining and understanding this type of trauma. (Cantu & Voy, 1995; Cantu, 1998, 2007).
The above reported numbers give the evidence that albeit the incidence of mTBI is tremendously high, the mortality caused by this type of trauma appears to be rather low (6-10 per 100,000/year). To reinforce this concept it has been reported that only 0.2% of all

*Roberto Vagnozzi[1], Stefano Signoretti[2], Massimo Manara[3], Roberto Floris[4], Angela M. Amorini[5], Andrea Ludovici[4], Simone Marziali[4], Tracy K. McIntosh[6] and Barbara Tavazzi[5]
[1]*Department of Neurosurgery, Chair of Neurosurgery, University of Rome "Tor Vergata", Rome, Italy*
[2]*Division of Neurosurgery, Department of Neurosciences-Head and Neck Surgery, San Camillo Hospital, Rome, Italy*
[3]*Association of Sports Physicians Parma, F.M.S.I., Parma, Italy*
[4]*Department of Diagnostic Imaging and Interventional Radiology, University of Rome "Tor Vergata", Rome, Italy*
[5]*Institute of Biochemistry and Clinical Biochemistry, Catholic University of Rome "Sacro Cuore", Rome, Italy*
[6]*Media NeuroConsultants Inc., Media, PA 19063, USA*
[7]*Department of Biology, Geology and Environmental Sciences, Division of Biochemistry and Molecular Biology, University of Catania, Catania, Italy*

patients attending the Emergency Departments (EDs) will die as a direct result of this head injury (Vos et al., 2002).
With this in mind, it is evident that this situation represents a really serious public health concern of our time: in fact, the EDs are required to see a massive number of patients, with the most challenging task of identifying the small number of them that will progress to have serious acute intracranial complications. Statistics demonstrate that only 1-3% of all mTBI patients admitted to the hospital with a Glasgow Coma Scale (GCS) score of 14-15 will subsequently develop life-threatening intracranial pathology (the remaining 97-99% of them will be discharged home within 48 hours) (Livingston et al., 2000; Lloyd et al., 1997; Shackford et al., 1992; Swann et al., 1981; Taheri et al., 1993). Furthermore, when a mTBI patient is presenting with a GCS score of 15 and no additional associated risk factors the probability that he may suffer from intracranial hemorrhage, requiring neurosurgical intervention, is below 0.1% (Fabbri et al., 2010).
As a consequence of this very low mortality rate, it is generally and widely accepted that mTBI represents a nosological entity with high frequency but that it does *not* represent a real serious injury. The common feeling that consequences of mTBI are only transient disturbances has slowly grown, mainly supported by the absence of structural lesions on traditional neuroimaging. Therefore, the general idea is that mTBI-affected patients need no intervention other than observation, with no additional clinical examinations nor specific recommendations for the post-injury period. However, a recent report revealed that the diagnosis of an intracranial hematoma in such patients was made with a median delay of 18 hours (Yates et al., 2007), strongly suggesting that the quality of the "observation" that mildly-injured patients receive while in hospital is of utmost concern. If in the USA it has been established that only 50% of mildly head injured patients admitted to the hospital had documentation of neurological observations (Yates et al., 2007) in Europe the situation is even much worse, with mTBI patients who have historically been observed by non-specialist wards by nurses and doctors not experienced in neurological observations. The fundamental issues of whether to image, observe and discharge, each one of these endless patients, focused essentially on identifying those at risk for a hemorrhagic complications has completely obscured the real dilemma of the concussive injury, whose early and late symptoms and sequelae are continuously underreported, underinvestigated, underdiagnosed, and mainly underestimated by the majority of physicians.

1.1 Background: Sports-related concussion

If mTBI were as "mild" as we might think, it would be difficult to explain the actual complex management of these patients that may involve various health care professionals including family practice physicians, behavioral psychologists, clinical psychologists, neuropsychologists, neurologists, psychiatrists, neuro-ophthalmologists, neurosurgeons, physiatrists, nurses, occupational therapists, and physical therapists. Furthermore, long beyond the typical recovery interval of one week to three months, at least 15% of persons with a history of mTBI continue to see their primary care physician because of persistent problems (Alexander, 1995; Bigler, 2003; Gouvier et al., 1992; Kay et al., 1992; Ingebrigtsen et al., 2000). From the aforementioned picture and reminding that the majority of mTBIs are unreported, it is clearly evident how much difficult would be to select a homogeneous population for a study on mTBI, just on the basis of those who refer to the hospital.
Although certainly blended in to the vast world of mTBI, by definition, brain concussion should be considered a discrete and distinct entity, since not all mTBI are truly

"concussive"; thus the two terms refer to different constructs and should not be used interchangeably (McCrory et al., 2009). Despite recent efforts, a unanimous definition of concussion has not yet been widely accepted (Cantu, 2007). It is possible to define concussion as a traumatic insult capable of provoking an acceleration-deceleration phenomenon within the skull (Barth et al., 2001). From a clinical point of view, concussion is not necessarily accompanied by loss of consciousness and is associated with various physical (headache, equilibrium, vision disturbances, etc.), cognitive (memory, concentration, etc.), emotional (behaviour) and sleep alterations (Gosselin et al., 2009; Hunt & Asplund, 2010; Randolph et al., 2009). These symptoms are included in the well known post-concussive syndrome and can affect, to various degrees, everyday life, resolving spontaneously within 7-10 days post-injury, in the majority of cases. In concussed subjects, the pathobiology of mTBI can not be defined by classical imaging techniques such as CT scan and MR (Kurca et al., 2006), as it occurs in all mTBI patients. This fact, coupled to the faintness and variability of symptoms, mainly assessed by the patient's self-evaluation, is not certainly of help in diagnosing and monitoring concussed patients.
It is generally accepted that at least 20% of all mTBI are sports-related injuries (concussions), of which 30-45% receives no medical care (McCrea et al., 2004). Athletes, therefore, represent a population at great risk of occurrence of concussive episodes and are, for several reasons, the population of choice with which to undertake trials to study the pathobiology of mTBI (Meehan & Bachur, 2009). It is well established that after the first traumatic episode the probability of recurrence of concussion in athletes increases by 3 times (Cantu, 2003), that currently, there is no agreement as to how many concussions are too many (Guskiewicz et al., 2003; Pellman et al., 2004), nor is there an unanimously approved diagnostic approach to monitor concussed athletes (Delaney et al., 2005; Kissick & Johnstone, 2005; Ponsford, 2005), consequently the criteria to assess a safe return of concussed athletes to play remain unclear (Guskiewicz et al., 2006; McClincy et al., 2006; Lovell et al., 2004).

1.2 Background: Metabolic changes characterizing brain vulnerability following concussion

An increasing number of studies on mTBI have focused attention on concussion-induced changes in brain metabolism (Bergsneider et al., 2000; Praticò et al., 2002) including those related to cerebral energy state (Vagnozzi et al., 1999). Hovda and colleagues first suggested the concept of metabolic vulnerability occurring in brain tissue after any concussive episode (Giza & Hovda, 2001; Hovda et al., 1993). During this transient period of altered brain metabolism and function, a second concussive episode of even modest entity may cause significantly addition and/or dramatic brain damage (Longhi et al., 2005), thereby underlying the so called second impact syndrome (SIS), encountered occasionally in sports medicine (Saunders & Harbaugh, 1984). In fact, the possibility of having a second concussive injury within a not yet defined period of time from the first (i.e., days or weeks) has been reported to be even fatal in some instances (Bowen, 2003; Cantu, 1998; Cobb & Battin, 2004; Logan et al., 2001; Mori et al., 2006; Saunders & Harbaugh, 1984). SIS is clinically characterized by untreatable malignant edema with devastating consequences for the patient up to death. Notwithstanding these reported cases, concerns still exist about the real occurrence of this peculiar pathological condition (McCrory, 2001; McCrory & Berkovic, 2001; Nugent, 2006).
By using a rodent model of closed diffuse mild head injury (Foda & Marmarou, 1994; Marmarou et al., 1994), data from our laboratories confirmed the concept of metabolic

vulnerability (Tavazzi et al., 2007; Vagnozzi et al., 2007) and have also produced solid experimental evidence linking the severity of brain injury and recovery with the extent of ATP and N-acetylaspartate (NAA) decrease and recovery (Tavazzi et al., 2007; Vagnozzi et al., 2005, 2007). NAA is the most prominent compound detectable with proton Magnetic Resonance Spectroscopy (^{1}H-MRS) in human brain, making it one of the most reliable molecular markers for brain ^{1}H-MRS studies. Although an exact role of this compound remains to be established, brain NAA was found present in concentrations a hundred fold higher than in non-nervous system tissue and therefore considered a brain-specific metabolite (Miyake et al., 1981; Truckenmiller et al., 1985) and as an in vivo marker of neuronal density. NAA metabolism involves different brain compartments, with neuronal mitochondria taking care of its biosynthesis via the activity of aspartate N-acetyltransferase (ANAT) and oligodendrocytes contributing to its degradation via the activity of N-acetylaspartate acylase (ASPA). NAA homeostasis is finely regulated by three different velocities: 1) rate of neuronal biosynthesis, 2) rate of neuronal outflow in the extracellular space, and 3) rate of oligodendrocyte uptake and degradation (Baslow, 2003a, 2003b). Furthermore, NAA biosynthesis is also strictly dependent on the neuronal energy state and therefore on the correct mitochondrial functioning. In fact, NAA synthesis necessarily requires the availability and the energy of hydrolysis of acetyl-CoA (ΔG = -31.2 kJ/mol), working as the acetyl group and energy donor in the acetylation reaction of aspartate catalyzed by ANAT. It is fundamental to understand that when acetyl-CoA is used for NAA synthesis there is an indirect high energy cost to the cell. In fact, since in this case acetyl - CoA will not enter the citric acid cycle (Krebs' cycle) there will be a decrease in the production of reducing equivalents (3 NADH and 1 $FADH_2$) as the fuel for the electron transport chain. Since the oxidative phosphorylation is stoichiometrically coupled to the amount of electron transferred to molecular oxygen by the electron transport chain, the final result will be a net loss of 11 ATP molecules for each NAA molecule newly synthesized. NAA concentration within neurons is comparable to that of glutamate (~10 mmol/l brain water) but, notwithstanding such a relevant amount and in spite of NAA is known since late '50s, there is no unanimity on the biochemical functions of this still enigmatic molecule. According to different studies, NAA may act as storage form for aspartate, protein synthesis regulator, shuttle of acetate and "amino-nitrogen" from the mitochondria to the cytoplasm, breakdown product or precursor of the neurotransmitter N-acetylaspartylglutamate (NAAG), metabolically inert pool regulating the anion deficit balance, metabolically active pool involved in the production of glutamate. Although NAA might play a marginal role in any of the aforementioned processes, recent studies have suggested that the potentially main roles of NAA might be to participates an acetyl group donor in brain lipid biosynthesis (production of myelin) (Moffett et al., 1991) and to act as a neuronal osmoregulator against cytotoxic swelling (molecular water pump) (Baslow, 2003a). Physiologically, NAA concentration is kept within a strict oscillation range even though NAA is regenerated 1.8 times/24 hours (with a calculated turnover rate of approximately 0.75 mmol/l water/hour). It is therefore evident that a perfect balance of the different velocities involved in NAA homeostasis, should exist. NAA decrease has been observed in association with many neurological diseases causing neuronal and axonal degeneration such as tumors, epilepsy, dementia, stroke, hypoxia, multiple sclerosis, and many leukoencephalopathies. Vice versa, the only known pathologic state characterized by a dramatic increase in cerebral NAA is the autosomic genetic leukodystrophy caused by the synthesis of a defective form of the

enzyme responsible for the NAA degradation (N-acetyl-asparto-acylase, ASPA), known as Canavan disease. Due to the enzymatic defect in the NAA degradation, Canavan-affected patients are also characterized by large urinary excretion of intact NAA, up to 2000-fold the normal physiologic excretion (Tavazzi et al., 2005). The morphological alterations in Canavan-affected patients, characterized at MRI by spongi degeneration and structurally defective myelin, can well account for the most accredited roles of NAA: the incapacity to degrade NAA would generate dramatic changes in water extrusion from the brain compartment with consequent spongi degeneration, as well as a drastic decrease in the availability of acetate groups for olygodendrocytes thereby causing deficits in lipid myelin biosynthesis.

1.3 Background: NAA and ^{1}H-MRS as a diagnostic tool to study in vivo brain vulnerability

As already mentioned, the relevance of NAA as a biochemical marker of the metabolic neuronal "wealth" is related to the possibility to measure NAA concentration non-invasively "in vivo" by ^{1}H-MRS. When subtracting the spectral signal of ^{1}H from water (the most abundant molecule containing an atom with an unpaired proton, which is a prerequisite to allow to detect a molecule by MRS), the ^{1}H-NAA resonance returns one the best defined peaks at approximately 2 ppm. Using the clinically safe magnetic fields of the MRS apparatuses (1.5 or 3.0 Tesla) in the range 1.8 to 3.4 ppm to additional, well-resolved, resonance peaks are obtained, one at approximately 3.2 ppm referring to choline (Cho) and the other at approximately 2.9 ppm referring to creatine (Cr). It is worth recalling that the relatively low magnetic fields with no side effects (1.5-3.0 T) do not allow to discriminate in the multitude of compounds that are physiologically present within the brain tissue (acetyl-choline, cytidinediphospho-choline, phospho-choline, glycerophospho-choline, phosphatidyl-choline, free-choline, etc.) having this quaternary ammonium derivative as a characterizing chemical group. The same can be applied either in the case of the creatine-containing compounds (creatine and creatinephosphate) or in the case of the NAA-containing compounds (NAA and NAAG). Actually, in a ^{1}H-MRS the Cho peak represents the choline-containing compounds (with phosphatidyl-choline representing the 90% of the signal intensity), the Cr peak represents creatine-containing compounds (with creatine accounting for the 90% of the intensity signal) and the NAA peak the NAA-containing compounds (with NAA being 12 to 15 fold more concentrated than NAAG). Unless determining the water content within the voxel(s) for a calculation of the absolute concentrations of the different metabolites, it is necessary to quantify at the same time the peak areas of the spectroscopic intensity signals of both Cr and Cho-containing compounds and to then refer to the NAA/Cr and NAA/Cho ratios for the semi-quantitative NAA evaluation in physiopathological conditions (Barker et al., 1993; Brooks et al., 2001; Friedman et al., 1999; Garnett et al., 2000; Mitsumoto et al., 2007). This type of approach assumes Cr and Cho as two unvarying parameters to which relate the NAA changes. The results of a multicenter clinical trial (Vagnozzi et al., 2010) involving 40 concussed athletes and 30 healthy volunteers have been recently published, revealing that, despite different combinations of field strengths (1.5 or 3.0 T) and modes of spectrum acquisition (single- or multi-voxel) among the scanners currently in use in most neuroradiology centers, NAA determination represents a quick (15-minute), easy-to-perform, noninvasive tool to accurately measure changes in cerebral biochemical damage occurring after a concussion.

Patients exhibited the most significant alteration of metabolite ratios at day 3 post-injury, showing a gradual recovering, initially in a slow fashion and, following day 15, more rapidly. At 30 days post-injury, all subjects showed complete recovery, having metabolite ratios similar to values detected in controls. Interestingly, all these 40 patients self-declared symptom clearance between 3 and 15 days after concussion strongly demonstrating differential times of disappearance of clinical gross signs and of normalization of brain energy metabolism. In a previous pilot study carried out in a cohort of singly and doubly-concussed athletes, examined by ^{1}H-MRS for their NAA cerebral content at different time points after concussive events, we demonstrated that the recovery of brain metabolism is not linearly related to time. In this study, two athletes experienced a second concussion between the 10th and the 13th day after the first insult. Although they were not affected by SIS nor showed signs of sTBI, they however had a significant delay in both symptom resolution and NAA normalization (Vagnozzi et al., 2008).

According to the aforementioned data it is clear that concussion causes a reversible change of brain metabolism that can be monitored by ^{1}H-MRS in terms of NAA variations. Furthermore, data recorded in the two doubly concussed athletes suggest that a second concussion might compromise rescue of cerebral metabolism and delaying its normalization. This would in turn cause delaying in return of athletes to play. In the present study, we report data referring to six concussed athletes who received a second concussion at different times during the period of alteration of brain metabolism. The time course changes in their cerebral NAA, evaluated by ^{1}H-MRS up to complete normalization, are presented.

2. Materials and methods

Six non-professional male athletes from different sport disciplines who had suffered from a sport-related concussion, defined as a traumatically induced transient alteration in mental status, not necessarily accompanied with loss of consciousness, were considered in this study. At the clinical examinations they had a GCS greater or equal to 14, normal neurological objective signs and an age ranging between 20 and 33 years (mean age = 26 ± 5 years). According to our previous observations (Vagnozzi et al., 2008, 2010), they were required to restrain from further physical activity during the entire observational period, which lasted until cerebral metabolic normalization. None of the patients had positive MRI for post-traumatic anatomical lesions (the presence of blood, etc.), or suffered from polytrauma, or presented with risk factors for subsequent complications (coagoulopathy, epilepsy, former neurosurgical interventions, alcohol or drug abuse, disabilities). The first clinical evaluation, MRI and MRS were carried out at 3 days post injury; follow-up MRI and MRS analyses, as well as clinical evaluation of concussion-associated symptoms, were performed at different times up to metabolic normalization (15, 22, 30, 45, 60, 90, 120 days post-injury). A group of 10 healthy male subjects matched for age (mean age = 25 ± 6 years) was subjected to MRS and used as the control group.

In controls and athletes, semi-quantitative analysis of NAA relative to creatine (Cr) and choline containing compounds (Cho) was performed in the single voxel (SV) mode, after obtaining proton spectra using a 3.0 T system (Philips, Intera Achieva). For conventional MRI studies, T1 and T2 weighted TSE images were acquired in axial coronal and sagittal planes and, in order to rule out even the smallest amount of intra-cerebral blood, Fast Field Echo (FFE) T2* sequences were used. A multi channel coil (8 ch.) Sense-Head with 4 mm

slice thickness, 1 mm gap and a FOV of 230 mm, was used for all MRI sequences. Following localized shimming and water suppression, the spectroscopic examination was carried out using a PRESS (Point Resolved Spectroscopy Sequence) pulse sequence, with the following settings: TE = 144 msec; TR = 2,000 msec; Spectral Bandwidth = 2,000 Hz; acquisition cycles = 128. The optimal positioning of the voxel was determined using the MR images acquired on axial, coronal and sagittal planes to facilitate its three-dimensional placement, adjacent to the cortical-subcortical junction in order to include only the white matter of the frontal lobes, bilaterally and the choice of this location as the region of interest, was made to obtain the most homogeneous data as possible. To this end, a spectrum from a single voxel (SV) customized to sample a volume of interest (VOI) of 3.375 cm^3 (1.5 x 1.5 x 1.5 cm), was obtained (acquisition time about 5 min for each voxel). In follow-up studies, the exact repositioning of the voxel on the same acquisition plane obtained in the previous MRI study was achieved by using dedicated software (SameScan, Philips Medical Systems). Metabolite intensity ratios (NAA-to-Cr and Cho-to-Cr) were automatically calculated at the end of each acquisition using dedicated software (SpectroView; Philips Medical Systems), by which gaussian-fitted peak areas relative to a baseline computed from a moving average of the noise regions of each spectrum were determined. Post-processing of spectral data, using a homemade computed program, allowed us to render uniform calculations of the area under the peaks of NAA, Cho and Cr, using common criteria for peak integration. In the case of a single, well-defined peak (typically the NAA peak), a valley-to-valley integration was performed to obtain the area under the peak. In the case of not fully resolved peaks (frequently the Cho and Cr peaks), a horizontal baseline between the start of the first peak to the end of the second peak was selected; the grouped peaks were then split by a vertical line, drawn from the median point of the common valley between peaks to the horizontal baseline and the area under the peaks calculated. These values were used to determine the metabolite ratios NAA/Cho, NAA/Cr and Cho/Cr.

3. Results

Table 1 summarizes the clinical features of the 6 doubly concussed athletes, including the time interval between concussion and the type and duration of the post-concussive self-reported clinical symptoms after both the first and the second head injury.

Table 1. Demographic data, sport activity and clinical features of 6 non professional athletes suffering from double concussion.

None of these concussions was characterized by loss of consciousness. All athletes declared to suffer from headache following the first concussive episode and each of them suffered from at least one additional post-concussive disturbance. Disappearance of self-reported clinical symptoms after the first concussion ranged between 3 to 8 days (mean duration = 5.8 ± 2.1 days). In two athletes the second concussive event occurred between the first and second ^{1}H-MRS (mean interval between the two concussions = 9.5 ± 0.7 days), whilst in the remaining 4 athletes the second concussion took place between the second and the third second ^{1}H-MRS (mean interval between the two concussions = 18.5 ± 2.1 days). In athletes 1 and 2, the second concussions were characterized by loss of consciousness (< 2 min). Following the second concussive episode, all athletes again declared to suffer from headache and each of them suffered from at least four additional post-concussive disturbances. Self-reported clinical symptoms following the second concussion disappeared with a mean duration = 41.2 ± 13.0 days ($p < 0.001$ when compared to duration of symptoms observed

after the first concussion). Figures 1 and 2 illustrate the time course changes of NAA (reported in the Figures as the NAA/Cr ratio) in the two doubly concussed athletes (Patients 1 and 2) receiving the second head injury between the 1st and the 2nd ^{1}H-MRS, both showing loss of consciousness < 2 min on field.

CASE	AGE	SEX	SPORT PRACTICED	SYMPTOMS AFTER 1ST IMPACT	PERSISTENCE OF SYMPTOMS AFTER 1ST IMPACT (DAYS)	TIME INTERVAL BETWEEN CONCUSSIONS (DAYS)	SYMPTOMS AFTER 2ND IMPACT	PERSISTENCE OF SYMPTOMS AFTER 2ND IMPACT (DAYS)
1	20	M	Boxe (amateur)	Headache, amnesia	3	10	Loss of consciousness, headache, difficulty in concentrating, irritability, sleep disturbances	52
2	24	M	Rugby	Headache, nausea, retrograde amnesia	4	9	Loss of consciousness, headache, nausea, retrograde amnesia, sleep disturbances, irritability	59
3	32	M	Soccer	Headache, fatigue, nervousness	8	18	Headache, irritability, difficulty in concentrating, "foggy vision", nausea	44
4	27	M	Soccer	Headache, troubling falling asleep	7	16	Headache, nausea, sleep disturbances, irritability, dizziness	35
5	20	M	Kick boxing (light contact)	Headache, sleep disturbances	8	21	Headache, retrograde amnesia, troubling falling asleep, difficulty in concentrating	24
6	33	M	Boxe (amateur)	Headache, anterograde amnesia	5	19	Headache, fatigue, irritability, dizziness, tingling	33

Table 1. Clinical features of doubly concussed athletes. The mean duration of symptom persistence lasted 5.8 ± 2.1 days after the 1st injury and 41.2 ± 13.0 days after the 2nd concussion ($p < 0.001$ when compared to duration of symptoms observed after the first concussion). In both cases, symptoms disappeared much earlier than the time needed for complete NAA restoration.

At the time of the 1st resonance spectrum acquisition (3 days post-injury) both subjects showed a consistent decrease in the NAA/Cr ratio. When effecting the 2nd MRS, notwithstanding athletes were both initially advised to restrain from physical activity, they both declared to have started again their respective sport discipline because of symptom disappearance and to have received a second concussion few days later (mean value between repeat concussions = 9.5 ± 0.7). At this 2nd MRS analysis, the NAA/Cr ratio fell slightly below 1.6 (-23.6% with respect to value in controls), a value very close to that observed in patients suffering from sTBI (Signoretti et al., 2010). In both these athletes, the second concussive episode produced a prolonged loss of consciousness (< 2 min). Both subjects admitted to have experienced, from the beginning up to clinical healing, much more severe and prolonged post concussive symptoms (mean value of symptom persistence = 55.5 days) than those lived following the first impact.
Figures 3, 4, 5 and 6 illustrate the NAA/Cr ratio recorded in four athletes receiving the second concussion between the 2nd and the 3rd MRS.

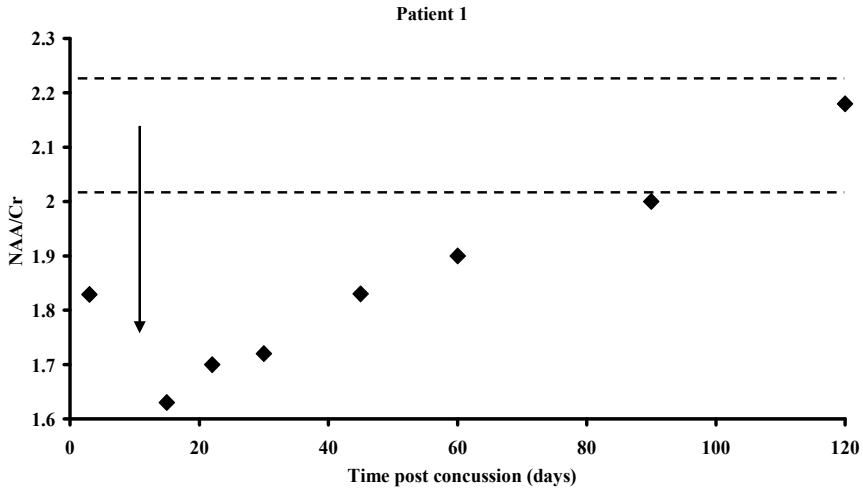


Fig. 1. Change of NAA in doubly concussed athlete. NAA relative to Cr (NAA/Cr ratio) was measured by ^{1}H-MRS in voxels properly positioned in the frontal lobes. The arrow indicates the approximate time of occurrence of the 2nd concussive episode (see Table 1). Dotted lines represent the range interval of the NAA/Cr ratio recorded in control healthy subjects. Notwithstanding prohibition to sustain physical activity, patient 1 restarted physical training immediately after symptom clearance (3 days after the 1st concussion), when NAA/Cr was about 16% below the value recorded in controls.

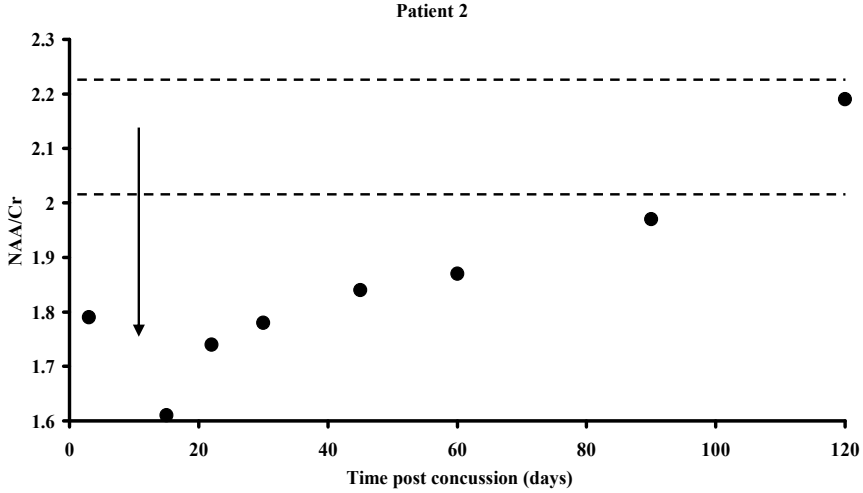


Fig. 2. Change of NAA in doubly concussed athlete. NAA relative to Cr (NAA/Cr ratio) was measured by ^{1}H-MRS in voxels properly positioned in the frontal lobes. The arrow indicates the approximate time of occurrence of the 2nd concussive episode (see Table 1). Dotted lines represent the range interval of the NAA/Cr ratio recorded in control healthy subjects. Notwithstanding prohibition to sustain physical activity, patient 2 restarted physical training immediately after symptom clearance (4 days after the 1st concussion), when NAA/Cr was about 17% below the value recorded in controls.

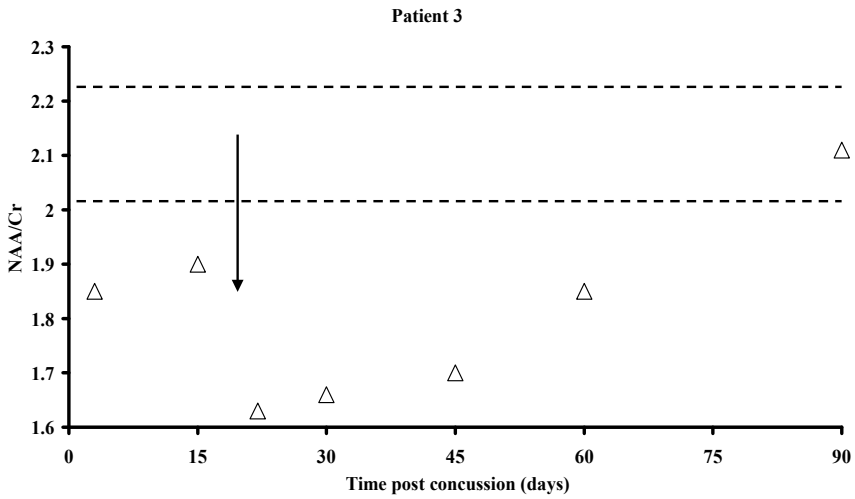


Fig. 3. Change of NAA in doubly concussed athlete. NAA relative to Cr (NAA/Cr ratio) was measured by ^{1}H-MRS in voxels properly positioned in the frontal lobes. The arrow indicates the approximate time of occurrence of the 2nd concussive episode (see Table 1). Dotted lines represent the range interval of the NAA/Cr ratio recorded in control healthy subjects. Notwithstanding prohibition to sustain physical activity, patient 3 restarted physical training immediately after symptom clearance (8 days after the 1st concussion), when NAA/Cr was about 13% below the value recorded in controls.

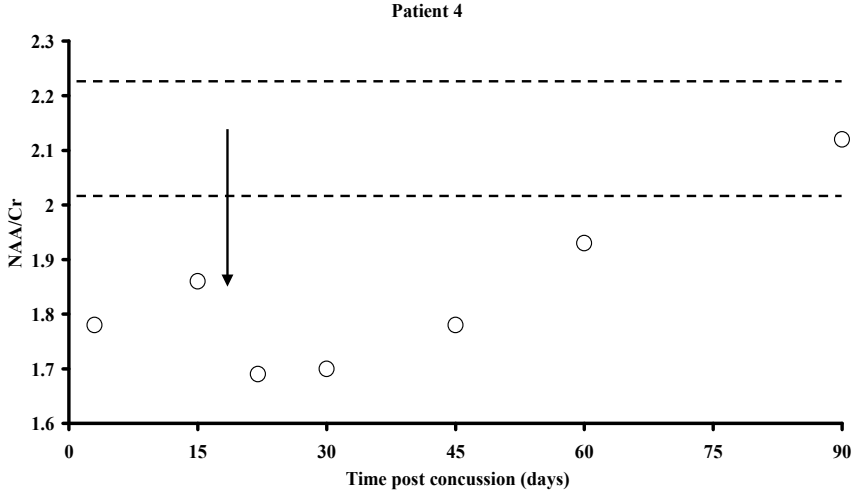


Fig. 4. Change of NAA in doubly concussed athlete. NAA relative to Cr (NAA/Cr ratio) was measured by ^{1}H-MRS in voxels properly positioned in the frontal lobes. The arrow indicates the approximate time of occurrence of the 2nd concussive episode (see Table 1). Dotted lines represent the range interval of the NAA/Cr ratio recorded in control healthy subjects. Notwithstanding prohibition to sustain physical activity, patient 4 restarted physical training immediately after symptom clearance (7 days after the 1st concussion), when NAA/Cr was about 14% below the value recorded in controls.

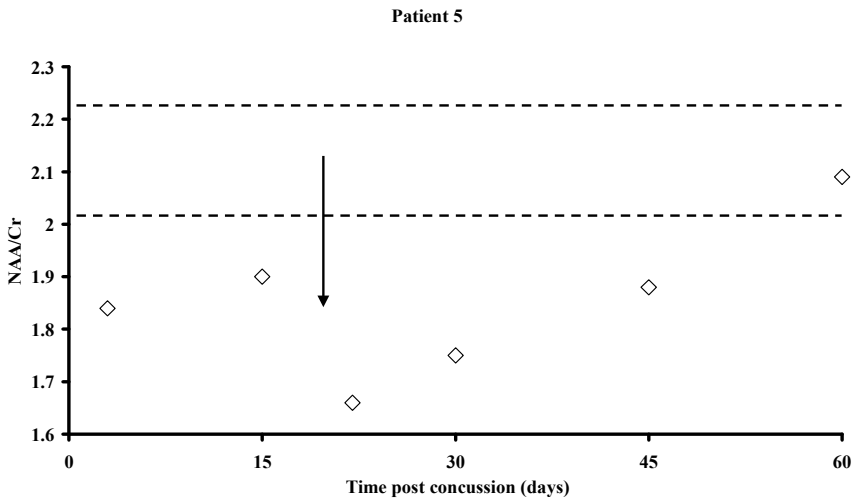


Fig. 5. Change of NAA in doubly concussed athlete. NAA relative to Cr (NAA/Cr ratio) was measured by ^{1}H-MRS in voxels properly positioned in the frontal lobes. The arrow indicates the approximate time of occurrence of the 2nd concussive episode (see Table 1). Dotted lines represent the range interval of the NAA/Cr ratio recorded in control healthy subjects. Notwithstanding prohibition to sustain physical activity, patient 5 restarted physical training immediately after symptom clearance (8 days after the 1st concussion), when NAA/Cr was about 14% below the value recorded in controls.

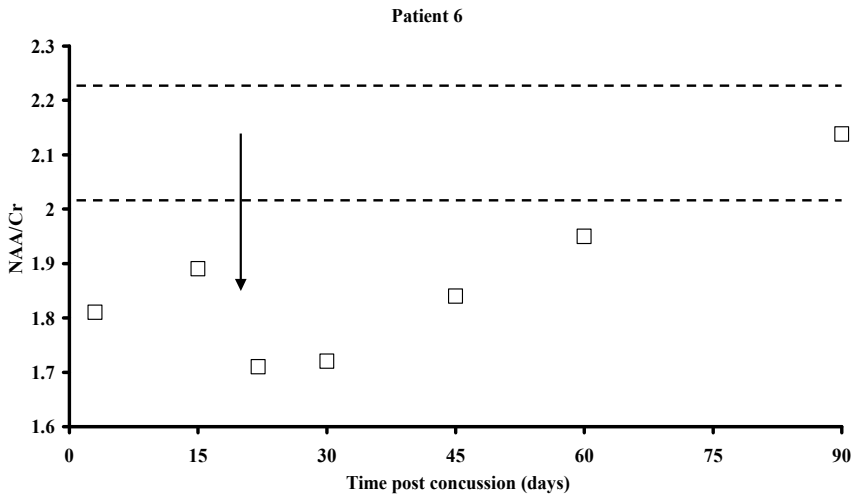


Fig. 6. Change of NAA in doubly concussed athlete. NAA relative to Cr (NAA/Cr ratio) was measured by ^{1}H-MRS in voxels properly positioned in the frontal lobes. The arrow indicates the approximate time of occurrence of the 2nd concussive episode (see Table 1). Dotted lines represent the range interval of the NAA/Cr ratio recorded in control healthy subjects. Notwithstanding prohibition to sustain physical activity, patient 6 restarted physical training immediately after symptom clearance (5 days after the 1st concussion), when NAA/Cr was about 16% below the value recorded in controls.

At the time of the 2nd MRS, the four patients showed a recovery of NAA and affirmed to be symptomless. Both these phenomena allowed athletes to violate the ban on sports so that they started practicing their respective disciplines before completion of brain metabolic recovery. At 22 days post-impact, we found in these subjects a significant further decline in the NAA/Cr ratio, the value of which was even lower than that recorded 3 days post-injury (Figures 3, 4, 5, 6 and 8). During the clinical consult, the four patients declared to have suffered from a second concussion (mean interval between the two concussions = 18.5 ± 2.1 days), interpreted on the field of minor relevance but being of surprisingly remarkable clinical severity and duration (mean value of symptom persistence = 34.0 days). It is worth underlining that in patient 5 completion of brain metabolic recovery was observed at the time of the 6th MRS, i.e. 39 days after the 2nd insult and 15 days after symptom disappearance.

4. Discussion

According to our opinion, data reported in the present study strongly demonstrate that the occurrence of repeat concussion produced a significant increase in the time of recovery of brain metabolism (as evaluated in terms of NAA/Cr variations determined by ^{1}H-MRS), coupled to the appearance of clinical symptoms with increased severity and duration with those reported after a single concussive event (Vagnozzi et al., 2008, 2010). In sports medicine, this finding implies that it should be mandatory for concussed athletes to observe a period of restriction from physical activity until the process of normalization of brain metabolism is completed. Since also in these subjects the clearance of post-concussive clinical symptoms took place much before than the return of NAA to physiological values (Vagnozzi et al., 2008, 2010) it is our advise that monitoring alterations in the biochemistry of post-concussed neurons (NAA changes) by ^{1}H-MRS should be considered a fundamental tool to evaluate recovery of post concussed athletes for their safe return to play.

Supported by abundant literature, it is nowadays worldwide accepted that concussion triggers a cascade of molecular events that transiently alter the biochemistry of the post-concussed neurons, with particular involvement for mitochondrial-dependent energy metabolism. This condition prompted Hovda and coll. to hypothesize the insurgence of a period of brain vulnerability during which a second concussive event may have fatal consequences for the neuronal vitality (Giza & Hovda, 2001; Hovda et al., 1993). Our previous researches in rats undergoing repeat mTBI, using the closed-head weight-drop model of diffuse injury set up by Marmarou and coll. (Foda & Marmarou 1994; Marmarou et al., 1994), clearly demonstrated that, depending on the time interval between injuries, two repeat concussions may cause metabolic cerebral irreversible alterations typical of single sTBI (3 days between concussions) (Tavazzi et al., 2007; Vagnozzi et al., 2007), i.e. cumulative effect of the two concussions. If the two repeat injuries were viceversa spaced by 5 days the changes of brain metabolism are fully reversible and comparable to those recorded in single mTBI, i.e. the two concussions acted as separate events (Tavazzi et al., 2007; Vagnozzi et al., 2007). This strongly indicates the existence of a window of metabolic brain vulnerability during which neurons, when receiving a second insult of even very modest entity, can suffer from dramatic impairment of cell functions. This phenomenon, can be explained by hypothesizing that neurons, after the first mTBI, are deeply involved in the energy-consuming processes to restore cell homeostasis, therefore rendering cells more susceptible to injuries of even very modest entity. The duration for the completion of these

"repairing processes" corresponds to the window of brain vulnerability. In a pilot study in a restricted group of concussed athletes, we first monitored the time course of NAA decrease and recovery following concussion, thereby demonstrating the occurrence of the metabolic brain vulnerability status after an mTBI also in human beings (Vagnozzi et al., 2008). In the same study, we also described 2 cases of doubly concussed athletes who received a second impact during the period of energy metabolism recovery and who therefore underwent to a 15 days delay in complete NAA restoration (Vagnozzi et al., 2008). Recently, we provided unquestionable evidences indicating that the determination of NAA by ^{1}H-MRS is a reliable tool with which monitoring post-concussive periods in athletes. In this last study we demonstrated that results of the MRS analyses were independent on the MR apparatus (different MR suppliers), the field strength adopted (1.5 or 3.0 T) and the mode of spectra acquisition (Vagnozzi et al., 2010). Furthermore, the number of athletes enrolled (n = 40) and serially analyzed allowed to demonstrate that it is possible to determine the period of metabolic brain vulnerability for a safe return of athletes to play. Recently, different research groups confirmed our findings and successfully applied NAA evaluation by MRS to monitor the metabolic recovery of mildly-injured patients (Henry, 2010; Gasparovic et al., 2009; Sarmento et al., 2009; Yeo et al., 2011), thereby strongly corroborating the concept that methods capable of investigating at the molecular level are of great clinical relevance in the surveillance of post-concussed patients. On the other hand, the vast data in literature obtained in different models of mTBI (Barkhoudarian et al, 2011; Signoretti et al., 2010) clearly showed that post-concussive brain modifications are caused by a cascade of molecular events involving cerebral metabolism and, more in general, cerebral biochemistry. At present, in addition to the subjective indication of the patient, cognitive neuropsychological tests are widely used to assess the condition of mildly injured athletes. This type of monitoring has been considered one of the cornerstones for return to play after a concussion (Maroon et al, 2000; McClincy et al., 2006; McCrea et al., 2003; Schatz et al., 2006), even though concerns have been raised, including the question of when they should be used in the management and assessment of concussion (Collie et al., 2006; Gosselin et al., 2006; Randolph et al., 2005). Furthermore, none of the currently available diagnostic tests (Broglio et al., 2007; McCrea et al., 2003; Schatz et al., 2006; Register-Mihalik et al., 2008) are capable of measuring the unique, transient and potentially dangerous state of metabolic vulnerability experienced by the post-concussed brain tissue. Therefore, the need to find objective parameters to evaluate the extent of and recovery from concussion-induced cerebral damage has been stressed recently (Cantu, 2000). Our previous studies (Tavazzi et al., 2005; Vagnozzi et al., 2005, 2008, 2010) and the present research demonstrate that ^{1}H-MRS is capable of detecting significant neurochemical changes present in the injured brain despite the normal appearance of neuroimaging, absence of symptoms and normal neurological examination, i.e. we validated the use of a rapid, objective and sensitive diagnostic tool with which evaluating normalization of cerebral metabolism for a safe return of concussed athletes to play outside the window of metabolic brain vulnerability. Therefore, restraint from physical activity following concussion should be mandatory to avoid the risk of insurgence of SIS, with SIS being interpreted as an acute, fatal disease caused by uncontrolled brain swelling (Bowen, 2003; Cantu, 1998; Cobb & Battin, 2004; Logan et al., 2001; Mori et al., 2006; Saunders & Harbaugh, 2006). Results of the present study suggest that the concept of SIS might certainly be revised and could be broaden to any case of repeat concussion in which, after the second injury, a clear disproportion among the

entity of the concussive event, the post-concussive clinical symptoms and the cerebral metabolic recovery indeed exists. This restricted cohort of doubly concussed athletes could be included within the aforementioned definition of SIS. In fact, notwithstanding all athletes received two repeat concussions (for each athlete, both events were characterized by the same acute symptoms with no change in GCS and negative MRI), clinical symptoms after the 2nd impact lasted much longer than the 1st one, satisfying our first proposed criterium to diagnose SIS. Moreover, in our previous studies we showed that the time to return the NAA/Cr to normal cerebral levels in singly concussed athletes is within 30 days post-impact. In the present cohort of doubly concussed athletes, the time required to measure values of the NAA/Cr ratio similar to those recorded in controls after the second concussion was of 81.2 ± 24.4 days. This much longer time for NAA recovery satisfy the second criterium we proposed to diagnose SIS. Independently on the inclusion in the SIS category, these athletes definitely showed a prolonged time of clearance of clinical symptoms and brain metabolism normalization. In our opinion, monitoring of brain metabolism in singly concussed athletes should drive the timetable of return to physical activity, especially for those practicing sports at risk of recurrent concussions (American football, boxe, ice hockey, rugby, alpine skiing, martial arts, soccer, etc.), according to this possible steps: 1) if upon first examination, NAA is below the value of healthy controls, i.e. altered energy metabolism, the athlete should rest with no physical activity (approximate post-concussion time interval of 1–15 days); 2) if, at the second examination, MRS suggests an initiation of the process of NAA recovery (i.e. quasi-normal energy metabolism), it is advisable that the athlete begin physical activity of increasing intensity (approximate post-concussion time interval of 16–22 days); 3) if, at the third MRS, progressive NAA replenishment is observed (i.e. normalized energy metabolism), then physical activity might be intensified to a 'return to play' level of conditions (approximate post-concussion time interval of 23–30 days); 4) if, at the fourth MRS, normal NAA, i.e. normal energy metabolism, has been determined, it is suitable that athletes be permitted to return to play (approximate post-concussion time interval 30 days). Such a timetable could be adapted to any post-concussed, non-athlete patient and translated into recommendations differing on personal lifestyle during the recovery of NAA post-concussion: 1) NAA below control values (prolonged altered energy metabolism) would recommend rest, with no physical activity and sedentary lifestyle (approximate post-concussion time interval of 1–15 days); 2) signs of initiation of NAA recovery (i.e. quasi-normal energy metabolism) would suggest normal working activity and moderate physical activity (approximate post-concussion time interval of 16–22 days); 3) normal NAA at MRS (i.e. normal energy metabolism re-established) would implicate return to full normal lifestyle (approximate post-concussion time interval of 23–30 days). Results of this and of previous studies (Vagnozzi et al., 2008, 2010) indicated that the kinetic of NAA recovery, following a single concussion, is non-linear, with a very slow phase of about 15-20 days and a second faster period of 10-15 days. We have recently demonstrated that this non-linear time-course of post-traumatic NAA recovery may be due to the cerebral energy imbalance, assessed by high-energy phosphate quantification (ATP, ADP, AMP, etc.), caused mainly by mitochondrial malfunctioning, as indicated by altered mitochondrial phosphorylating capacity (measured by the ATP/ADP ratio) (Signoretti et al., 2010; Tavazzi et al., 2005). Under these conditions, the remarkable decrease in cerebral NAA, which mirrors the changes in brain ATP, may possibly be attributed to the general energy depression consequent to impaired mitochondrial functions (Lifshitz et al., 2003; Robertson

et al., 2006). Incorporating the data obtained in preclinical studies on mTBI, demonstrating decreased ATP concentration for a given period of time post-injury (Tavazzi et al. 2007; Vagnozzi et al., 2005, 2007), it is conceivable that the process of NAA normalization is markedly hindered by an imbalance of neuronal energy metabolism induced by concussion. In fact, NAA synthesis necessarily requires the availability and the energy of hydrolysis of acetyl-CoA (ΔG = -31.2 kJ/mol), working as the acetyl group and energy donor in the acetylation reaction of aspartate catalyzed by ANAT. It is fundamental to understand that when acetyl-CoA is used for NAA synthesis there is an indirect high energy cost to the cell. In fact, since in this case acetyl -CoA will not enter the citric acid cycle (Krebs' cycle) there will be a decrease in the production of reducing equivalents (3 NADH and 1 $FADH_2$) as the fuel for the electron transport chain. Since the oxidative phosphorylation is stoichiometrically coupled to the amount of electron transferred to molecular oxygen by the electron transport chain, the final result will be a net loss of 11 ATP molecules for each NAA molecule newly synthesized. Experimental studies (Signoretti et al., 2010) have shown that spontaneous re-synthesis of NAA occurs only after recovery of mitochondrial dysfunction with consequential return to normal ATP levels; therefore, it appears possible that normalization of NAA concentrations may occur only after the cerebral energy state has fully recovered. The slow normalization of the cell energetic could also be attributed to the drastic decrement of the nicotinic coenzyme pool that was observed in rat models of graded injury. In fact, previous studies (Tavazzi et al., 2005; Vagnozzi et al., 2007) showed the net diminution of the nicotinic coenzyme pool (NAD^+ + NADH and $NADP^+$ + NADPH) that certainly plays a pivotal role in the final result of general depression of cell energy metabolism. This depletion jeopardizes either the reducing equivalent supply to mitochondrial oxidative metabolism, or the catalytic activity of dehydrogenase-mediated oxidoreductive reactions. To date, possible mechanisms for this phenomenon are the hydroxyl radical-induced hydrolysis of the N-glycosidic bond of the reduced forms of the nicotinic coenzymes NADH and NADPH and the activation of the enzyme NAD-glycohydrolase (Lautier et al., 1994). Both mechanisms cause the hydrolysis of these coenzymes and give rise to the same end products, i.e., ADP-ribose(P) and nicotinamide. Independently of the predominant mechanism, the final result is certainly deleterious for the correct functioning of cell metabolism. Finally, the augmentation of poly-ADP ribosylation reactions through the activation of the enzyme poly-ADP ribose polymerase (Du et al., 2003; Nanavaty et al., 2002; Pacher et al., 2002), has been demonstrated to trigger the mechanisms of apoptotic induction (Yu et al., 2002). The overall result should be to significantly contribute to the decrease in the rate of NAA recovery during the time period close to the head insult, when cells are more "metabolically vulnerable" and physical restriction is mandatory to avoid catastrophic consequences.

5. Conclusion

This and previous data (Vagnozzi et al., 2008, 2010) demonstrated that this process can be non-invasively followed in vivo by ^{1}H-MRS giving clinically relevant information concerning the duration of the window of metabolic brain vulnerability. This time interval should be characterized by restriction of physical activity to avoid the occurrence of second concussion with unpredictable consequences, from the delay in cerebral metabolic normalization (such a delay being not yet defined in duration) to the onset of uncontrolled brain edema (i.e., the current definition of SIS). In our opinion, we again provided the

experimental evidence in the dramatic discrepancy between the time required for the clearance of post-concussive clinical symptoms and the time needed to restore concussion-perturbed brain metabolism. Since ^{1}H-MRS is the analytical method of choice and NAA the biochemical parameter indirectly representing the brain energy metabolism, it should be strongly suggest ed to determine healing of post-concussed athletes and patients using this potent diagnostic tool. In light of the consequences of a second concussive event during the window of brain vulnerability, potentially catastrophic, it should be strongly recommended that the restriction of physical activity is mandatory and that the removal of this restriction is submitted to the full recovery of the NAA physiological level. Due to the potential catastrophic consequences of repeat concussions and the need to have clear diagnostic tools and protocols to study recovery of the post-concussed brain it is fundamental to undertake further studies to better understand these topics.

6. Acknowledgement

This work has been supported in part by research funds of the three Universities involved (Catania, Rome "Tor Vergata", Rome Catholic "Sacro Cuore").

7. References

Alexander, M.P. (1995). Mild traumatic brain injury: pathophysiology, natural history, and clinical management. *Neurology*, Vol. 45, No.7, (July 1995), pp. 1253–1260, ISSN 0028-3878

Barker P.B.; Soher, B.J.; Blackband, S.J.; Chatham, J.C.; Mathews, V.P. & Bryan, R.N. (1993). Quantitation of proton NMR spectra of the human brain using tissue water as an internal concentration reference. *NMR in Biomedicine*, Vol.6, No.1, (January-February 1993), pp. 89–94, ISSN 0952-3480

Barkhoudarian, G.; Hovda, D.A. & Giza, C.C. (2011). The molecular pathophysiology of concussive brain injury. *Clinics in sports medicine,* Vol.30, No.1, (January 2011), pp. 33-39, ISSN 0278-5919

Barth, J.T.; Freeman, J.R.; Broshek, D.K. & Varney, R.N. (2001). Acceleration-deceleration sport-related concussion: the gravity of it all. *Journal of Athletic Training*, Vol.36, No.3, (September 2001), pp. 253–256, ISSN 1062-6050

Baslow, M.H. (2003a). Brain N-acetylaspartate as a molecular water pump and its role in the etiology of Canavan disease: A mechanistic explanation. *Journal of Molecular Neuroscience*, Vol.21, No. 3, pp. 185–190, ISSN 0895-8696

Baslow, M.H. (2003b). N-acetylaspartate in the vertebrate brain: Metabolism and function. *Neurochemical Research*, Vol.28, No.6, (June 2003), pp. 941–953, ISSN 0364-3190

Bergsneider, M.; Hovda,. D.A.; Lee, S.M.; Kelly, D.F.; McArthur, D.L.; Vespa, P.M.; Lee, J.H.; Huang, S.C.; Martin, N.A.; Phelps, M.E. & Becker, D.P. (2000). Dissociation of cerebral glucose metabolism and level of consciousness during the period of metabolic depression following human traumatic brain injury. *Journal of Neurotrauma*, Vol.17, No.5, (May 2000), pp. 389–401, ISSN 0897-7151

Bigler, E.D. (2003). Neurobiology and neuropathology underlie the neuropsychological deficits associated with traumatic brain injury. *Archives of Clinical Neuropsychology*, Vol.18, No.6, (August 2003), pp. 595–627, ISSN 0887-6177

Bowen, A.P. (2003). Second impact syndrome: A rare; catastrophic; preventable complication of concussion in young athletes. *Journal of Emergency Nursery*, Vol.29, No.3, (June 2003), pp. 287–289, ISSN 0099-1767

Broglio, S.P.; Ferrara, M.S., Macciocchi, S.N.; Baumgartner, T.A. & Elliott, R. (2007). Test-retest reliability of computerized concussion assessment programs. *Journal of athletic training*, Vol.42, No.4, (October-December 2007), pp. 509-514, ISSN 1062-6050

Brooks, W.M.; Friedman, S.D. & Gasparovic, C. (2001). Magnetic resonance spectroscopy in traumatic brain injury. *Journal of Head Trauma Rehabilitation*, Vol.16, No.2, (April 2001), pp. 149–164, ISSN 0885-9701

Bruns, J. Jr. & Hauser, W.A. (2003). The epidemiology of traumatic brain injury: a review. *Epilepsia*, Vol.44, Suppl.10, (September 2003), pp. 2–10, ISSN 0013-9580

Cantu, R.C. (1998). Second-impact syndrome. *Clinics in Sports Medicine*, Vol.17, No.1, (January 1998), pp. 37–44, ISSN 0278-5919

Cantu, R.C. & Voy, R. (1995). Second impact syndrome: a risk in any contact sport. *The Physician and Sport Medicine,* Vol.23, No.6, (June 1995), pp. 27-34, ISSN 0091-3847

Cantu, R.C. (2000). Malignant brain edema and Second Impact Syndrome. In: *Neurologic Athletic Head and Spine Injuries*, R.C. Cantu, (Ed.), 132-137, ISBN 072-1683-39-8, WB Saunders Company, Michigan , USA

Cantu, R.C. (2003). Recurrent athletic head injury: risks and when to retire. *Clinics in Sports Medicine*, Vol.22, No.3, (July 2003), pp. 593–603, ISSN 0278-5919

Cantu, R.C. (2007). Athletic concussion: current understanding as of 2007. *Neurosurgery,* Vol.60, No.6, (July 2007), pp. 963–964, ISSN 1524-4040

Cobb, S. & Battin, B. (2004). Second-impact syndrome. *Journal of School Nursing*, Vol.20, No.5, (October 2004), pp. 262–267, ISSN 1050-8405

Collie, A.; Makdissi, M.; Maruff, P.; Bennell, K. & McCrory, P. (2006). Cognition in the days following concussion: comparison of symptomatic versus asymptomatic athletes *Journal of Neurology, Neurosurgery, and Psychiatry*, Vol.77, No.2, (February 2006), pp. 241-245, ISSN 0022-3050

Delaney, J.S.; Abuzeyad, F.; Correa, J.A. & Foxford, R. (2005). Recognition and characteristics of concussions in the emergency department population. *The Journal of Emergency Medicine*, Vol.29, No.2, (August 2005), pp. 189–197, ISSN 0736-4679

Du, L.; Zhang, X.; Han, Y.Y.; Burke, N.A.; Kochanek, P.M.; Watkins, S.C.; Graham, S.H.; Carcillo, J.A.; Szabó, C. & Clark, R.S. (2003). Intra-mitochondrial poly(ADP-ribosylation) contributes to NAD+ depletion and cell death induced by oxidative stress. *The Journal of Biological Chemistry*, Vol.278, No.20, (May 2003), pp. 18426–18433, ISSN 0021-9258

Fabbri, A.; Servadei, F.; Marchesini, G.; Negro, A. & Vandelli, A. (2010). The changing face of mild head injury: temporal trends and patterns in adolescents and adults from 1997 to 2008. *Injury*, Vol.41, No.9, (September 2010), pp. 913-917, ISSN 1879-0267

Foda, M.A. & Marmarou, A. (1994). A new model of diffuse brain injury in rats: part II. Morphological characterization. *Journal of Neurosurgery*, Vol.80, No.2, (February 1994), pp. 301-313, ISSN 0022-3085

Friedman, S.D.; Brooks, W.M.; Jung, R.E.; Chiulli, S.J.; Sloan, J.H.; Montoya, B.T.; Hart, B.L. & Yeo, R.A. (1999). Quantitative proton MRS predicts outcome after traumatic brain injury. *Neurology*, Vol.52, No.7, (April 1999), pp. 1384–1391, ISSN 0028-3878

Garnett, M.R.; Blamire, A.M.; Corkill, R.G.; Cadoux-Hudson, T.A.; Rajagopalan, B. & Styles, P. (2000). Early proton magnetic resonance spectroscopy in normal-appearing brain correlates with outcome in patients following traumatic brain injury. *Brain*, Vol.123, No.10, (October 2000), pp. 2046–2054, ISSN 0006-8950

Gasparovic, C.; Yeo, R.A.; Mannell, M.; Ling, J.; Elgie, R.; Phillips, J.; Doezema, D. & Mayer, A.R. (2009). Neurometabolite concentrations in gray and white matter in mild traumatic brain injury: an H-1-magnetic resonance spectroscopy study. *Journal of Neurotrauma*, Vol.26, No.10, (October 2009), pp. 1635-1643, ISSN 0897-7151

Giza, C.C. & Hovda, D.A. (2001). The neurometabolic cascade of concussion. *Journal of Athletic Training*, Vol.36, No.3, (September 2001), pp. 228-235, ISSN 1062-6050

Gosselin, N.; Lassonde, M.; Petit, D.; Leclerc, S.; Mongrain, V.; Collie, A. & Montplaisir, J. (2009). Sleep following sport-related concussions. *Sleep Medicine*, Vol.10, No.1, (January 2009), pp. 35–46, ISSN 1389-9457

Gosselin, N.; Theriault, M.; Leclerc, S.; Montplaisir, J. & Lassonde, M. (2006). Neurophysiological anomalies in symptomatic and asymptomatic concussed athletes. *Neurosurgery*, Vol.58, No.6, (June 2006), pp. 1151-1161, ISSN 1524-4040

Gouvier, W.D.; Cubic, B.; Jones, G.; Brantley, P. & Cutlip, Q. (1992). Postconcussion symptoms and daily stress in normal and head-injured college populations. *Archives of Clinical Neuropsychology*, Vol.7, No.3, (March 1992), pp. 193–211, ISSN 0887-6177

Guskiewicz, K.M.; Bruce, S.L.; Cantu, R.C.; Ferrara, M.S.; Kelly, J.P.; McCrea, M. & National Athletic Trainers' Association. (2006). Research based recommendations on management of sport related concussion: summary of the National Athletic Trainers' Association position statement. *British Journal of Sports Medicine*, Vol.40, No.1, (January 2006) , pp. 6–10, ISSN 0306-3674

Guskiewicz, K.M.; McCrea, M.; Marshall, S.W.; Cantu, R.C.; Randolph, C.; Barr, W.; Onate, J.A. & Kelly, J.P. (2003). Cumulative effects associated with recurrent concussion in collegiate football players: the NCAA Concussion Study. *JAMA*, Vol.290, No.19, (November 2003), pp. 2549–2555, ISSN 0098-7484

Henry, L.C.; Tremblay, S.; Boulanger, Y.; Ellemberg, D. & Lassonde, M. (2010). Neurometabolic changes in the acute phase after sports concussions correlate with symptom severity. *Journal of Neurotrauma*, Vol.27, No.1, (January 2010), pp. 65-76, ISSN 0897-7151

Hovda, D.A.; Badie, H.; Karimi, S.; Thomas, S.; Yoshino, A. & Kawamata T. (1993). Concussive brain injury produces a state of vulnerability for intracranial pressure perturbation in the absence of morphological damage. In: *Intracranial pressure VIII*. C.J.J. Avezaat, J.H.M. van Eijndhoven, A.I.R. Maas & J.T. Tans, (Eds.), 469–472, ISBN 038-7559-46-9, Springer-Verlag, Berlin

Hunt, T. & Asplund, C. (2010). Concussion assessment and management. *Clinics in Sports Medicine*, Vol.29, No.1, (January 2010), pp. 5–17, ISSN 0278-5919

Ingebrigtsen, T.; Romner, B. & Kock-Jensen, C. (2000). Scandinavian guidelines for initial management of minimal, mild, and moderate head injuries. *The Journal of Trauma*, Vol.48, No.4, (April 2000), pp. 760–766, ISSN 0022-5282

Kay, T.; Newman, B.; Cavallo, M.; Ezrachi, O. & Resnick, M. (1992). Toward a neuropsychological model of functional disability after mild traumatic brain injury. *Neuropsychology*, Vol.6, No.4, (October 1992), pp. 371–384, ISSN 0894-4105

Kissick, J. & Johnstone, K.M. (2005). Return to play after concussion: principles and practice. *Clinical Journal of Sport Medicine*, Vol.15, No.6, (November 2005), pp. 426–431, ISSN 1536-3724

Kurca, E.; Siva, K.S. & Kucera, P. (2006). Impaired cognitive functions in mild traumatic brain injury patients with normal and pathologic magnetic resonance imaging. *Neuroradiology*, Vol.48, No.9, (September 2006), pp. 661–669, ISSN 0028-3940

Lautier, D.; Hoflack, J.C.; Kirkland, J.B.; Poirier, D. & Poirier, G.G. (1994). The role of poly(ADP-ribose) metabolism in response to active oxygen cytotoxicity. *Biochimica et biophysica acta*, Vol.1221, No.3, (April 1994), pp. 215–220, ISSN 0006-3002

Lifshitz, J.; Sullivan, P.G.; Hovda, D.A.; Wieloch, T. & McIntosh, T.K. (2004). Mitochondrial damage and dysfunction in traumatic brain injury. *Mitochondrion*, Vol.4, No.5-6, (September 2004), pp. 705-713, ISSN 1567-7249

Livingston, D.H.; Lavery, R.F.; Passannante, M.R.; Skurnick, J.H.; Baker, S.; Fabian, T.C.; Fry, D.E. & Malangoni, M.A. (2000). Emergency department discharge of patients with a negative cranial computed tomography scan after minor head injury. *Annals of Surgery*, Vol.232, No.1, (July 2000), pp. 126-132, ISSN 0003-4932

Lloyd, D.A.; Carty, H.; Patterson, M.; Butcher, C.K. & Roe, D. (1997). Predictive value of skull radiography for intracranial injury in children with blunt head injury. *Lancet*, Vol.349, No.9055, (March 1997), pp. 821-824, ISSN 0140-6736

Logan, S.M.; Bell, G.W. & Leonard, J.C. (2001). Acute subdural hematoma in a high school football player after 2 unreported episodes of head trauma: A case report. *Journal of Athletic Training*, Vol.36, No.4, (December 2001), pp. 433–436, ISSN 1062-6050

Longhi, L.; Saatman, K.E.; Fujimoto, S.; Raghupathi, R.; Meaney, D.F.; Davis, J.; McMillan, B.S.A.; Conte, V.; Laurer, H.L. Stein, S.; Stocchetti N. & McIntosh, T.K. (2005). Temporal window of vulnerability to repetitive experimental concussive brain injury. *Neurosurgery*, Vol.56, No.2, (February 2005), pp. : 364–374, ISSN 1524-4040

Lovell, M.; Collins, M. & Bradley, J. (2004). Return to play following sports-related concussion. *Clinics in Sports Medicine*, Vol.23, No.3, (July 2004), pp. 421-441, ISSN 0278-5919

Marmarou, A.; Foda, M.A.; van den Brink, W.; Campbell, J.; Kita, H. & Demetriadou, K. (1994). A new model of diffuse brain injury in rats: part I. Pathophysiology and biomechanics. *Journal of Neurosurgery*, Vol.80, No.2, (February 1994), pp. 291–300, ISSN 0022-3085

Maroon, J.C.; Lovell, M.R.; Norwig, J.; Podelek, K.; Powell, J.W. & Hartl, R. (2000). Cerebral concussion in athletes: evaluation and neuropsychological testing. *Neurosurgery*, Vol.47, No.3, (September 2000), pp. 659-669, ISSN 1524-4040

McClincy, M.P.; Lovell, M.R.; Pardini, J.; Collins, M.W. & Spore, M.K. (2006). Recovery from sports concussion in high school and collegiate athletes. *Brain Injury*, Vol.20, No.1, (January 2006), pp. 33–39, ISSN 0269-9052

McCrea, M.; Guskiewicz, K.M.; Marshall, S.W.; Barr, W.; Randolph, C.; Cantu, R.C.; Onate, J.A.; Yang, J. & Kelly, J.P. (2003). Acute effects and recovery time following concussion in collegiate football players: the NCAA Concussion Study. *JAMA*, Vol.290, No.19, (November 2003), pp. 2556-2563, ISSN 0098-7484

McCrea, M.; Hammeke, T.; Olsen, G.; Leo, P. & Guskiewicz, K. (2004). Unreported concussion in high school football players: implications for prevention. *Clinical Journal of Sport Medicine*, Vol.14, No.1, (January 2004), pp. 13–17, ISSN 1536-3724

McCrory, P.R.; Meeuwisse, W.; Johnston, K.; Dvorak, J.; Aubry, M.; Molloy, M. & Cantu, R.C. (2009). Consensus statement on Concussion in Sport 3rd International Conference on Concussion in Sport held in Zurich, November 2008. *Clinical Journal of Sport Medicine,* Vol.19, No.3, (May 2009), pp. 185-200, ISSN 1536-3724

McCrory, P.R. & Berkovic, S.F. (2001). Concussion: The history of clinical and pathophysiological concepts and misconceptions. *Neurology*, Vol.12, No.12, (December 2001), pp. 2283–2289, ISSN 0028-3878

McCrory, P.R. (2001). Does second impact syndrome exist? *Clinical Journal of Sport Medicine*, Vol.11, No.3, (July 2001), pp. 144–149, ISSN 1536-3724

Meehan, W.P. 3rd & Bachur, R.G. (2009). Sport-related concussion. *Pediatrics,* Vol.123, No.1, (January 2009), pp. 114–123, ISSN 0031-4005

Mitsumoto, H.; Ulug, A.M.; Pullman, S.L.; Gooch, C.L.; Chan, S.; Tang, M.X.; Man, X.; Hays, A.P.; Floyd, A.G.; Battista, V.; Montes, J.; Hayes, S.; Dashnaw, S.; Kaufmann, P.; Gordon, P.H.; Hirsch, J.; Levin, B.; Rowland L.P. & Shungu, D.C. (2007). Quantitative objective markers for upper and lower motor neuron dysfunction in ALS. *Neurology*, Vol.68, No.17, (April 2007), pp. 1402–1410, ISSN 0028-3878

Miyake, M.; Kakimoto, Y. & Sorimachi, M. (1981). A gas chromatographic method for the determination of N-acetyl-L-aspartic acid; N-acetyl-alpha-aspartylglutamic acid and beta-citryl-L-glutamic acid and their distributions in the brain and other organs of various species of animals. *Journal of Neurochemistry*, Vol.36, No.3, (March 1981), pp. 804–810, ISSN 0022-3042

Moffett, J.R.; Namboodiri, M.A.; Cangro, C.B. & Neale, J.H. (1991). Immunohistochemical localization of *N*-acetylaspartate in rat brain. *Neuroreport*, Vol.2, No.3, (March 1991), pp.131–134; ISSN 0959-4965

Mori, T.; Katayama, Y. & Kawamata, T. (2006). Acute hemispheric swelling associated with thin subdural hematomas: Pathophysiology of repetitive head injury in sports. *Acta Neurochirurgica Supplement,* Vol.96, pp. 40–43; ISSN ISSN 0001-6268

Nanavaty, U.B.; Pawliczak, R.; Doniger, J.; Gladwin, M.T.; Cowan, M.J.; Logun, C. & Shelhamer, J.H. (2002). Oxidant-induced cell death in respiratory epithelial cells is due to DNA damage and loss of ATP. *Experimental lung research*, Vol.28, No.8, (December 2002), pp. 591–607, ISSN 0190-2148

Nugent, G.R. (2006). Reflections on 40 years as a sideline physician. *Neurosurgical Focus*, Vol.21, No.4, (October 2006), pp. E2, ISSN 1092-0684

Pacher, P.; Liaudet, L.; Mabley, J.; Komjáti, K. & Szabó, C. (2002). Pharmacologic inhibition of poly(adenosine diphosphate-ribose) polymerase may represent a novel therapeutic approach in chronic heart failure. *Journal of the American College of Cardiology*, Vol.40, No.5, (September 2002), pp. 1006–1016, ISSN 0735-1097

Pellman; E.J.; Viano, D.C.; Casson, I.R.; Tucker, A.M.; Waeckerle, J.F.; Powell, J.W. & Feuer, H. (2004). Concussion in professional football. Repeat injuries—Part 4. *Neurosurgery*, Vol.55, No.4, (October 2004), pp. 860–873, ISSN 1524-4040

Ponsford, J. (2005). Rehabilitation interventions after mild head injury. *Current Opinion in Neurology*, Vol.18, No.6, (December 2005), pp. 692–697, ISSN 1350-7540

Praticò, D.; Reiss, P.; Tang, L.X.; Sung, S.; Rokach, J. & McIntosh, T.K. (2002). Local and systemic increase in lipid peroxidation after moderate experimental traumatic brain injury. *Journal of Neurochemistry*, Vol.80, No.5, (March 2002), pp. 894–898, ISSN 0022-3042

Randolph, C.; McCrea, M. & Barr, W.B. (2005). Is neuropsychological testing useful in the management of sport-related concussion? *Journal of Athletic Training*, Vol.40, No.3, (July-September 2005), pp. 139-152, ISSN 1062-6050

Randolph, C.; Millis, S.; Bar, W.B.; McCrea, M.; Guskiewicz, K.M.; Hammeke, T.A. & Kelly, J.P. (2009). Concussion symptom inventory: an empirically derived scale for monitoring resolution of symptoms following sport-related concussion. *Archives of Clinical Neuropsychology*, Vol.24, No.3, (May 2009), pp. 219–229, ISSN 0887-6177

Register-Mihalik, J.K.; Mihalik, J.P. & Guskiewicz, K.M. (2008). Balance deficits after sports-related concussion in individuals reporting posttraumatic headache. *Neurosurgery*, Vol.63, No.1, (July 2008), pp. 76-80, ISSN 1524-4040

Robertson, C.L.; Soane, L.; Siegel, Z.T. & Fiskum, G. (2006). The potential role of mitochondria in pediatric traumatic brain injury. *Developmental Neuroscience*, Vol.28, No.4-5, pp. 432–446, ISSN 0378-5866

Sarmento, E.; Moreira, P.; Brito, C.; Souza, J.; Jevoux, C. & Bigal, M. (2009). Proton spectroscopy in patients with post-traumatic headache attributed to mild head injury. *Headache,* Vol.49, No.9, (October 2009), pp. 1345-1352, ISSN 0017-8748

Saunders, R.L. & Harbaugh, R.E. (1984). The second impact in catastrophic contact-sports head trauma. *JAMA*, Vol.252, No.4, (July 1984), pp. 538–539, ISSN 0098-7484

Schatz, P.; Pardini, J.E.; Lovell, M.R.; Collins, M.W. & Podell, K. (2006). Sensitivity and specificity of the ImPACT Test Battery for concussion in athletes. *Archives of Clinical Neuropsychology*, Vol.21, No.1, (January 2006), pp. 91-99, ISSN 0887-6177

Shackford, S.R.; Wald, S.L.; Ross, S.E.; Cogbill, T.H.; Hoyt, D.B.; Morris, J.A.; Mucha, P.A.; Pachter, H.L.; Sugerman, H.J.; O'Malley, K. et al. (1992). The clinical utility of computed tomographic scanning and neurologic examination in the management of patients with minor head injuries. *The Journal of Trauma,* Vol.33, No. 3, (September 1992), pp. 385-394, ISSN 0022-5282

Signoretti, S.; Di Pietro, V.; Vagnozzi, R.; Lazzarino, G.; Amorini, A.M.; Belli, A.; D'Urso, S. & Tavazzi, B. (2010). Transient alterations of creatine, creatine phosphate, N-acetylaspartate and high-energy phosphates after mild traumatic brain injury in the rat. *Molecular and Cellular Biochemistry,* Vol. 333, No.1-2, (January 2010), pp. 269-277, ISSN 0300-8177

Swann, I.J.; MacMillan, R. & Strong, I. (1981). Head injuries at an inner city accident and emergency department. *Injury*, Vol.12, No.4, (January 1981), pp. 274-278, ISSN 1879-0267

Tagliaferri, F.; Compagnone, C.; Korsic, M.; Servadei, F. & Kraus, J. (2006). A systematic review of brain injury epidemiology in Europe. *Acta Neurochirurgica (Wien),* Vol.148, No.3, (November 2005), pp. 255–268, ISSN 0001-6268

Taheri, P.A.; Karamanoukian, H.; Gibbons, K.; Waldman, N.; Doerr, R.J. & Hoover, E.L. (1993). Can patients with minor head injuries be safely discharged home? *Archives of Surgery*, Vol.128, No.3, (March 1993), pp. 289-292, ISSN 0272-5533

Tavazzi, B.; Lazzarino, G.; Leone, P.; Amorini, A.M.; Bellia, F.; Janson, C.G.; Di Pietro, V.; Ceccarelli, L.; Donzelli, S.; Francis, J.S. & Giardina, B. (2005). Simultaneous high performance liquid chromatographic separation of purines; pyrimidines; N-acetylated amino acids; and dicarboxylic acids for the chemical diagnosis of inborn errors of metabolism. *Clinical Biochemistry*, Vol.38, No.11, (November 2005), pp. 997–1008, ISSN 0009-9120

Tavazzi, B.; Vagnozzi, R.; Signoretti, S.; Amorini, A.M.; Belli, A.; Cimatti, M.; Delfini, R., Di Pietro, V.; Finocchiaro, A. & Lazzarino, G. (2007). Temporal window of metabolic brain vulnerability to concussions: oxidative and nitrosative stresses-part II. *Neurosurgery*, Vol.61, No.2, (August 2007), pp. 390–396, ISSN 1524-4040

Truckenmiller, M.E.; Namboodiri, M.A.; Brownstein, M.J. & Neale, J.H. (1985). N-Acetylation of L-aspartate in the nervous system: differential distribution of a specific enzyme. *Journal of Neurochemistry,* Vol.45, No.5, (November 1985), pp. 1658–1662, ISSN 0022-3042

Vagnozzi, R.; Marmarou, A.; Tavazzi, B.; Signoretti, S.; Di Pierro, D.; del Bolgia, F.; Amorini, A.M.; Fazzina, G.; Sherkat, S. & Lazzarino, G. (1999). Changes of cerebral energy metabolism and lipid peroxidation in rats leading to mitochondrial dysfunction after diffuse brain injury. *Journal of Neurotrauma*, Vol.16, No.10, (October 1999), pp. 903–913, ISSN 0897-7151

Vagnozzi, R.; Signoretti, S.; Tavazzi, B.; Cimatti, M.; Amorini, A.M.; Donzelli, S.; Delfini, R. & Lazzarino, G. (2005). Hypothesis of the post-concussive vulnerable brain: experimental evidence of its metabolic occurrence. *Neurosurgery*, Vol.57, No.1, (July 2005), pp. 164–171, ISSN 1524-4040

Vagnozzi, R.; Tavazzi, B.; Signoretti, S.; Amorini, A.M.; Belli, A.; Cimatti, M.; Delfini, R.; Di Pietro, V.; Finocchiaro, A. & Lazzarino, G. (2007). Temporal window of metabolic brain vulnerability to concussions: mitochondrial-related metabolic impairment-part I. *Neurosurgery,* Vol.61, No.2, (August 2007), pp. 379–389, ISSN 1524-4040

Vagnozzi, R.; Signoretti, S.; Tavazzi, B.; Floris, R.; Ludovici, A.; Marziali, S.; Tarascio, G.; Amorini, A.M.; Di Pietro, V.; Delfini, R. & Lazzarino, G. (2008). Temporal window of metabolic brain vulnerability to concussion: a pilot ^{1}H-MRS study in concussed athletes-part III. *Neurosurgery*, Vol.62, No.6, (June 2008), pp. 1286-1295, ISSN 1524-4040

van der Naalt, J. (2001). Prediction of outcome in mild to moderate head injury: a review. *Journal of Clinical and Experimental Neuropsychology*, Vol.23, No.6, (December 2001), pp. 837–851, ISSN 1380-3395

Vos, P.E.; Battistin, L.; Birbamer, G., Gerstenbrand, F.; Potapov, A.; Prevec, T.; Stepan, Ch.A.; Traubner, P.; Twijnstra, A.; Vecsei, L. & von Wild, K. (2002). EFNS guideline on mild traumatic brain injury: report of an EFNS task force. *European Journal of Neurology*, Vol.9, No.3, (May 2002), pp. 207-219, ISSN 1468-1331

Yates, D.; Aktar, R.; Hill, J. & Guideline Development Group. (2007). Assessment, investigation, and early management of head injury: summary of NICE guidance. *British Medical Journal*, Vol.335, No.7622, (October 2007), pp. 719-720, ISSN 0959-8146

Yeo, R.A.; Gasparovic, C.; Merideth, F.; Ruhl, D.; Doezema, D. & Mayer, A.R. (2011). A longitudinal proton magnetic resonance spectroscopy study of mild traumatic brain injury. *Journal of Neurotrauma*, Vol.28, No.1, (January 2011), pp. 1-11, ISSN 0897-7151

Yu, S.W.; Wang, H.; Poitras, M.F.; Coombs, C.; Bowers, W.J.; Federoff, H.J.; Poirier, G.G.; Dawson, T.M. & Dawson, V.L. (2002). Mediation of poly(ADP-ribose) polymerase-1-dependent cell death by apoptosis-inducing factor. *Science,* Vol.297, No.5579, (July 2002), pp. 259–263, ISSN 0036-8075

Edited by Amit Agrawal

The present two volume book "Brain Injury" is distinctive in its presentation and includes a wealth of updated information on many aspects in the field of brain injury. The Book is devoted to the pathogenesis of brain injury, concepts in cerebral blood flow and metabolism, investigative approaches and monitoring of brain injured, different protective mechanisms and recovery and management approach to these individuals, functional and endocrine aspects of brain injuries, approaches to rehabilitation of brain injured and preventive aspects of traumatic brain injuries. The collective contribution from experts in brain injury research area would be successfully conveyed to the readers and readers will find this book to be a valuable guide to further develop their understanding about brain injury.

ISBN 978-953-51-5259-0

IntechOpen