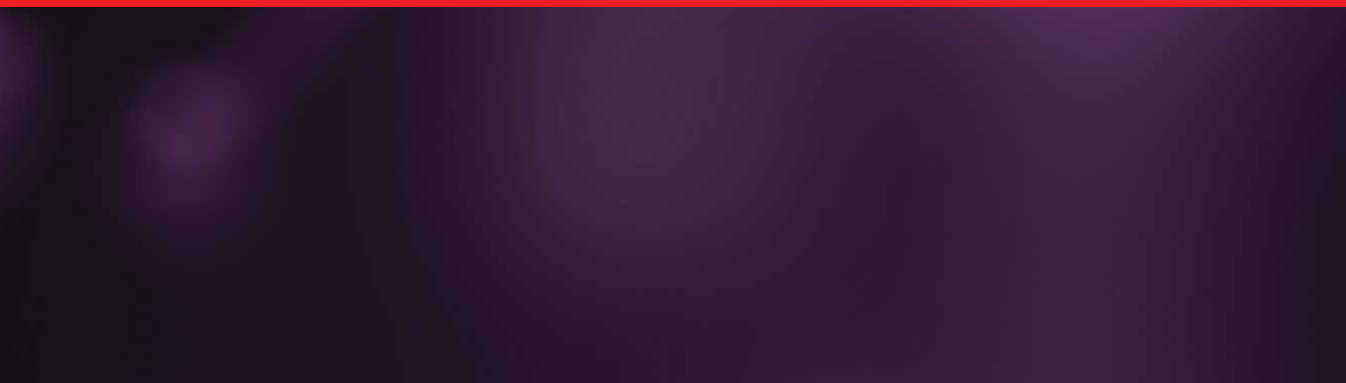
IntechOpen

IntechOpen Series
Biochemistry, Volume 28

Reactive Oxygen Species

Edited by Rizwan Ahmad



Reactive Oxygen Species

Edited by Rizwan Ahmad

Published in London, United Kingdom

Reactive Oxygen Species
http://dx.doi.org/10.5772/intechopen.94870
Edited by Rizwan Ahmad

Contributors
Pavel Krystynik, Lokesh Gambhir, Neha Kapoor, Richa Bhardwaj, Gaurav Sharma, Garima Tyagi, Johnson Olaleye Oladele, Adenike T. Oladiji, Oluwaseun Titilope Oladele, Oyedotun M. Oyeleke, Radheshyam Maurya, Madhulika Namdeo, Pambu Lelo Aaron, Zakuani Luzinga Nadege, Fabrice Ndayisenga, Bongo Ngiala Gedeon, Rafael Ovalle, Giuseppe Gallo, Sung Soo Kim, Dongjin Yeo, Tae Gyu Choi, Nurhayat Atasoy, Ufuk Mercan Yücel, Jyotsnarani Pradhan, Bristy Ganguly, Manisha Hota, Joshua Banda, Alan K. Chang, Vladimir Djordjevic, Manoj Parihar, Asha Kumari, Mahendar Singh Bhinda, Sachin Sharma, Manoj Kumar Chitara, Ashim Debnath, Chandan Maharana, Binny Sharma, ARNAB MAITY, Thiago Gomes Gomes Heck, Pauline Brendler Goettems Fiorin, Mirna Stela Stela Ludwig, Matias Nunes Frizzo, Rizwan Ahmad, Mohammad Yusuf Hasan, Haseeb Ahsan

First published in London, United Kingdom, 2022 by IntechOpen
IntechOpen is the global imprint of INTECHOPEN LIMITED, registered in England and Wales, registration number: 11086078, 5 Princes Gate Court, London, SW7 2QJ, United Kingdom
Printed in Croatia

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

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

Reactive Oxygen Species
Edited by Rizwan Ahmad
p. cm.

This title is part of the Biochemistry Book Series, Volume 28
Topic: Cell and Molecular Biology
Series Editor: Miroslav Blumenberg
Topic Editor: Rosa María Martínez-Espinosa

Print ISBN 978-1-83968-281-0
Online ISBN 978-1-83968-282-7
eBook (PDF) ISBN 978-1-83968-283-4
ISSN 2632-0983

IntechOpen Book Series

Biochemistry

Volume 28

Aims and Scope of the Series

Biochemistry, the study of chemical transformations occurring within living organisms, impacts all of the life sciences, from molecular crystallography and genetics, to ecology, medicine and population biology. Biochemistry studies macromolecules - proteins, nucleic acids, carbohydrates and lipids –their building blocks, structures, functions and interactions. Much of biochemistry is devoted to enzymes, proteins that catalyze chemical reactions, enzyme structures, mechanisms of action and their roles within cells. Biochemistry also studies small signaling molecules, coenzymes, inhibitors, vitamins and hormones, which play roles in the life process. Biochemical experimentation, besides coopting the methods of classical chemistry, e.g., chromatography, adopted new techniques, e.g., X-ray diffraction, electron microscopy, NMR, radioisotopes, and developed sophisticated microbial genetic tools, e.g., auxotroph mutants and their revertants, fermentation, etc. More recently, biochemistry embraced the 'big data' omics systems. Initial biochemical studies have been exclusively analytic: dissecting, purifying and examining individual components of a biological system; in exemplary words of Efraim Racker, (1913 –1991) "Don't waste clean thinking on dirty enzymes." Today, however, biochemistry is becoming more agglomerative and comprehensive, setting out to integrate and describe fully a particular biological system. The 'big data' metabolomics can define the complement of small molecules, e.g., in a soil or biofilm sample; proteomics can distinguish all the proteins comprising e.g., serum; metagenomics can identify all the genes in a complex environment e.g., the bovine rumen.

This Biochemistry Series will address both the current research on biomolecules, and the emerging trends with great promise.

Meet the Series Editor

Miroslav Blumenberg, Ph.D., was born in Subotica and received his BSc in Belgrade, Yugoslavia. He completed his Ph.D. at MIT in Organic Chemistry; he followed up his Ph.D. with two postdoctoral study periods at Stanford University. Since 1983, he has been a faculty member of the RO Perelman Department of Dermatology, NYU School of Medicine, where he is codirector of a training grant in cutaneous biology. Dr. Blumenberg's research is focused on the epidermis, expression of keratin genes, transcription profiling, keratinocyte differentiation, inflammatory diseases and cancers, and most recently the effects of the microbiome on the skin. He has published more than 100 peer-reviewed research articles and graduated numerous Ph.D. and postdoctoral students.

Meet the Volume Editor

Dr. Rizwan Ahmad is a University Professor and Coordinator, Quality and Development, College of Medicine, Imam Abdulrahman bin Faisal University, Saudi Arabia. Previously, he was Associate Professor of Human Function, Oman Medical College, Oman, and SBS University, Dehradun. Dr. Ahmad completed his education at Aligarh Muslim University, Aligarh. He has published several articles in peer-reviewed journals, chapters, and edited books. His area of specialization is free radical biochemistry and autoimmune diseases.

Contents

Preface

The term "reactive oxygen species" (ROS) refers to a group of reactive molecules and free radicals produced by molecular oxygen. The production of ROS is a bane to all aerobic species. ROS formed as a byproduct of the mitochondrial electron transport in aerobic respiration tends to cause several harmful events. Only phagocytic cells are responsible for ROS production as part of the host cell defense systems. Recent work has demonstrated that ROS has a role in cell signaling, including apoptosis, gene expression, and the activation of cell signaling cascades. ROS are oxygen-containing molecules with high chemical activity. The existence of an electron with only a single bond gives these molecules, which can take a variety of shapes, their reactivity. In this condition, electrons possess an affinity to make stronger bonds, resulting in chemical reactions. ROS might be as simple as superoxide (O_{2-}.) molecules or as complex as hydrogen peroxide (H_2O_2).

The book begins with a chapter summarizing the history of the Fenton reaction. The chapter provides insights into the Fenton reaction and highlights its importance in creating three fields of peroxide oxidation chemistry: hydroxyl radicals, ferryl-oxo-ions, and perferryl-oxoions. Singlet oxygen, a highly reactive form of molecular oxygen, is essential in environmental and biomedical processes. The detection and quantification of singlet oxygen species provide critical information to understand their involvement and mechanism in many processes. Superoxide dismutases (SODs) of intracellular pathogens are the main determinants of their survival inside the host niche. Furthermore, they also play a vital role in the severity of disease and virulence of these pathogens by protecting them from extracellular host-derived ROS activity. The chapter on SOD in psychiatric disorders provides a significant account of modulation of SOD activity in schizophrenia, bipolar disorder, depression, and Alzheimer's disease. The discussion shows the significance of SOD in preventing oxidative stress and initiation of apoptosis. The authors of the chapter on ROS in autoimmune (AI) diseases elucidate the protective roles of ROS in various disorders despite its proven role in exacerbation of AI diseases. ROS plays a complex role in AI responses, and they have been linked to the initiation, generation, and amplification of novel epitopes. The chapter discusses the important role of ROS in the pathophysiology of rheumatoid arthritis (RA) involved in the initiation of various signaling pathways.

A chapter on aging summarizes the various mechanisms underlying skin aging in terms of UV radiation and ROS as well as the role of antioxidants in impeding these processes. Furthermore, it hints at potential future explorations and challenges in the field of skin aging. In the chapter on epigenetic modifications in the aging process, the authors investigate the role of histone methylation in the process of aging and oxidative stress with an emphasis on the role of the SET-18 gene in the aging process.

Excess ROS in the brain generates oxidative stress that causes damage to the CNS, ultimately leading to neurodegeneration and incompetence. One chapter examines the possible mechanisms and inhibition to check oxidative stress and its associated symptoms. Alzheimer's disease (AD) is a major neurodegenerative disease worldwide. A chapter on AD deals with the role of metal chelation therapy as a possible treatment for the disease. This chapter focuses on how metal ion imbalance causes oxidative stress and affects AD pathology.

The role of nuclear factor erythroid 2-related factor 2 (Nrf2) as a dual-edged sword has been a majorly researched topic over the last decade. The crosstalk between Nrf2 and nuclear factor kappa B (NF-B) is central to the construction of the redox response network. The chapter on Nrf2 gives an idea about the therapeutic potential of targeting Nrf2 under multiple clinical settings. ROS typically accumulate in plants during abiotic and biotic stress conditions, resulting in oxidative damage and, eventually, programmed cell death. One chapter provides an overview of ROS regulation in plants and the key enzymes involved in abiotic stress tolerance mechanisms. The chapter on reducing agents provides conclusive evidence on oxidative stress and discusses how variations in agents' levels could cause reversal.

Particulate matter (PM) is a kind of air pollutant that consists of a mixture of solid particles and liquid droplets. Thus, an organism's ability to respond to PM inhalation necessitates anti-oxidative, anti-inflammatory, and cellular stress defenses, which can be compromised in susceptible people with chronic diseases such as diabetes and obesity. A chapter on this topic elucidates the mechanisms through which PM affects health and gives an account of research models in particle inhalation studies. The mechanisms and applicability of advanced oxidation processes for hydroxyl radicals and singlet oxygen species for water treatment are discussed. According to research, free radicals have a significant impact on aging, the damage of which can be corrected by adequate antioxidant defense and optimal antioxidant nutrient intake. This chapter emphasizes the importance of alternative antioxidants in the body for eliminating free radicals and their harmful effects. It explains the importance of alternative antioxidants (from plant sources) in the body for quenching free radicals and their harmful effects.

In recent decades, there has been a surge of interest in the role of ROS in various diseases. From basic science research to clinical trials, the biomedical community has made rapid progress toward a better understanding of ROS-metabolizing systems and their role in health and diseases. The reduced antioxidant defense may mimic the effect of increased ROS generation, and the dominant mechanism may vary depending on an individual's genetic predisposition. This book provides insights into ROS on research-based innovations and the mechanisms of antioxidants in combating them.

I am grateful to Prof. Mahdi Abumadni and Dr. Bassam Awary for their support and encouragement throughout the project. My friend Prof. Haseeb Ahsan needs special mention, as he is always available whenever needed for proofreading and corrections. My mother and family members are the pillars behind me and without their support, this work would have been next to impossible. I am very thankful to the staff at IntechOpen, particularly Author Service Manager Ms. Romina Rovan for her timely assistance throughout this project.

Rizwan Ahmad
College of Medicine,
Imam Abdulrahman Bin Faisal University,
Dammam, Saudi Arabia

Chapter 1

A History of the Fenton Reactions (Fenton Chemistry for Beginners)

Rafael Ovalle

Abstract

A deceptively simple mixture, ferrous sulfate ($FeSO_4$), hydrogen peroxide (H_2O_2), tartaric acid ($C_4H_6O_6$), and water (H_2O), initiated a century-long argument and a convoluted hunt to understand the oxidation mechanism(s) initiated by the combination of these components. Fenton's discovery rallied a legion of scientists, including two Nobel Winners, to find an explanation for the chemistry discovered when a graduate student mixed a couple of random chemicals, producing a molecule that became purple in strong base. Those investigation uncovered three separate branches of iron/oxygen chemistry, the Hydroxyl Radical [HO•], the Ferryl-Oxo Ion $[Fe = O]^{+2}$, and the Perferryl-Oxo Ion $[Fe = O]^{+3}$. Today their uses include chemical modifications [either untargeted and random [HO•] or targeted and selective $[Fe = O]^{+2}$, $[Fe = O]^{+3}$ dehydrogenations and/or oxygen additions] to effective and green oxidation and mineralization of persistent organic wastes.

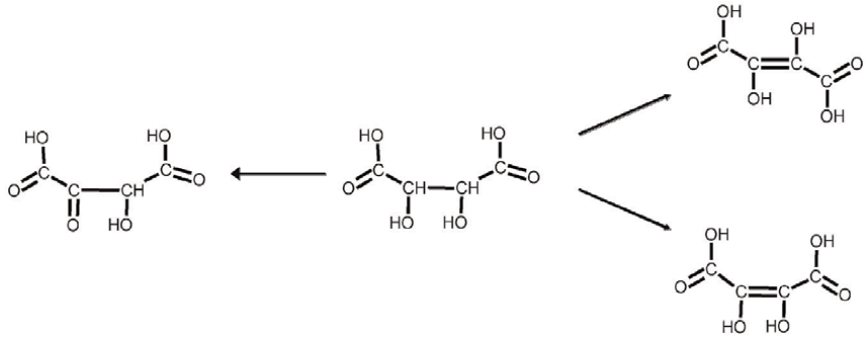
Keywords: Fenton chemistry, ferryl-oxo ion, perferryl-oxo ion, hydroxyl radical, hydrocarbons (alkanes), alcohols, polyols, carbohydrates, reactions, history, biology

1. Introduction

The Initial Experiments. In 1876, Henry John Horstman Fenton first discovered the enhanced oxidizing power of ferrous ions (Fe^{+2}), hydrogen peroxide (H_2O_2) on tartaric acid ($C_4H_6O_6$). When Fenton added sodium hydroxide (NaOH) to the mixture, the solution became bright purple [1]. Fenton made a decision to find out what that purple molecule was. That goal became his career and immortalized his name in the annals of chemistry [2].

Eighteen years later, Fenton repeated the experiment, again adding to a tartaric acid solution, a catalytic amount of $FeSO_4$, followed by H_2O_2 with the molar ratio of each factor: $C_4H_6O_6$ / H_2O_2 / Fe^{+2} = 1.0: 1.0: 'catalytic'. Fenton then isolated the reaction product by sequentially precipitating the acid with heavy metal ions, weighing the salt to calculate the molar formula, re-purifying the acid, then repeating the process with a different cation, thus calculating the empirical formula of the new acid. The new acid bound one divalent cation ion or two monovalent cations ions per molecule, and thus was a di-acid. Fenton determined: 1) the molecule had the empirical formula $C_4H_4O_6$; 2) was a 4-carbon di-acid; 3) produced by abstraction of two hydrogen atoms from tartaric acid [3].

Fenton (1896) assumed that the 4-chain backbone was not severed, limiting his options to three possible structures: 1) loss of two hydrogen from a single middle carbon, forming a hydroxy-, keto-, di- carboxylic acid: (2-hydroxy-3-oxosuccinic acid); or 2) loss of two hydrogen from the internal adjacent carbon atoms forming a double bond with the HO- groups either in: 2a) *trans-* conformation: 2-, 3-, di-hydroxyfumaric acid, or 2b) *cis-* conformation: 2-, 3-, di-hydroxymaleic acid (Eq. (1)).



Fenton's Initial Guess for $C_6H_4O_6$. (1)

The first structure was eliminated when the di-acid failed to form a hydrazone with phenylhydrazine (an aldehyde/ketone reactive agent). The assumption that the molecule had two internal hydroxyl groups was verified when the molecule formed a 4-carbon di-ester, di-anhydride with either acetyl chloride or acetic anhydride.

The structure of the molecule was finalized by reaction with aniline. Fenton knew (from literature) that the 1:1 product of aniline and fumaric acid ($C_4H_2O_4$: $-C_2H = HC_3-$ in *trans-*) was soluble in water, whereas the 1:1 product of aniline and maleic acid ($C_4H_2O_4$, $-C_2H=HC_3-$ in *cis*) was insoluble in water. The aniline derivative of the unknown acid was also insoluble in water. Fenton concluded that Fe^{+2}/H_2O_2 oxidized tartaric acid to 2-, 3-, di-hydroxy-maleic acid: a loss of two H• atoms in *cis-* orientation, forming a double bond and creating a previously unknown molecule [4].

2. Early Fe^{+2}/H_2O_2 investigations

Following the initial discovery, Fenton tested the range of his new reagent. Fenton & Jackson (1899) oxidized aliphatic alcohols, polyalcohols, and benzoic, then $FeSO_4$ followed by H_2O_2, added stepwise, in molar ratios of (1: 0.1–0.25: 1), adding the peroxide gradually in small amounts.

The aliphatic alcohols: (CH_3OH, CH_2CH_5OH, *n*-C_3H_7OH, *i*-C_3H_7OH, and n-$C_5H_{11}OH$), did not produce any visible changes in temperature or precipitates with phenylhydrazine, therefore Fenton assumed that these molecules were non-reactive [5]. Merz & Waters (1947) commented that the 1:1 alcohol: H_2O_2 ratio used in the experiments by Fenton & coworkers was so high that the alcohols were oxidized directly to organic acids. Fenton & coworkers would have discovered this result if: a) they had assayed their samples for acids, and/or b) assayed the alcohols with different concentrations of H_2O_2] [6].

On the other hand, the polyalcohols ($C_2H_6O_2$, $C_3H_8O_3$, $C_4H_{10}O_4$, $C_5H_{12}O_5$, and $C_6H_{14}O_6$) showed temperature increases and release of gases, whereas the H_2O_2 only controls showed no reaction for either group. When the oxidized polyalcohols were

reacted with phenylhydrazine forming osazones, indicating that hydroxyl groups of the polyols were oxidized to carbonyls, forming aldoses and/or ketoses. The oxidation of hydroxyl groups in the polyalcohols to aldehydes or ketones required a loss of two hydrogen atoms (H•) from the molecular formula.

Benzoic acid ($C_7H_6O_2$) was oxidized to salicylic acid (*o*-$C_7H_6O_3$) as determined by a robust violet color when ferric ion was added to the solution indicating that an exchange of a -H atom with an -OH group adjacent to the -COOH on the benzene ring [5].

Fenton & Jones (1900) repeated testing the oxidizing abilities of Fe^{+2}/H_2O_2 on a larger set of aliphatic and polyhydroxy acids. Their method was to prepare a 1 M solution of reagent in H_2O at 0°C, add $FeSO_4$ to 0.125 M, then add H_2O_2 to 0.25 M (-$FeSO_4$ was the control). The authors again reported that aliphatic acids appeared non-reactive while polyhydroxy acids showed vigorous and energetic reactions. The oxidized acids reacted with phenylhydrazine, and the precipitates were purified by crystallization, confirming that hydroxyl groups were oxidized to ketones or aldehydes and the molecules identified by melting point determinations. The oxidation of benzoic acid to salicylic acid was also confirmed [7].

Collectively, Fenton (1896) and Fenton et al. (1899, 1900) presented evidence for three different reactions: 1) carbon–carbon double bond formation with loss of 2 H-; 2) carbon–oxygen double bond formation with loss of 2 H- (both aldehydes and ketones; and 3) oxygen addition to phenol.

2.1 Isolation of glucosones

Following Fenton's lead, Cross, Bevan, & Smith (1898) oxidized glucose with $FeSO_4$ / H_2O_2 and isolated "glucosone" (2-keto-, glucose). The experiment consisted of: 1) in 100 mL H_2O, 4% or 10% glucose; 2) $FeSO_4$ added to a concentration of $1/10^4$ ratio with glucose, and 3) H_2O_2 was gradually added to a final 1:1 molar glucose/ H_2O_2 with stirring on ice [8]. Theorizing that 2-keto-, glucose would be indigestible, the authors used yeast to scavenge unoxidized glucose. After filtering out the yeast, the authors found that the solution still contained carbonyl molecules, as indicated by reduction of CuO. The solution retained 20% of the reducing power of the original glucose solution. The oxidized glucose solution also increased in acidity. After drying (105°F / 40.6°C), the dried residue comprised 88% of the weight of glucose including 3.8% furfural.

The solids were resuspended in chilled water and reacted with phenylhydrazine (PHZ), a reversible carbonyl-reactive label). The rationale was that while glucosone and glucose are likely to be equally soluble in most solvents, the double substituted glucosazone was expected to have a different solubility profile from the glucohydrazone. The glucosone reacted with 2 moles of PHZ, whereas glucose reacts with only one mole of PHZ, therefore their solubility in differences in organic solvents would be greater than the unlabeled molecules. After purification, PHZ-labeling was reversed with H_2SO_4, allowing analysis of purified glucosone. The authors repeated their method with fructose and sucrose recovering only glucosone from all three sugars implying: 1) fructose oxidized to glucosone; and 2) sucrose hydrolyzed to glucose and fructose.

Morell *et al.* (1899–1905) conducted a larger and more detailed survey of the oxidation of monosaccharides to corresponding -osones with Fenton's reagent. Following Cross & Bevan's method [9–14]. Morrell et al. (1899a) expanded the study of the osones to include galactose, rhamnose, arabinose, and mannose [9].

Morrell et al. (1899b) increased glucose to glucosone yields by: 1) slow addition of H_2O_2, 2) controlling temperature with refrigeration, 3) controlling pH and precipitating

organic acid with ($Pb(OAc)_2$). This method increased glucosone yield to 10%. Morell et al. purified PHZ-glucosazone and PHZ-mannosazone from their corresponding PHZ-hydrazones, but were not able to purify PHZ-arabinosazone, PHZ-rhamnosazone, and PHZ-galactosazone from their PHZ-hydrazone contaminants [10].

Morrell *et al*. (1900) oxidized glucose, fructose, arabinose, rhamnose, galactose, maltose, lactose and sucrose, then labeled both aldoses and osones with PHZ. The authors increased purity by precipitating the saccharic acids with $Pb(OAc)_2$, while controlling pH with $Ba(OH)_2$. PHZ-rhamnosazone was also separated from its PHZ-hydrazone, but not galactose or arabinose [11].

Morrell *et al*. (1902) found that reacting a glucosone with bromine severed the C2-C3 bond and oxidized C3 to a carboxylic acid, the final product being erythronic acid (trihydroxy-butyric acid), which was then dehydrated to butyric acid with nitric acid. The identity of erythronic acid was confirmed by calculating formula weights of the lead and barium salts [12].

Morell et al. (1903) oxidized aldoses with Fenton's reagent, precipitate organic acids with $Pb(OAc)_2$ and $Ba(OH)_2$, then label the oxidation mixture for carbonyl groups with methyl-, phenyl-hydrazine (MPHZ). However, galactose and arabinose MPHZ-osazones were not separable from their MPHZ-hydrazones. The authors tested bromo-, phenylhydrazine (BPHZ) and found that BPHZ-arabinosazone was easily separable from BPHZ-arabinose hydrazone using benzene as the solvent [13].

Morrell & Bellars (1905) retested purification of all the aldose osazones with BPHZ. BPHZ-labeling after Fenton oxidation allowed sharp separations of BPHZ -osazones from the corresponding hydrazones in benzene, thus achieving the goal of acquiring pure osazones after Fe^{+2}/H_2O_2 oxidation [14]. [Considering that both Cross & Bevan (1898) and Morell et al. (1899) stated that the purified osones were tasted, the implication is that both groups were investigating the osones as non-caloric sweeteners].

2.2 Isolation of aldonic acids and other byproducts

Cross & Bevan (1898) surveyed the by-products yielded by Fenton oxidation of aldoses to glucosones. The secondary products included: tartronic acid, ($\sim$ 8%), acetic acid ($\sim$5%), formic acid ($\sim$15%), and furfural(s) ($\sim$ 4%). Missing carbohydrate mass was assumed to be lost as carbon dioxide (CO_2). The authors noted that Fenton oxidation of glucose produced furfural, but fructose did not; on the other hand, glucose produced lower amounts of dicarboxylic acids and pentoses [8]. Cross & Bevan (1899) oxidized a 2% solution of furfural with H_2O_2 and a catalytic amount of $FeSO_4$. Formic, acetic acid, and a red precipitate identified as pyromucic acid were isolated. The authors also reported that Fe^{+2}/H_2O_2 oxidized benzene to phenol, followed by additional hydroxylations [15].

Morrell et al. (1903) used $Pb(OAc)_2$ and $Ba(OH)_2$ to precipitate the sugar acid impurities in the quest to purify the osones. From the oxidation of glucose and fructose, the experimenters recovered the several polyhydroxy acids after precipitation with Pb^{+2} or Ba^{+2} ions. After removal of Pb^{+2} and Ba^{+2} ions with H_2SO_4, the solubilized acids were identified as glycolic and oxalic. The Pb^{+2} / Ba^{+2} soluble acids were separately precipitated as calcium salts and identified as glyoxylic and trihydroxy-butyric acids [13].

In sum, Cross & Bevan (1898, 1899) and Morrell et al. (1899–1903) confirmed that Fenton's reagent was responsible for the following reactions: dehydrations producing C=C bonds, C-C bond cleavages, and oxygen atom additions forming hydroxyl, aldehyde, ketone, and carboxylic acid groups, creating new classes of organic molecules.

3. Joint history of the hydroxyl radical and ferryl-oxo ion

Prof. Henry J. H. Fenton died in 1929 without knowing the mechanism of the reagent that he discovered. Within three years of his death, two competing mechanisms naming two different intermediate molecules were published.

3.1 Hydroxyl radical

In 1932, Fritz Haber & Joseph Weiss (1932) published in *Naturwissenschaften* (Science of Nature), and again in Proceedings of the Royal Society of London: A (1934), that the hydroxyl radical (HO•) is the oxidative intermediate responsible for Fenton's observation.

The authors proposed that the Fe^{+2} ion donates an electron to the peroxide molecule, cleaving the O-O bridge producing a hydroxyl radical (HO•) and a hydroxide ion (HO^-). The hydroxyl radical then attacks another peroxide molecule, forming superoxide, eventually generating oxygen [16, 17].

3.2 Ferryl-oxo ion

On the other side of the Atlantic Ocean, William Bray & H. M. Gorin published in Journal of the American Chemical Society (1932) that oxygen production after addition of Fe^{+2} ions to H_2O_2 in H_2O is due to creation of the ferryl-oxo ion $(Fe = O)^{+2}$ (**Figure 2**). After creation, the ferryl-oxo ion then reacts with another ferryl-oxo molecule to produce oxygen gas, recycling the ferrous ion. The authors proposed that an oxygen atom (•O•) is abstracted by a Fe^{+2} ion from the peroxide molecule, forming the ferryl-oxo ion ($Fe = O^{+2}$) (no net change in charge) and H_2O [18].

[Bray & Gorin named the molecule 'ferryl ion', but the term is currently used for the Fe^{+4} ion (without oxygen) [19]. To avoid confusion, the Bray & Gorin molecule is named here 'ferryl-oxo' ion. For similar reason, Barton's 'perferryl ion' will be named 'perferryl-oxo ion'].

These two papers divided the scientific community into partisan camps with sports-like fanaticism that continues today. Champions of the hydroxyl radical theory include: JD Rush, WH Koppenol [20–23], C. Walling [24–26], M. Kremer [27–29], JH Merz, WA Waters, [6, 30, 31], as well as many others. The scientists who argued for the existence of the ferryl-oxo ion included JT Groves [32–35], DA Wink [36–38], and DT Sawyer [39], among many others.

3.3 Oxidative behavior of the hydroxyl radical

Only exceeded by a fluorine (F^0) atom, the powerful hydroxyl (HO•) radical is the second strongest electrophile, and will even oxidize chlorine ion(s) (Cl^-) to elemental chlorine (Cl^0) or gas (Cl_2) [40]. A hydroxyl radical will strip an electron from an element (except F^-) or H• from a hydrocarbon (**Figure 1**).

Hydroxyl radicals (HO•: **Figure 1**) are created by:

1. Donation of an electron to H_2O_2 from a transition metal ion (**Figure 1a**) (with *exception* of iron and copper ions [41]) produces HO• radicals via secondary electron transfer from the ion to peroxide. The O-O bond of peroxide scissions, producing a hydroxyl ion (HO^-) and hydroxyl radical (HO•);

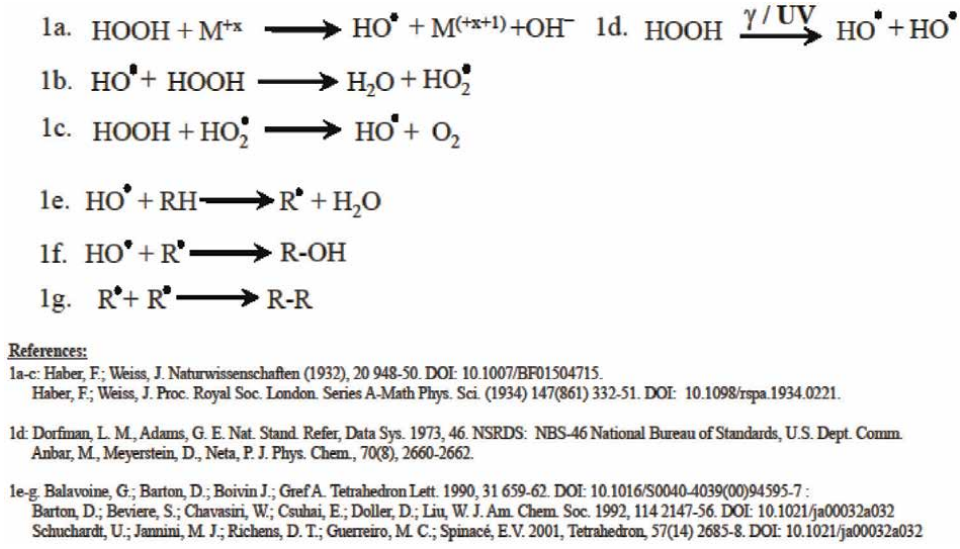


Figure 1.
Hydroxyl radical reactions.

2. Splitting H_2O_2 with UV (λ = 253.7 nm) or γ- radiation (from ^{60}Co) produces two hydroxyl radicals:

$$H\text{-}O\text{-}O\text{-}H + (UV) \rightarrow HO\bullet$$

Hydrogen Peroxide is Cleaved by UV Rays to Hydroxyl Radicals (2)

(See (Eq. (2)) (**Figure 1d**) [42];

3. UV flash irradiation of oxygen donating molecules, such as N2O to create oxygen atoms (•O•) that react with water molecules:

$$H_2O + \bullet O\bullet \rightarrow 2\,HO\bullet$$

Water Molecule and Oxygen Atom Form Hydroxyl Radicals (3)

(Eq. (3)) [43, 44]; or.

4. Ionizing water with electrically produced β-rays to produce radicals

$$H_2O + e^- \rightarrow HO\bullet + H^-/H\bullet + HO^-$$

Water Molecule is Cleaved by High Energy Electrons to Hydroxyl Radicals, Hydroxyl Ions, Hydride Radicals, and Hydrogen Atoms (4)

(Eq. (4)) [42];

(H• radicals likely combine with each other, as also H^- & H^+ ions; thus escaping as H_2 gas) [42, 45].

Once created, hydroxyl radicals (HO•) will oxidize an element, ion, or compound, by extracting an electron to form HO^- and a cation, increase the valence of another ion by +1, or abstract H- from an X-H bond of organic molecule, forming H_2O and an organic radical (**Figure 1e**).

3.3.1 Oxidation of hydrocarbons (alkanes) by hydroxyl radicals

A hydroxyl radical abstracts H• from an alkane to create a C• radical and water (**Figure 1e**) [46, 47]. A second HO• is required to collide with C• to form an alcohol (**Figure 1f**), thus a high HO•/ substrate ratio is required to produce alcohols. Subsequent HO• hydroxyl attacks to the same carbon atom progressively oxidizes and adds oxygen atoms, producing aldehydes/ketones, then organic acids, and finally, carbon dioxide [31, 42, 48]. An example of a hydroxyl radical reaction sequence for oxidation of methane:

$$\begin{aligned}
&(1)\mathrm{HO}\bullet + \mathrm{CH_4} \rightarrow \bullet\mathrm{CH_3} + \mathrm{H_2O};\\
&(2)\mathrm{HO}\bullet + \bullet\mathrm{CH_3} \rightarrow \mathrm{CH_3OH}\\
&(3)\mathrm{CH_3OH} + \mathrm{HO}\bullet \rightarrow \mathrm{CH_2O}\\
&(4)\mathrm{CH_2O} + \mathrm{HO}\bullet \rightarrow \mathrm{HCO_2}\\
&(5)\mathrm{HCO_2} + \mathrm{HO}\bullet \rightarrow \mathrm{CO_2} + \mathrm{H_2O}
\end{aligned} \tag{5}$$

Sequential Oxidation of Methane to Carbon Dioxide by Hydroxyl Radicals

(Eq. (5)) (**Figure 1f**) [25].

There is no guarantee that a second HO• will collide with a carbon radical to make an alcohol.

Under low HO• concentrations, long-lived hydrocarbon radicals fuse to each other to make large complex hydrocarbons via $R_1C\bullet\bullet CR_2$ fusions (**Figure 1g**). The hydroxyl radical oxidation of methane can also follow:

$$\begin{aligned}
&(1)\mathrm{HO}\bullet + \mathrm{CH_4} \rightarrow \bullet\mathrm{CH_3} + \mathrm{H_2O};\\
&(2)\mathrm{CH_3}\bullet + \bullet\mathrm{CH_3} \rightarrow \mathrm{CH_3} - \mathrm{CH_3}
\end{aligned} \tag{6}$$

Partial Oxidation of Methane by Hydroxyl Radicals Allow C-C Fusions

(Eq. (6)) (**Figure 1g**) [25].

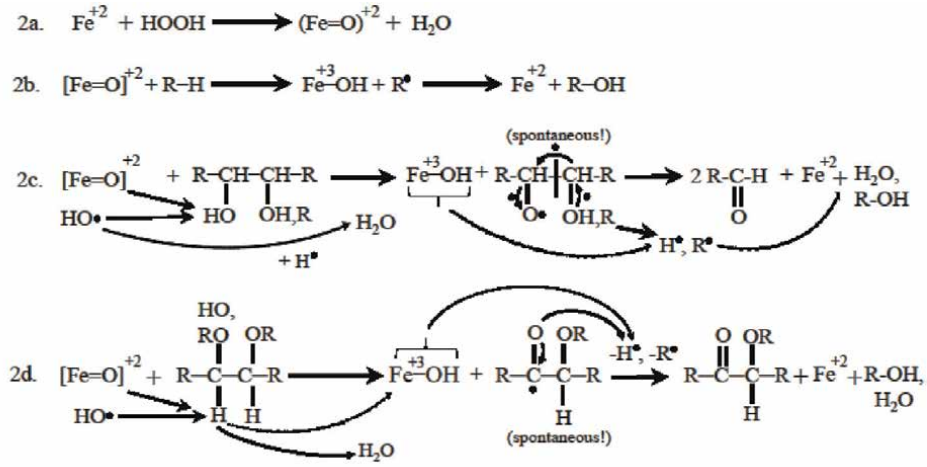
Thus carbon–carbon fusions are a hallmark of hydroxyl radical reactions [42, 49].

The HO• radical is: 1) uncharged, and 2) will abstract an electron any atom (except F^-) or H• from a molecule it collides with, thus the (HO•) radical is an indiscriminant oxidant. Its oxidation profile determined by accessibility and rate of diffusion. In the oxidation of simple alkanes, the oxidation preference is: 1° H > 2° H > 3 H°.

Hydroxyl radicals (HO•) [17] can be created by H_2O_2 receiving an electron from a transition metal ion [41], or by splitting an oxygen donating molecule, either H_2O_2, with UV light or radiation [42, 50] or N_2O (aq.) with UV light [43, 44]. Hydroxyl radicals can be quenched/scavenged by reducing agents [51] including aliphatic alcohols [50], DMSO [52], acetate ions [22], polyols [53], H_2S [54], and NO [55]. These reagents are included as radical traps where HO• radical oxidations are undesirable.

3.3.2 Hydroxyl radical oxidation of alcohols

Waters (1946) reported that HO• radical oxidation of ethylene glycol (CH_2(OH)-CH_2(OH)) produced both glycoaldehyde (CH(O)-CH_2(OH)) and formaldehyde (2x: CH_2O). To determine which H• abstraction caused C-C bond cleavage, Waters



References:
2a. Bray, W.; Gorin M. J Am Chem Soc. 1932, 54 2124-25. DOI: doi.org/10.1021/ja01344a505

2b. Groves, J. T.; van der Puy, M. J. Am. Chem. Soc. 1974, 96 5274-75. DOI: 10.1021/ja01344a505 :
Groves, J. T.; van der Puy, M. J. Am. Chem. Soc. 1976, 5290-7. DOI: 10.1021/ja00433a039 :
Groves J. T.; McClusky G. A. J. Am. Chem. Soc. 1976, 98(3) 859–861 DOI:/10.1021/ja00419a049

2c,d. [HO]: Waters, W. Nature 1946, 158(4011)380. DOI: 10.1038/158380b0:
[Fe=O+2]: Okamoto, T.; Sasaki, K.; Oka, S. 1988. J Am. Chem. Soc. 1988, 110 1187-96. DOI: 10.1021/ja00212a030
Sugimoto, H.; Sawyer, D. (b) J. Org. Chem. 1985, 50 1784-6. DOI: 10.1021/jo00210a053

Figure 2.
Ferryl-Oxo ion reactions.

oxidized pinacol, which has no available O-**C-H** bonds, to acetone ($(CH_3)_2C=O$) as the only product. Thus, Waters (1946) proved that H• abstraction from the hydroxyl oxygen (H-C-***O-H***) bond of a 1-, 2-, diol causes C-C bond cleavage, and H• abstraction from an ***H-C***-O-H bond of a 1-, 2-, diol causes localized C=O bond formation [56] (see ferryl-oxo ion: **Figure 2c** and **d**).

Droege & Tully (1986a,b) oxidized gaseous ethane (^1H, ^2H, and mixed) (46)] and propane (^1H, ^2H, and mixed) [47] with UV-activated N_2O & H_2O to compare oxidation rates of the terminal vs. center carbons, and test the isotope effect on HO• oxidation for different positions of the ethane and propane molecules. The authors found that there was no difference in positional abstraction for hydrogen vs. deuterium at 1° (ethane & propane) or 2° positions (propane only); however C-C chain fusions increased with temperature.

Baxley & Wells (1998) oxidizing tertiary alcohols with HO• radicals in air. HO• radicals were generated by UV activation of CH_3ONO, NO, and O_2 gases. H-abstraction from the sole -OH group caused C-C cleavage producing a ketone, a hydrocarbon and water. Abstractions from C-H bonds produced either addition of a second hydroxyl group or fusions producing long chain diols, however the authors noted that the hydroxyl group of 2-butanol was targeted more frequently than of 2-pentanone [48].

3.3.3 Hydroxyl radical oxidation of diols, polyols, and carbohydrates

Gilbert and King (1981, 1984) oxidized glucose with HO• generated by Ti^{+3}/H_2O_2. Using electron spin resonance (ESR) the authors concluded that HO• radical produced

carbon (C•) radicals at all positions in equal ratios, indicating distributed attack by HO• toward all carbon positions in glucose, the established signature of HO• oxidations [57, 58].

Dizdaroglu & Von Sonntag reacted glucose [43] and cellobiose (44) with HO• generated from UV irradiation of N_2O saturated H_2O. By mass spectroscopy, the authors identified several 6-carbon derivatives of glucose including gluconic and glucuronic acids, several hexosuloses, hexodialdose, and. Several dehydrohexoaldoses, proposing that addition of H• or HO• radicals occurred after abstraction of –H or –OH groups from glucose. The authors determined for both carbohydrates, all carbons were oxidized equally.

In addition to 6-carbon molecules, Von Sonntag and coworkers reported fragmentation of glucose into various aldoses, formaldehyde, formic acid, carbon dioxide, and carbon monoxide, representing different C-C bond cleavages. The authors did not explain the origins of the C-C bond cleavage products.

In summary, the hydroxyl radical (HO•) is a powerful but non-selective oxidant. It can abstract electrons from any molecule or element with exception of fluorine. Hydroxyl radicals will abstract hydrogen atoms (H•) from organic molecules from any accessible C-H, O-H, or N-H (59) bond at rates proportional to accessibility by simple diffusion [43, 46, 57].

3.4 Oxidative behavior of the ferryl-oxo ion

The ferryl-oxo [$(Fe = O)^{+2}$] ion, a less powerful oxidant than the HO• radical, is created by oxygen abstraction from H_2O_2 (**Figure 2a**). The oxidizing power of the $(Fe = O)^{+2}$ ion is moderately stronger than the strength of C-H bond of an alkane and roughly equivalent to the C-H bond strength of benzene; the $(Fe = O)^{+2}$ ion is reported not to abstract H• from anhydrous acetonitrile (CH_3-C ≡ N) [59]. Though weaker than the HO• radical, the $(Fe = O)^{+2}$ ion is a discriminatory oxidant, abstracting H from the weakest X-H bond in a molecule and oxidizing molecules with the weakest X-H bonds in a mix of molecules (**Figure 2b**) [32–35]. In the oxidation of simple alkanes, the oxidation preference is: 3° H > 2° H > 1° H (**Figure 2**).

3.4.1 Ferryl-oxo ion oxidation of hydrocarbons (alkanes)

Groves & coworkers demonstrated that oxidation of alkanes by $(Fe = O)^{+2}$ in non-aqueous environments produced alcohols without carbon–carbon fusions. Addition of -OH groups to alkanes was both *regio-* and *stereo*-selective. Using isotopic $H_2{}^{18}O_2$ / $H_2{}^{16}O$, Groves and coworkers found that the peroxyl oxygen was incorporated as the hydroxyl oxygen 90% of the time. Groves et al. termed the effect 'oxygen rebound' [32–35].

Groves et al. proposed a two-step mechanism to explain their results (**Figure 2b**):

1. The ferrous ion abstracts oxygen from peroxide forming the ferryl-oxo ion with a coordinate double bond;

2. The ferryl-oxo ion [$(Fe = O)^{+2}$] abstracts H• from a C-H bond creating a C• radical and ferric hydroxide [$(Fe^{+3}OH)$ or $(Fe\text{-}OH)^{+3}$] (**Figure 2b**);

3. The oxygen of ferric hydroxide ($Fe^{+3}OH$) reacts with the C• radical, producing an alcohol (RC-OH), and regenerating the Fe^{+2} ion.

Unlike HO•, the $[Fe = O]^{+2}$ is both stereo- and regio- selective. For hydrocarbons, the H-C oxidation preference order is: 3° C-H > 2° C-H > 1° C-H. The basis of the rebound effect is likely due to attraction of the electrophile C• radical and the nucleophile oxygen (•O•) of the $(Fe\text{-}OH)^{+3}$ ion. The oxygen is added to the same bond position on the oxidized carbon.

[Fenton's original reaction: (Eq. (7)) violates Groves' model because tartaric acid oxidation follows a different pathway:

$$C_4H_6O_4 + FeSO_4 + H_2O_2 + H_2O \rightarrow C_4H_4O_4 + 2H_2O$$

Fenton's First Reaction: Oxidation of Tartaric Acid by $FeSO_4/H_2O_2$ (7)

Erik Hückel's double bond resonance theory states that molecules with 4N + 2 unpaired electrons in conjugated (staggered) double bonds are extraordinarily stable. In the oxidation of tartaric acid, the abstraction of the first H• from C2 is followed by ejection of the second H• from C3 to form a C=C bond, because the central C=C bond is conjugated to the flanking C=O bonds of the terminal carboxylic acids. Thus, Fenton's molecule was resistant to further oxidations, allowing him to discover it].

3.4.2 Ferryl-oxo ion oxidization of alcohols

Ferryl-oxo ion $[(Fe = O)^{+2}]$ oxidation of oxygen-containing organic molecules behaves differently from hydrocarbon oxidations (**Figure 2c** and **d**). Carbon and hydrogen have similar affinity for electrons, therefore the electron pair is shared equally and in a hydrocarbon, hydrogen-carbon all bonds are about equal strengths. Oxygen (O) heteroatoms have a higher affinity for electrons than carbon or hydrogen atoms, making the C-O bond stronger than a C-C bond, while weakening other bonds extending from the hydroxyl carbon significantly [19, 60–62].

As an example, when (HO•) oxidizes ethanol, H• abstraction occurs indifferently from any of the six C-H positions, producing roughly 50% ethylene glycol and 50% acetaldehyde yield. On the other hand, when ethanol is oxidized by $(Fe = O)^{+2}$ ion, the bond strengths of the methyl C-H bonds are ~96 kCal/mole, whereas the hydroxyl C-H bond strengths are ~81.6 kCal/mol and the O-H bond strength is ~104 kCal/mole (60). Because the $(Fe = O)^{+2}$ ion has the higher probability of abstracting H• from a hydroxyl carbon (due to bond strength) or hydroxyl oxygen (due to charge attraction) rather methyl carbons, acetaldehyde will be formed in preference to ethylene glycol [60, 63–66].

3.4.3 Ferryl-oxo ion oxidation of diols, polyols, and carbohydrates

Following the Coon & White (1977) discovery of the Fe^{+3}-heme core in cytochrome P450 and its ability to sever the O-O bond, and oxidize $NADPH_2$ [67, 68], Okamoto et al. (1985) mimicked the ability of enzyme $P450_{scc}$ to split a C-C bond of a diol in 1-, 2-, bis-(4-methoxyphenyl)ethane-l,2-diol using Fe^{+3} ion, O_2, and a reductant (N-benzyl-3-carbamoyl-1,4-dihydropyridine) [69].

Okamoto et al. (1988) found that Fe^{+2} + H_2O_2 could substitute for Fe^{+3} and O_2 to cleave diols to paired aldehydes. Using various inhibitors and/or substituting ferric for ferrous ion, the authors concluded that $(Fe = O)^{+2}$ was created and was the oxidant that cleaved the 1-, 2-, diols (**Figure 2c**). The authors also discovered that when one

hydroxyl group was substituted, paired aldehydes formed, but when both hydroxyl groups were blocked, no aldehydes were produced (**Figure 2d**) [70].

Sugimoto and Sawyer (1985a) reported that Fe^{+2} and two moles of H_2O_2 oxidized alkenes (hydrocarbons with double bonds) to paired aldehydes formed by $(Fe = O)^{+2}$. The authors proposed that Fe^{+2} ion and H_2O_2 caused dioxygen addition to a double bond, forming a dioxetane, that then scissioned to a diol; a second oxidation $(Fe = O)^{+2}$ scissioned the diol to paired aldehydes. The authors saw similar oxidative behavior when CH3-O-O-H and *p*-Cl-Ph-O-O-H were substituted for H_2O_2 [71].

Thus 1-, 2-, diols produce the same products when oxidized by either HO• (56) or $(Fe = O)^{+2}$ [68] oxidants indicating that the formation of paired aldehydes is faster than oxygen addition reactions of alkanes. [Though contemporary, the Sawyer's and Oka's research teams did not appear to be aware of each other, or of Waters (1946)].

The rationale for asymmetric cleavage of diols (**Figure 2c** and **d**) is due to the additive weakening of the C-C bond between the two hydroxyl groups [19, 60, 61, 65]. When H• is abstracted from a hydroxy oxygen of a diol pair, the weakest bond of the oxygen-centered (H-C-O•) carbonyl radical is the C-C bond between the diol pair (•O-R_1CH ~ R_2CH-OH); electron abstraction from the C ~ C bond produces paired aldehydes (**Figure 2c**). However, when H• is abstracted from a C-H bond of a hydroxyl carbon, the weakest bond of the diol group is the O-H bond opposite the C• radical (H ~ O-R_1C•); the hydrogen atom is ejected from carbon-centered (H ~ O-C•) carbonyl radical to form the carbonyl bond. Abstraction of H• from a tertiary -OH group can cause ejection of a C• radical to form the C=O bond (**Figure 2d**) [60–66].

[Fenton's oxidation of tartaric acid should have produced two products: 2-, 3-, dihydroxy-, maleic acid [HOOC-C(OH) = C(OH)-COOH] *and* glyoxalic acid [HO-C(O)-C(O)H. Waters (1946), Okamoto et al. (1988), and Sugimoto et al. (1984, 1985a) indicates that Fenton could have discovered both oxidation products].

3.5 Comparison of ferryl-oxo ion and hydroxyl radical oxidizations

Though HO• radical and $(Fe = O)^{+2}$ ion both create a C• radical, the differences between the two oxidants is: 1) HO• is a 1 e^- oxidant, whereas $(Fe = O)^{+2}$ ion is a 2 e^- oxidant, thus two independent HO• oxidations are required to make a hydroxyl group; and 2) reducing agents that trap HO• radicals and thus halt HO•-based oxidations, do not disrupt ferryl-oxo ion oxidations. The most likely explanation radical quenching by the ferryl-oxo ion is the proximate distance of Fe^{+3}-O-H and C• radical is coupled with likely nucleophile / electrophile attraction, allowing rapid re-reaction to occur [36–38].

The noted crypto-HO• positional effect seen in Fe^{+2}/H_2O_2 catalyzed oxidations is likely due to localized binding of Fe^{+2} ions to a substrate that has O heteroatoms when it added to the substrate prior to H_2O_2 [22, 23, 72] addition.

4. Perferryl-oxo ion

4.1 Early history of the perferryl-oxo ion

Fenton conducted Fe^{+3}/H_2O_2 experiments but did not note any reactions and assumed that no reaction(s) had occurred [5, 7]. However, on the other side of the English Channel, Fenton's contemporaries found contrary evidence.

Spring (1895) mixed H_2O_2 solutions with different pure chemical substances noting which substances caused oxygen gas release. Spring noted that both ferrous and ferric chlorides decomposed H_2O_2 and released oxygen gas [40].

Ruff (1898) used basic ferric acetate and H_2O_2 to oxidize gluconic acid to arabinose and carbon dioxide, a C_1-C_2 bond cleavage with oxidations of both C_1 and C_2, the reaction now known as 'Ruff's degradation' [73].

Bohnson (1921) noted that a solution of a ferric salt in water, dilute enough to show only very slight color, turns brightly lavender with '1 or 2 drops' of 30% H_2O_2 followed by O_2 gas release from the solution. After bubbling ends, no residual H_2O_2 remained in the solution, indicating complete decomposition. The author observed that when H_2O_2 is added to Fe^{+3} salts, a lavender color appears briefly. Bohnson speculated that the color represented a transient higher Fe oxidation state. Bohnson trapped the lavender pigment with cold KOH coloring the solution red, then $Ba(OH)_2$, forming a red gelatinous precipitate. Washing the precipitate with HCl released chlorine gas. Bohnson determined the empirical formula of the precipitate: barium ferrate ($BaFeO_4$), thus isolating the Fe^{+6} oxidation state as FeO_4^{-2} (ferrate) ion. Bohnson also prepared potassium ferrate (K_2FeO_4) by bubbling chlorine gas through a solution of $Fe(OH)_3$/KOH solution, producing a deep lavender color; addition of $Ba(Cl)_2$ to the lavender solution again formed the red precipitate: $BaFeO_4$ [74].

Bohnson (1921) also demonstrated direct conversion of ethanol to acetic acid. Bohnson noted that addition of Fe^{+3} ions to an H_2O_2 solution produced oxygen gas, but addition of ethanol to the H_2O_2 solution prior addition of Fe^{+3} ions disrupted oxygen evolution, leading the author speculated that ethyl alcohol was oxidized to acetaldehyde or acetic acid. Bohnson also compared of oxidation by ethanol by Fe^{+2}/H_2O_2 vs. Fe^{+3}/H_2O_2 and found that Fe^{+2}/H_2O_2 produces acetaldehyde, then acetic acid, whereas Fe^{+3}/H_2O_2 oxidized ethanol to acetic acid primarily, with only trace amounts of acetaldehyde detected. Bohnson proposed that Fe^{+3}/H_2O_2 oxidized ethanol directly to acetic acid, bypassing acetaldehyde formation [74].

Walton & Christensen (1926) compared the oxidation of ethanol with $FeSO_4$/H_2O_2 or $Fe_2(SO_4)_3$/H_2O_2 under anhydrous conditions. Separately assaying for acetaldehyde and acetic acid, the authors noted that when ethanol is oxidized with $FeSO_4$/H_2O_2 acetaldehyde appeared before acetic acid, whereas when ethanol is oxidized by $Fe_2(SO_4)_3$/H_2O_2 acetic acid appears long before acetaldehyde, proving that Fe^{+3}/H_2O_2 oxidation exhibits non-Fenton-like behavior, thus confirming Bohnson (1921) [75].

Wieland & Franke (1928) reported that under strong acidic conditions Fe^{+3}/H_2O_2 oxidized formic acid (HCOOH) to CO_2 and H_2O, and dihydroxymaleic acid (HOOC-(OH)C=C(OH)-COOH) to 2,3 dioxo-propanoic acid (HOOC-C(O)-C(O)-COOH) and CO_2 [76].

Goldschmidt & Pauncz (1933) investigated the $Fe_2(SO_4)_3$/H_2O_2/ethanol reaction and confirmed that ethanol was oxidized directly to acetic acid. The authors also explained that Fenton & Jackson (1899) and Fenton & Jones (1900) did not detect aldehydes from aliphatic alcohols because the 1:1 molar ratio of H_2O_2 and alcohol was sufficient to oxidize all the alcohol to organic acids [77].

Even as late as 1989, Fe^{+3}/H_2O_2 oxidation articles appeared noting unusual oxidations. Sanderson et al. (1989) submitted a patent for co-synthesis of *t*-butanol and *t*-butyl peroxide from *t*-butane by Fe^{+3}/H_2O_2, showing addition of either a hydroxyl or a peroxyl group to the 3° carbon without explanation of mechanism [78].

4.2 Oxidative behavior of the perferryl-oxo ion

White & Coon (1977) summarized the discovery of the mechanism of respiration by mitochondrial enzyme cytochrome P450. Cytochrome P450 uses a Fe^{+3} ion chelated in a heme ring to conduct the reduction: (Eq. (8)) [67, 68].

$$\text{P450} - \left(\text{Fe}^{+3}\right) + \text{NADPH}_2 + {}^1\!/_2\text{O}_2 \rightarrow \text{P450} - \left(\text{Fe}^{+3}\right) + \text{NADP}^+ + \text{H}_2\text{O}$$

Cytochrome P450 Reduction of $NADPH_2$ with Oxygen (8)

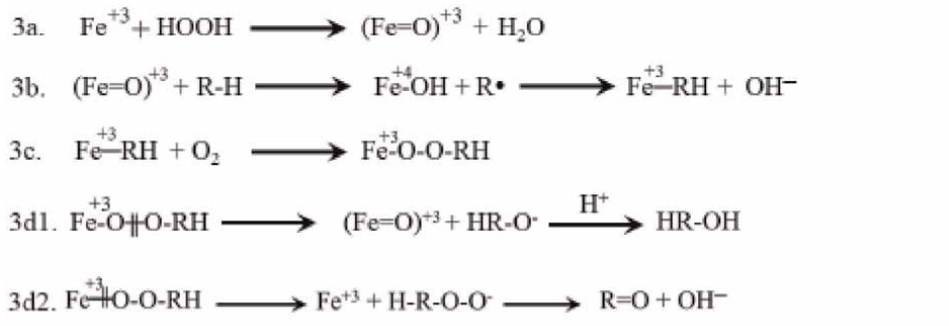
Responding to the discovery that the critical enzyme of respiration forms a Fe^{+3} = O intermediate to split the dioxygen molecule, Barton et al. (1983) sought to mimic the biological reaction using chelated Fe^{+3} ions and peroxide ion (O_2^{-2}) instead of oxygen as a new process to oxidize hydrocarbons to alcohols. Working with alkanes (R1-CH_2-R2), Barton expected that pyridine-chelated Fe^{+3} ions and potassium peroxide (K_2O_2) would produce alcohols (**Figure 3**) [54].

What Barton did not expect was that the reaction produced a mix of alcohols [R1-HC(OH)-R2] *and ketones* (R1-(C=O)-R2). Direct oxidation of hydrocarbons to ketones, a single step $4e^-$ oxidation and oxygen addition, was new to organic chemistry. For the oxidation of simple alkanes, the oxidation preference of the Fe^{+3}/H_2O^2 oxidant is: 3° H > 2° H > 1 H°. Barton et al. (1983) also found that they could manipulate the alcohol/ketone ratio by choosing iron chelators with different N/O ratios.

To understand the reaction mechanism, Barton and co-workers studied the oxidation of adamantine ($C_{10}H_{16}$, 4 tertiary, 6 secondary, 0 primary C-H groups). Despite the preponderance of secondary carbons, Barton's reactant favored oxidation of tertiary vs. secondary carbons by a 5:1 margin indicating that the oxidant behaved similar to the ferryl-oxo ion, but single step ketone addition was never reported for $(Fe = O)^{+2}$ oxidations [54].

Sugimoto & Sawyer (1985b) confirmed and extended Barton's findings by using Fe^{+3} and H_2O_2 to oxidize hydrocarbons molecules with double and triple bonds, isolating epoxides (R1-C-^{O}-C-R2) and oxetanes (R1-C-$^{O-O}$-C-R2) [79].

Seven years elapsed until Barton and coworkers resolved the perferryl-oxo structure and oxidation mechanism (**Figure 3**).



References:
3a. Barton, D.; Gastiger, M.; Motherwell, W. J. Chem. Soc. Chem. Comm. 1983, 41-3. DOI: 10.1039/C39830000041 : Barton, D.; Csuhai, E.; Doller, D.; Ozbalik, N.; Balavoine, G. PNAS 1990, 87 3401-04. DOI: 10.1073/pnas.87.9.3401.

3b, 3c. Barton, D.; Doller, D. Acc. Chem. Res. 1992, 25, 504-12. DOI: 10.1021/ar00023a004 : Schuchardt, U.; Jannini, M. J.; Richens, D. T.; Guerreiro, M. C.; Spinacé, E.V. 2001, Tetrahedron, 57(14) 2685-8. DOI: 10.1021/ja00032a032 : Barton, D.; Beviere, S.; Chavasiri, W.; Csuhai, E.; Doller, D.; Liu, W. J. Am. Chem. Soc. 1992, 114 2147-56. DOI: 10.1021/ja00032a032 :

3d.1,2. Barton, D.; Doller, D. Acc. Chem. Res. 1992, 25, 504-12. DOI: 10.1021/ar00023a004 : Schuchardt, U.; Jannini, M. J.; Richens, D. T.; Guerreiro, M. C.; Spinacé, E.V. 2001, Tetrahedron, 57(14) 2685-8. DOI: 10.1021/ja00032a032 : Sugimoto, H.; Spencer, L.; Sawyer, D. PNAS 1987, 84 1731-33. DOI: 10.1073/pnas.84.7.1731

Figure 3.
Perferryl-Oxo ion reactions.

Couching their model on the accepted behavior of Fe^{+3} nucleus of cytochrome P450 [80–82], Barton et al. (1990) proposed $(Fe = O)^{+3}$ as the reaction product of Fe^{+3} and H_2O_2 or Fe^{+2} and $O_2^{\bullet -}$ (superoxide anion) (**Figure 3a**). [Barton et al. (1990–8) wrote the structure of the perferryl-oxo ion as $[Fe^{V}{=}O]$. The $(Fe^{V}{=}O^{(-2)})$ and $(Fe = O)^{+3}$ formulas are equivalent for atoms, bonds, and net charge].

Barton et al. (1990): 1) proposed a bifurcated pathway leading either to alcohol or ketone formation, showing that the alcohol/ketone ratio could be varied with addition of dianisyl telluride, and 2) determined that both alcohol and ketone formation occurred in two steps, choosing different non-reversible paths at the second reaction [83, 84].

Barton and Doller (1992) mapped out steps of the pathway of perferryl-oxo ion oxidation of hydrocarbons (**Figure 3b–d**):

Step 1 (**Figure 3b**): Formation of Fe^{+4}-C-R intermediate. Using diphenyl-diselenide (Ph-Se-Se-Ph), or phenyl selenol (Ph-Se-H), Barton trapped the Fe^{+4}-C-R intermediate as a stable (Fe^{+3}-Ph-Se-C-R) intermediate as detected by mass spectroscopy (structure not specified).

Step 2 (**Figure 3c**): Oxygen Insertion to form Fe^{+3}-O-O-C-R intermediate. Comparing $^{16}O_2$ and $^{18}H_2O_2$, the authors detected primarily ^{16}O-labeled alcohols and ketones indicating that O_2 (not O_2^{-2}) formed the dioxygen bridge. The authors proposed that in an anoxic environment, peroxide is oxidized to dioxygen by ferric ions in sufficient quantities to complete the reaction as follows: [Eq. (9)]

$$2\,Fe^{+3} + O_2^{-2} \rightarrow 2\,Fe^{+2} + O_2$$

Reduction of H_2O_2 to O_2 by Ferric Ions (9)

Step 3 (**Figure 3d** (1 & 2)): Bifurcated Pathways Arise from Differential Cleavage of the O-O Bridge. The Fe^{+3}-O-O-C-R intermediate is the branch point between the 2e- and 4e- oxidative pathways: a) scission of the Fe^{+3}-O-|-O-R bond produces an alkoxide ($R\text{-}O^{-}$) and the $(Fe = O)^{+3}$ ion (**Figure 3d**.1); b) scission of the Fe^{+3}-|-O-O-R bond produces Fe^{+3} ion and a peroxyl ($^{-}$O-O-R) ion which then degrades to a ketone (R = O), and an oxide ion (O^{-2}) (**Figure 3d**.2).

Barton and Doller (1992) trapped the ferric-peroxy-carbon (Fe^{+3}-O-O-C-R) cleavage intermediates with tri-methoxy phosphine ($P(OMe)_3$). $P(OMe)_3$ reacted with either oxygen (R-C-O*-O-Fe^{+3} or R-C-O-O*-Fe^{+3}) trapping potential oxygen bridge cleavage products as R-C-O-$P(OMe)_3$ and R-C-O-O-$P(OMe)_3$ respectively. Thus Barton and Doller (1992) explained the mechanism of bifurcated production of alcohols or ketones from alkanes by perferryl-oxo $(Fe = O)^{+3}$ ion (85). Barton's oxidation scheme was confirmed by Schuchardt et al. (2001) [55].

Barton's perferryl-oxo ion oxidation theory explains Ruff's oxidation gluconic acid to arabinose (1898) [71] the one-step conversion of ethanol to acetic acid observed by Bohnson (1921) [72], Walton & Christensen (1926) [75], Goldschmidt & Pauncz (1933) [75], and the co-synthesis of *t*-butanol and *t*-butyl peroxide from *t*-butane by Sanderson (1989) [76].

4.3 Comparison of $(Fe = O)^{+2}$ and $(Fe = O)^{+3}$ ion chemistry

Both $(Fe = O)^{+2}$ (**Figure 2**) and $(Fe = O)^{+3}$ ions (**Figure 3**) abstract H• from the weakest C-H bond present in a molecule to form ferric ($Fe^{+3}OH$) or ferryl hydroxide ($Fe^{+4}OH$) and a C• radical respectively [85].

The electrophilic ferric and ferryl hydroxides react 'instantaneously' with the nucleophilic C• radical, but the resulting intermediates are different. Ferric hydroxide donates HO• to the C• radical, regenerating the ferrous ion, ending the cycle [33], however the ferryl atom attacks the C• radical (ejecting the hydroxyl group) to form the ferryl-carbon (Fe^{+4}-C) intermediate [83]. Oxygen (O_2) insertion into the (Fe^{+4}-C) bond creates the bifurcated oxidative pathways not available to either ferryl-oxo ion or hydroxyl radical [86].

Sugimoto et al. (1987), using ^{2}H and ^{18}O labeled ethanediols, determined that H• abstraction by $(Fe = O)^{+3}$ from the hydroxyl oxygen of a diol group [R_1-HC(O***H***)-HC(OH)-R_2] causes C-C bond cleavage, producing paired aldehydes [R_1-HC=O + R_2-HC=O], whereas H• abstraction from the carbon backbone produces hydroxy-ketones [R_1-C(O)-HC(OH)-R_2] [87].

5. Mixed Fenton oxidation systems

5.1 Untangling oxidation behaviors arising when Fe^{+2} ions, H_2O_2, and H_2O are present

The Fenton reaction (Fe^{+2} + H_2O_2 + H_2O) has been shown to generate three powerful oxidants: 1) (HO•) radical [16, 17]; 2) $(Fe = O)^{+2}$ ion [18]; and 3) $[Fe = O]^{+3}$ ion [86, 88].

Sugimoto & Sawyer (1985a & 1985b) proposed that both ferrous and ferric ions can abstract an •O• atom from H_2O_2, thus explaining how ferrous and ferric spontaneously reorganize to form the secondary oxidants ferryl $(Fe = O)^{+2}$ and perferryl $(Fe = O)^{+3}$ ions, respectively.

Sugimoto and Sawyer (1984) and (1985b) compared $(Fe = O)^{+2}$ and $(Fe = O)^{+3}$ oxidations, respectively, in anhydrous CH_3CN or 90% CH_3CN/10% H_2O with several organic and inorganic molecules. In anhydrous CH_3CN, ferryl-oxo ions oxidation produced only 2-electron oxidations, primarily dehydrations or hydroxyl additions, while perferryl-oxo ions produced both 2-, and 4- electron oxidations. Neither oxidant produced 1- electron oxidations.

In aqueous acetonitrile (CH_3CN/H_2O), single electron oxidations, characteristic of HO• were observed including: 1) carbon–carbon fusions; 2) oxidation of Fe^{+2} ions; and 3) reduction of Fe^{+3} ions to Fe^{+2} ions. The authors proposed that HO• radicals are created by ferryl-oxo and perferryl-oxo ions only when water is present, implying that H• abstraction from water produces HO• radicals via the formula: $(Fe = O)^{+2,+3}$ + H_2O• HO• + $Fe^{(+3,+4)}OH$ [59, 61].

Sawyer et al. (1993) tested the oxidizing capability of Fe^{+2} ions and organic peroxides (R-O-O-H) 1) under anhydrous conditions in the presence vs. absence of O_2, and 2) under anoxic conditions with anhydrous (Fe^{+2}) or partially hydrated ($Fe^{+2}(H_2O)_2$) conditions. The authors found evidence of $1e^{-}$ oxidations either when O_2 or H_2O_2 were present, indicating 1) that $(Fe = O)^{+2}$ reacted with H_2O to form HO• radicals, or 2) with O_2, creating O_2• [16], which then reacted with (R-O-O-H) to generate HO• radicals [39]. On the other hand, Hage et al. (1995) found that in the conversion of benzene to phenol, if a small amount of H_2O was added, the efficiency of conversion was increased, but other $1e^{-}$ signature products were not detected [89].

Sawyer et al. (1996) surveyed the oxidizing abilities of $Fe^{+2,+3}$, Cu^{+2}, Co^{+2}, and Mn^{+2} ions in anhydrous solvents with ROOH, with/out O_2. Under an argon atmosphere, only the hydroxyl radical sources produced chain fusion events, none of the

ions did. When air (20% O_2) was substituted, all of the ions showed oxidation patterns consistent with HO• radicals. The authors concluded that the metal ions, activated by peroxide, reacted with solubilized O_2, producing superoxide ($O_2^{\bullet -}$ or $HO_2^{\bullet}$), which in turn reacted with H_2O_2 to generate reactive singlet oxygen (•O•) which then reacts with R-C-H to produce HO• radicals [41].

Barton et al. (1995, 1996) seconded the research of Sawyer's group, confirming that absent H_2O, ferryl-oxo and perferryl-oxo ions perform distinct and distinctive 2- (and 4-) electron oxidations without mixing the unique chemistries of either ion [86, 90].

Mwebi (2005) also confirmed that when Fe^{+2} ions, H_2O_2, and H_2O are reacted in aqueous conditions, all three secondary oxidants [$(Fe = O)^{+2}$, $(Fe = O)^{+3}$, and HO•] arise in that either $(Fe = O)^{+2}$ and $(Fe = O)^{+3}$ ions can abstract H• from H_2O to create the HO• radical, the HO• radical can oxidize Fe^{+2} ions to Fe^{+3} ions, and H_2O_2 can reduce Fe^{+3} ions to Fe^{+2} ions [51].

5.2 Biological occurrence and utilization of the Fenton reagent

Oceans covered Earth 4.4 billion years ago [91], evidence of bacteria dates back 3.5 billion years ago (92), and evidence of oxygenic photosynthesis 2.3 billion years ago [91, 92]. From at least that time living organisms have evolved to defend against, and/or, utilize Fenton chemistry.

The use of the Fenton reagents to kill organisms or degrade biopolymers is widely distributed in the biosphere. Saprophytic fungi use Fenton reagents to degrade polysaccharides of woody plant tissues [93], including cellulose [93–96], callose [97], and hemicelluloses [98].

On the other side of the eukaryote kingdom, mammalian leukocytes and neutrophils pump Fe^{+2} ions [99, 100] and H_2O_2 into phagosomes to produce oxygen radicals [101] to effect pathogen killing [102–107]. For both nutrient mobilization and pathogen killing, these oxidants target external glycan including cell walls to cause cell lysis and/or internal glycans such DNA and RNA to facilitate death of bacteria and eukaryote parasites.

Moore and coworkers incubated *Saccharomyces cerevisiae* cells with an Fe^{+2}-chelating anti-cancer medication. Treated and control cells were stained, fixed, and thin-sectioned for electron microscopy. While studying chromosome damage the authors observed damage to the yeast cell walls by the anti-cancer drug [108–110].

Following Moore's lead, Lipke and coworkers treated ^{35}S -labeled *S. cerevisiae* cells with the an Fe^{+2}-binding anti-cancer medication, then compared protein levels release into growth media from treated and control cells [111], and cell lysis rates of treated and control cells after adding *Arthrobacter luteus* (Zymolyase) protease [112].

In Lim et al. (1995), the authors noted that pretreatment with yeast cells with an Fe^{+2}-binding anti-cancer agent increased cell lysis rates by Zymolyase protease with: 1) Fe^{+2} + O_2 or Fe^{+3} + O_2, but not Ca^{+2}, Co^{+2}, Cu^{+2}, Mn^{+2}, Mg^{+2}, and Zn^{+2} ions; 2) H_2O_2 could substitute for O_2; and 3) Fe^{+2}/H_2O_2 and Fe^{+3}/H_2O_2 alone also accelerated yeast cell lysis; 4) H_2O_2 only controls did not accelerate Zymolyase lysis rates [112].

To understand the basis of cell wall weakening by Fe^{+2}/H_2O_2, Ovalle et al. (2001) elected to separately test pure analogs of carbohydrates and proteins found in yeast wall [113]. Ovalle et al. (2001) assumed that partial oxidation of fungal wall monosaccharides would oxidize hydroxyl groups to aldehydes and/or carboxylic acids and developed a method for separating carbohydrates from 0 to 20 glucan units on polyacrylamide gels. Surveying the available literature of the time, the authors followed the method of Ahrgren et al. (1975) where dextran was preincubated with $FeSO_4$ prior to addition of H_2O_2 [114].

Ovalle et al. (2001) [113] labeled the aldehyde groups of glucose, maltose, maltotriose and enzymatically digested laminaran with 8- amino, 1-, 3-, 6-, naphthalene trisulfonate (ANTS) and NaCNBH3, by the method of Klock & Starr (1998) [115], to have glucan ladders to estimate degree of polymerization of carbohydrate chains separated by polyacrylamide gel electrophoresis. Ovalle et al. (2001) modified the method Klock & Starr (1998) to visualize carboxylic acids and by quenching aldehydes with NaBH4, then crosslinking ANTS to carboxylic acids with N-hydroxysuccinamide (NHS) and N-ethyl-N-(3-aminopropyl) carbodiimide (EDC) [116]. Ovalle et al. (2001) separately visualized de novo aldehydes and de novo carboxylic acids (after quenching aldehydes with $NaBH_4$) on 10% stacking/ 20% running acrylamide gels.

Ovalle et al. (2020) [117] used the method of Ovalle et al. (2001) to determine if Fe^{+2}/H_2O_2 would oxidize algal laminaran (d.p. ≈ 50–60 glucose units; 97–99% β1–3 glu / 1–3% β1–6 glu). To optimize metal ion-carbohydrate interactions, $FeSO_4$ was incubated with carbohydrate for 1 min prior to addition of H_2O_2. The final ratio (glucose monomer: Fe^{+2}: H_2O_2 = 10:1:1) was chosen to oxidize 10% of glucose monomers and reduce the likelihood of a secondary oxidation of glucose fragments to 1% maximum. Unoxidized laminaran did not enter that stacking gel. $NaIO4^{-}$ oxidized laminaran entered the stacking gel but stopped at the stacking gel/running gel interface. Fenton-oxidized laminaran produced smears, when labeled for either aldehyde or carboxylate groups. Enzyme- (Zymolyase) digested laminaran were used as glucan ladders when labeled for aldehydes or organic acids.

To label glucan fragments so as to be suitable for positive ion mass spectroscopy [118, 119], Ovalle et al. (2020) substituted tert-butyl ester of tyrosine (TBT) for ANTS with no other changes required. Ovalle et al. (2020) observed the elution of TBT-labeled glucan fragments with masses consistent with six classes of TBT-labeled molecules: aldoses, dialdoses, uronic acids, hexosuloses, aldonic acids (unlabeled), and hexulosonic acids (unlabeled) (**Figure 4**).

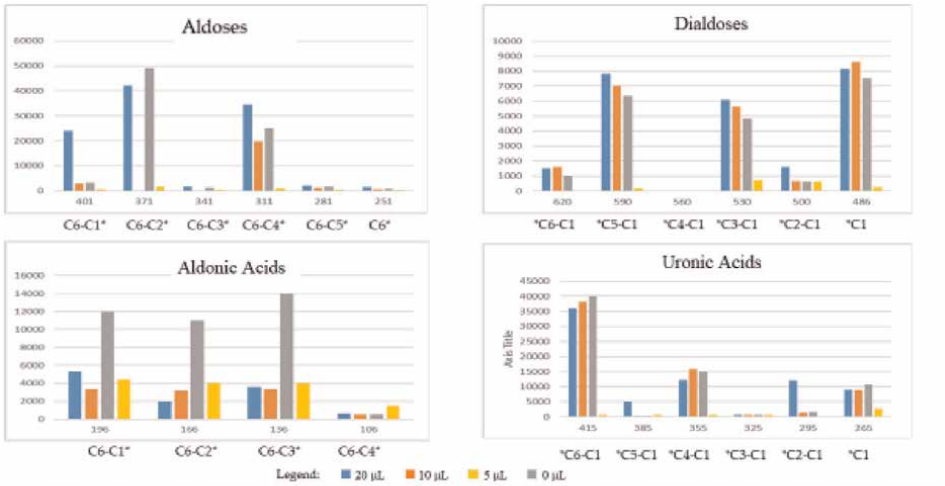


Figure 4.
Comparison of particles of four molecule classes from Laminaran after Fenton oxidation.

6. The authors concluded

1. Aldose / dialdose pairs arose from glucose by H• abstraction from an unsubstituted hydroxyl groups at O2, O4, or O6, and were mediated by $[Fe = O]^{+2}$ ion after Fe^{+2} ion was bound to a site where it was activated by H_2O_2. Diol-splitting reactions are consistent with oxidation by both HO• and $(Fe = O)^{+2}$ oxidants, however the ratios of the aldose / dialdose pairs were uneven, implying bias oxidations, hallmark of the $(Fe = O)^{+2}$ ion.

2. Uronic and aldonic acids were produced by ketone addition to a hydroxyl carbon (except at C1). The reaction is consistent with oxidation by Barton's perferryl-oxo ion.

3. As ferric ions were not added to the assay, Fe^{+2} ions must have been oxidized by HO• radicals.

4. Though present, the biased distribution of fragments excludes HO• radicals as the primary oxidant, HO• radicals are partially credited for non-zero values of infrequent carbohydrate fragments. Thus, Ovalle et al. (2020) observed all three Fenton oxidants directly or indirectly in the aqueous Fe^{+2}/H_2O_2 oxidation of laminaran.

6.1 Current applications of the Fenton oxidants

The Fenton Oxidants (HO•, $Fe = O^{+2}$, and $Fe = O^{+3}$) are being investigated as molecular scissors for insertion of reactive functional groups into otherwise inert substrates, such as carbohydrates. Oxidation of hydroxyl groups to carbonyl or carboxylic acid groups will allow them to act as carriers for various molecules with ramification in many sectors.

6.1.1 Hydroxyl radical oxidation of carbohydrates

Neyra et al. (2014, 2015) used a catalytic amount Fe^{+2} ions to produce HO• radicals from H_2O_2 to oxidize hydroxyl groups of acetylneuraminic acid monomers (2014) and tetramers (2015) to carbonyl and/or carboxylic groups. The goal of the experiment was to modify the sugars to create anchors for proteins so as to create vaccine adjuvant platforms [120, 121].

6.1.2 Perferryl-oxo oxidations of carbohydrates

Sorokin et al. (2004), using 'heme'-chelated Fe^{+3} ions, oxidized glucose monomers in starch fibers at C2 and C3 to produce acid / aldehyde pairs without hydrolyzing the flanking glycosidic bridges. The dual oxidations allow for two independent modifications of the glucose monomers in the starch chain [122–124].

6.1.3 Ozone-Fenton systems

Ozone (O_3) is being considered as an alternative to H_2O_2. Ozone gas can be activated by UV ($O_3 \rightarrow O_2 + \bullet O\bullet$) to produce oxygen radicals, or by reaction with iron

ions (Fe^{+2}, Fe^{+3} + $O^3 \rightarrow Fe = O^{+2}$, $Fe = O^{3+}$ + O_2, thus producing each Fenton oxidant without water as a byproduct.

Pestovsky (2004, 2005, 2006) reacted Fe^{+2} ion with O_3 in aqueous buffer as an alternative method of creating $(Fe = O)^{+2}$ ion. The signature of HO• radicals: 1 e^- oxidations, were not detected for the oxidation of several classes of organic molecules [125–127].

Bataineh (2015a), and Bataineh et al. (2012, 2015b) compared the oxidation of DMSO with Fe^{+2} and O_3 in aqueous phosphate vs. acetonitrile solvents. In acetonitrile the primary product was $DMSO_2$, an oxygen addition reaction. In buffered H_2O, ethane and methylsulfinate were the primary products, indicating fragmentation of DMSO occurred by HO• oxidation [128–130].

Enami et al. (2014) fired microjets of aqueous $FeCl_2$ into sprays of either O_2 or O_3/O_2 mixed gases. Particles detected by negative ion MS proved that Fe^{+2} and O_3 produces new particles not seen in $FeCl_2$ or $FeCl_2/O_2$ sprays [131].

6.1.4 Fenton systems for bioremediation

Fenton oxidants are gaining popularity as agents of bioremediation because of their ability to mineralize toxic organic molecules without contamination by ecologically damaging elements (halogens, heavy metal ions, etc.).

6.1.4.1 Ozone (O_3) for bioremediation with (HO•) radical or $(Fe = O)^{+3}$ ion

Turan-Ertas & Gurol (2002) compared ozone (O_3) against Fe^{+3}/H_2O_2 in the degradation of diethylene glycol [$(HO\text{-}CH_2\text{-}CH_2)O$], a toxic byproduct of the synthesis of ethylene glycol. The authors compared the diethylene glycol oxidation profile by O_3 and Fe^{+3}/H_2O_2. Both procedures were effective in degrading diethylene glycol, however the Fe^{+3}/H_2O_2 oxidation produced fewer and simpler products [132].

7. Fenton chemistry for beginners

1. In 1894, John HJH Fenton published his discovery that $FeSO_4$ and H_2O_2 produced oxidations not mimicked by other methods known at the time.

2. In 1932 & 1934, Fritz Haber & Joseph Weiss proposed the existence of HO• (hydroxyl radical) and $HO_2•^-$ (superoxide anion) as the principal oxidants of Fenton's reaction. Merz and Waters (1947) were among the first to propose that HO• radical oxidizes organic molecules by H• abstraction.

3. In 1932, William Bray & H. M. Gorin proposed $(Fe = O)^{+2}$ (ferryl-oxo ion) as the principal oxidant of the Fenton's reaction. Groves and coworkers proposed (in anhydrous solvents) oxygen rebound phenomena, to explain abstraction of H-, followed by addition of HO- to the same carbon to create of alcohols from alkanes in a single step. The debate raged for decades until umpired by D. T. Sawyer and coworkers.

4. Though $4e^-$ oxidations by Fe^{+3}/H_2O_2 were observed by Ruff in 1898, and thereafter for nearly 100 years, Derek H. R. Barton & coworkers proposed the

structure of the oxidant as $(Fe = O)^{+3}$ (perferryl-oxo ion) in 1990, and the bifurcated oxygen addition mechanism in 1992.

5. Donald T. Sawyer & coworkers investigated the behaviors of several transition metal ions in aqueous and anhydrous systems. Sawyer and coworkers proved: 1) H_2O is not a spectator molecule; in the absence of water, Fe^{+2} and Fe^{+3} ions do not produce HO• radicals, thus explaining why Groves saw only $2e^{-}$ oxidations in anhydrous media, while Rush and others observed $1e^{-}$ oxidations in aqueous systems; 2) in aqueous system HO• radicals can oxidize ferrous ions to ferric ions; and 3) $HO_2•^{-}$ radicals can reduce ferric ions to ferrous ions, thus in water all three oxidants are present.

6. Though each oxidant has a singular profile seen in the oxidation of hydrocarbons, different oxidative behaviors are seen with organic molecules containing oxygen. Oxygen causes $(Fe = O)^{+2}$ and $(Fe = O)^{+3}$ ions to target hydrogens that are bonded to hydroxyl carbons and hydroxyl oxygens. Abstraction of H• from an O-H bond in molecules with adjacent hydroxyl groups causes C-C cleavage of diols for all three oxidants.

7. Because of competition between the oxidants for targets, the order of addition of reagents alters the outcome of the assay. Fenton's method was to begin with substrate, add H_2O_2, and then $FeSO_4$. In this sequence, when Fe^{+2} (or Fe^{+3}) ions are activated by peroxide, ferryl-oxo (or perferryl-oxo) ions will react with adjacent H_2O molecules, producing HO• radicals, that then diffuse to the substrate, oxidizing H-X bonds by accessibility.

   Addition of Fe^{+2} (or Fe^{+3}) ions first allows the metal ions to associate with and/or chelate onto the substrate. Addition of H_2O_2 now creates the ferryl-oxo (or perferryl-oxo) ions adjacent to the substrate, increasing the likelihood of in situ oxidation at the ion's binding site, creating uneven product profiles, as observed in Ovalle et al. (2020). Allowing binding of metal ions to substrates before addition of H_2O_2 can explain observations of non-canonical "crypto-hydroxyl-" substrate oxidations previously observed by many authors.

8. This summary is not an exhaustive history, nor is it the full collection of all the articles I read. However, it took me many years to both acquire and understand the chemistry of each oxidant. I did not address other metal/peroxide systems (such as copper-Fenton chemistry) here as it was not relevant to either Ovalle et al. (2001) nor (2020).

   This article is written as a guide for newcomers so that they have a head start in finding the papers they need for their research. Welcome to the club!

8. Conclusions

John H. J. H. Fenton did not know that his discovery would enthrall a legion of researchers, be championed by two Noble laureates, and create three separate fields of peroxide oxidation chemistry: hydroxyl radicals (HO•), ferryl-oxo ions $(Fe = O)^{+2}$ and perferryl-oxo ions $(Fe = O)^{+3}$.

Fenton's successors required a full century to explain the ramification of these reactants. These three simple molecules continue to generate novel research investigations in chemistry, physics, and biology. I am proud to be among Fenton's successors.

Acknowledgements

I thank Professors Peter Lipke and Carol Moore for their insights and direction that led me to investigate Fenton chemistry and write Ovalle et al. (2001) and (2020), and Professor Clifford E Soll, who invested hundreds of hours devising the gradient and conditions for sharp separations of the TBT-labeled carbohydrate fragments, but did not live to see the fruition of our collaboration.

Special thanks to Mr. Lijie Chen who acted as my secretary, sounding board, and webmaster as I wrote Ovalle et al. (2020), helping me unearth 100+ years of Fenton literature including the personal histories of the many scientists that followed the path discovered by Prof. Fenton.

I also thank Professors Claude Brathwaite and Barbara Studamire for financial and moral support during the long writing of Ovalle et al. (2020). Finally, I thank Professors Rick Magliozzo, Richard Burger, and Alex Greer for insights into iron-oxygen interactions.

The works of Ovalle et al. (2001) and Ovalle et al. (2020) were supported by NIH GM47176 and RCMI RR03037 grants.

Conflict of interest

I declare I have no financial or other interests, aside from the telling of the history of, and unusual chemistry of the Fenton reaction and its investigators.

Author details

Rafael Ovalle
Graduate School of CUNY, New York City, N.Y., USA

*Address all correspondence to: ovalle.rafael1@gmail.com

References

[1] Fenton H. On a new reaction of tartaric acid. Chem News 1876;33(190):190.

[2] Barbusinski K. Henry John Horstman Fenton - short biography and brief history of Fenton reagent discovery. Chemistry-Didactics-Ecology-Metrology 2009;R. 14, NR 1-2:101-105.

[3] Fenton H. LXXIII.—Oxidation of tartaric acid in presence of iron. Journal of the Chemical Society, Transactions 1894;65:899-910.

[4] Fenton HJH. XLI.—The constitution of a new dibasic acid, resulting from the oxidation of tartaric acid. Journal of the Chemical Society, Transactions 1896;69: 546-562.

[5] Fenton HJH, Jackson HJ. I.—The oxidation of polyhydric alcohols in presence of iron. Journal of the Chemical Society, Transactions 1899;75:1-11.

[6] Merz J, Waters WA. A.—Electron-transfer reactions. The mechanism of oxidation of alcohols with Fenton's reagent. Discuss Faraday Soc 1947;2: 179-188.

[7] Fenton HJH, Jones H. VII.—The oxidation of organic acids in presence of ferrous iron. Part I. Journal of the Chemical Society, Transactions 1900;77: 69-76.

[8] Cross C, Bevan E, Smith C. XLIX.—Reactions of the carbohydrates with hydrogen peroxide. Journal of the Chemical Society, Transactions 1898;73: 463-472.

[9] Morrell, Robert Selby, and Crofts, J. M. Action of hydrogen peroxide on carbohydrates in the presence of iron. Proceedings of the Chemical Society (London) 1899;15(208):99-100.

[10] Morrell RS, Crofts JM. LXXV.—Action of hydrogen peroxide on carbohydrates in the presence of ferrous salts. Journal of the Chemical Society, Transactions 1899;75:786-792.

[11] Morrell RS, Crofts JM. CXV.—Action of hydrogen peroxide on carbohydrates in the presence of ferrous salts. II. Journal of the Chemical Society, Transactions 1900;77:1219-1221.

[12] Morrell RS, Crofts JM. LXX.—Action of hydrogen peroxide on carbohydrates in the presence of ferrous sulphate. III. Journal of the Chemical Society, Transactions 1902;81:666-675.

[13] Morrell RS, Crofts JM. CXXI.—Action of hydrogen peroxide on carbohydrates in the presence of ferrous sulphate. IV. Journal of the Chemical Society, Transactions 1903;83:1284-1292.

[14] Morrell RS, Bellars AE. XXXV.—Action of hydrogen peroxide on carbohydrates in the presence of ferrous sulphate. Part V. J. Chem. Soc., Trans., 1905,87, 280-293 1905;87: 280-293.

[15] Cross C, Bevan E, Heiberg T. LXXI.—Oxidation of furfuraldehyde by hydrogen peroxide. Journal of the Chemical Society, Transactions 1899;75: 747-753.

[16] Haber F, Weiss J. Über die katalyse des hydroperoxydes. Naturwissenschaften 1932;20(51): 948-950.

[17] The catalytic decomposition of hydrogen peroxide by iron salts. Proceedings of the Royal Society of London A: Mathematical, Physical and Engineering Sciences: The Royal Society; 1934.

[18] Bray WC, Gorin M. Ferryl ion, a compound of tetravalent iron. J Am Chem Soc 1932;54(5):2124-2125.

[19] Weast RC, Astle MJ, Beyer WH. CRC handbook of chemistry and physics.: CRC press, Boca raton FL; 1989.

[20] Rush JD, Koppenol WH. Oxidizing intermediates in the reaction of ferrous EDTA with hydrogen peroxide. Reactions with organic molecules and ferrocytochrome c. J Biol Chem 1986 May 25;261(15):6730-6733.

[21] Rush JD, Koppenol WH. Reactions of Fe(II)-ATP and Fe(II)-citrate complexes with t-butyl hydroperoxide and cumyl hydroperoxide. FEBS Lett 1990;275(1-2): 114-16.

[22] Rush JD, Koppenol WH. The reaction between ferrous polyaminocarboxylate complexes and hydrogen peroxide: An investigation of the reaction intermediates by stopped flow spectrophotometry. J Inorg Biochem 1987;29(3):199-215.

[23] Rush JD, Maskos Z, Koppenol WH. Distinction between hydroxyl radical and ferryl species. Meth Enzymol 1990; 186:148-156.

[24] Walling C, Kato S. Oxidation of alcohols by Fenton's reagent. Effect of copper ion. J Am Chem Soc 1971;93(17): 4275-4281.

[25] Walling C. Fenton's reagent revisited. Acc Chem Res 1975;8(4): 125-131.

[26] Walling C. Intermediates in the reactions of Fenton type reagents. Acc Chem Res 1998;31(4):155-157.

[27] Kremer SM, Wood PM. Production of Fenton's reagent by cellobiose oxidase from cellulolytic cultures of Phanerochaete chrysosporium. Eur J Biochem 1992;208(3):807-14.

[28] Kremer M. Mechanism of the Fenton reaction. Evidence for a new intermediate. Physical Chemistry Chemical Physics 1999;1(15):3595-3605.

[29] Kremer ML. Is OH the active Fenton intermediate in the oxidation of ethanol? J Inorg Biochem 2000;78(3):255-257.

[30] Merz J, Waters W. 511. The oxidation of aromatic compounds by means of the free hydroxyl radical. Journal of the Chemical Society (Resumed) 1949:2427-2433.

[31] Merz J, Waters WA. S 3. Some oxidations involving the free hydroxyl radical. Journal of the Chemical Society (Resumed) 1949:S15-S25.

[32] Groves JT, Van der Puy M. Stereospecific aliphatic hydroxylation by an iron-based oxidant. J Am Chem Soc 1974;96(16):5274-5275.

[33] Groves JT, McClusky GA. Aliphatic hydroxylation via oxygen rebound. Oxygen transfer catalyzed by iron. J Am Chem Soc 1976;98(3):859-861.

[34] Groves JT, Van der Puy M. Stereospecific aliphatic hydroxylation by iron-hydrogen peroxide. Evidence for a stepwise process. J Am Chem Soc 1976; 98(17):5290-5297.

[35] Groves JT. High-valent iron in chemical and biological oxidations. J Inorg Biochem 2006;100(4):434-447.

[36] Wink DA, Nims RW, Desrosiers MF, Ford PC, Keefer LK. A kinetic investigation of intermediates formed during the Fenton reagent mediated degradation of N-nitrosodimethylamine: evidence for an oxidative pathway not

involving hydroxyl radical. Chem Res Toxicol 1991;4(5):510-512.

[37] Wink DA, Wink CB, Nims RW, Ford PC. Oxidizing intermediates generated in the Fenton reagent: kinetic arguments against the intermediacy of the hydroxyl radical. Environ Health Perspect 1994;102 Suppl 3:11-15.

[38] Wink DA, Nims RW, Saavedra JE, Utermahlen WE,Jr, Ford PC. The Fenton oxidation mechanism: reactivities of biologically relevant substrates with two oxidizing intermediates differ from those predicted for the hydroxyl radical. Proc Natl Acad Sci U S A 1994 Jul 5;91 (14):6604-6608.

[39] Sawyer DT, Kang C, Llobet A, Redman C. Fenton reagents (1: 1 FeIILx/ HOOH) react via [LxFeIIOOH (BH)](1) as hydroxylases (RH. fwdarw. ROH), not as generators of free hydroxyl radicals (HO. bul.). J Am Chem Soc 1993;115(13):5817-5818.

[40] Spring W. Recherches sur les conditions dans lesquelles le peroxyde d'hydrogène se décompose. Bulletin de l'Académie Royale des Sciences, des Lettres et des Beaux-arts de Belgique. Sciences.3e série 1895;30(7):32-55.

[41] Sawyer DT, Sobkowiak A, Matsushita T. Metal [ML x; M= Fe, Cu, Co, Mn]/hydroperoxide-induced activation of dioxygen for the oxygenation of hydrocarbons: oxygenated Fenton chemistry. Acc Chem Res 1996;29(9):409-416.

[42] Dorfman LM, Adams GE. Reactivity of the hydroxyl radical in aqueous solutions. National Standard Reference Data System 1973;No. NSRDS-NBS-46.

[43] Dizdaroglu M, Henneberg D, Schomburg G, von Sonntag C. Radiation Chemistry of Carbohydrates, VI: γ-Radiolysis of Glucose in Deoxygenated N2O Saturated Aqueous Solution. Zeitschrift für Naturforschung B 1975;30 (5-6):416-425.

[44] Dizdaroglu M, Von Sonntag C. Strahlenchemie von Kohlenhydraten, IV. γ-Radiolyse von Cellobiose in N2O-gesättigter wäßriger Lösung/γ-Radiolysis of Cellobiose in N2O Saturated Aqueous Solution. Zeitschrift für Naturforschung B 1973;28(9-10):635-646.

[45] Thomas J. Rates of reaction of the hydroxyl radical. Transactions of the Faraday Society 1965;61:702-707.

[46] Droege AT, Tully FP. Hydrogen-atom abstraction from alkanes by hydroxyl. 3. Propane. J Phys Chem 1986; 90(9):1949-1954.

[47] Tully FP, Droege AT, Koszykowski M, Melius CF. Hydrogen-atom abstraction from alkanes by hydroxyl. 2. Ethane. J Phys Chem 1986;90(4):691-698.

[48] Baxley JS, Wells J. The hydroxyl radical reaction rate constant and atmospheric transformation products of 2-butanol and 2-pentanol. Int J Chem Kinet 1998;30(10):745-752.

[49] Bossmann SH, Oliveros E, Göb S, Siegwart S, Dahlen EP, Payawan L, et al. New evidence against hydroxyl radicals as reactive intermediates in the thermal and photochemically enhanced Fenton reactions. The Journal of Physical Chemistry A 1998;102(28):5542-5550.

[50] Anbar M, Meyerstein D, Neta P. Reactivity of aliphatic compounds towards hydroxyl radicals. Journal of the Chemical Society B: Physical Organic 1966:742-747.

[51] Mwebi NO. Fenton & Fenton-like Reactions: The Nature of Oxidizing

Intermediates Involved. University of Maryland, College Park, Md. 2005; Thesis.

[52] Eberhardt MK, Colina R. The reaction of OH radicals with dimethyl sulfoxide. A comparative study of Fenton's reagent and the radiolysis of aqueous dimethyl sulfoxide solutions. J Org Chem 1988;53(5):1071-1074.

[53] Yamazaki I, Piette LH. EPR spin-trapping study on the oxidizing species formed in the reaction of the ferrous ion with hydrogen peroxide. J Am Chem Soc 1991;113(20):7588-7593.

[54] Barton DH, Gastiger MJ, Motherwell WB. A new procedure for the oxidation of saturated hydrocarbons. Journal of the Chemical Society, Chemical Communications 1983(1): 41-43.

[55] Schuchardt U, Jannini MJ, Richens DT, Guerreiro MC, Spinacé EV. Gif chemistry: new evidence for a non-radical process. Tetrahedron 2001;57 (14):2685-2688.

[56] Waters WA. Glycol splitting by hydroxyl radicals. Nature 1946 Sep 14; 158:380.

[57] Gilbert BC, King DM, Thomas CB. Radical reactions of carbohydrates. Part 2. An electron spin resonance study of the oxidation of D-glucose and related compounds with the hydroxyl radical. Journal of the Chemical Society, Perkin Transactions 2 1981(8):1186-1199.

[58] Gilbert BC, King DM, Thomas CB. The oxidation of some polysaccharides by the hydroxyl radical: an ESR investigation. Carbohydr Res 1984;125 (2):217-235.

[59] Sugimoto H, Sawyer DT. Iron (II)-induced activation of hydrogen peroxide to ferryl ion (FeO^{+2}) and singlet oxygen ($^1O_2$) in acetonitrile: monoxygenations, dehydrogenations, and dioxygenations of organic substrates. J Am Chem Soc 1984;106(15):4283-4285.

[60] Kerr J, Stocker D. Strengths of chemical bonds. https://www.researchgate.net/ 1983:9-52-9-75.

[61] Blanksby SJ, Ellison GB. Bond dissociation energies of organic molecules. Acc Chem Res 2003 Apr;36 (4):255-263.

[62] Luo Y. Handbook of bond dissociation energies in organic compounds.: CRC press; 2002.

[63] Louwerse MJ, Baerends EJ. Oxidative properties of FeO^{+2}: electronic structure and solvation effects. Physical Chemistry Chemical Physics 2007;9(1):156-166.

[64] Louwerse MJ. Computational Modeling of Oxidation Catalysis: Studies concerning Fenton's reaction. 2008.

[65] Luo Y, Kerr J. Bond dissociation energies. CRC Handbook of Chemistry and Physics 2012;89.

[66] Kerr J, Stocker D. Bond dissociation energies by kinetic methods. Chem Rev 1983:9-52-9-75.

[67] Coon M, White R, Nordblom G, Ballou D, Guengerich F. Highly purified liver microsomal cytochrome P450: properties and catalytic mechanism. Croat Chem Acta 1977;49(2):163-177.

[68] Groves JT, McClusky GA, White RE, Coon MJ. Aliphatic hydroxylation by highly purified liver microsomal cytochrome P-450. Evidence for a carbon radical intermediate. 1978.

[69] Okamoto T, Sasaki K, Shimada M, Oka S. Catalysis of aerobic C–C bond

cleavage of 1, 2-bis (4-methoxyphenyl) ethane-1, 2-diol by meso-tetraphenylporphyrinatoiron (III). A model system for cytochrome P-450 scc-dependent glycol cleavage. Journal of the Chemical Society, Chemical Communications 1985(7):381-383.

[70] Okamoto T, Sasaki K, Oka S. Biomimetic oxidation with molecular oxygen. Selective carbon-carbon bond cleavage of 1, 2-diols by molecular oxygen and dihydropyridine in the presence of iron-porphyrin catalysts. J Am Chem Soc 1988;110(4): 1187-1196.

[71] Sugimoto H, Sawyer DT. Iron (II)-induced activation of hydroperoxides for the dehydrogenation and monooxygenation of organic substrates in acetonitrile. J Am Chem Soc 1985;107 (20):5712-5716.

[72] Youngman RJ, Elstner EF. Oxygen species in paraquat toxicity: the crypto-OH radical. FEBS Lett 1981;129(2): 265-268.

[73] Ruff O. Ueber die verwandlung der d-gluconsäure in d-arabinose. European Journal of Inorganic Chemistry 1898;31 (2):1573-1577.

[74] Bohnson VL. The catalytic decomposition of hydrogen peroxide by ferric salts. J Phys Chem 1921;25(1): 19-54.

[75] Walton JH, Christensen CJ. The catalytic influence of ferric ions on the oxidation of ethanol by hydrogen peroxide. J Am Chem Soc 1926 08/01;48 (8):2083-2091.

[76] Wieland H, Franke W. Über den mechanismus der oxydationsvorgänge. XII. Die aktivierung des hydroperoxyds durch eisen. European Journal of Organic Chemistry 1927;457(1):1-70.

[77] Goldschmidt S, Pauncz S. über die peroxydatische und katalatische wirkung von ferrosalzen. II. European Journal of Organic Chemistry 1933;502(1):1-19.

[78] Sanderson JR, Marquis ET, Lin J. Isobutane oxidation in the presence of a soluble iron complex as catalyst. United States Patent. 1989;#4,803,305.

[79] Sugimoto H, Sawyer DT. Ferric chloride induced activation of hydrogen peroxide for the epoxidation of alkenes and monoxygenation of organic substrates in acetonitrile. J Org Chem 1985;50(10):1784-1786.

[80] de Montellano, Paul R Ortiz, Correia MA. Inhibition of cytochrome P450 enzymes. Cytochrome P450: Structure, Mechanism, and Biochemistry. Springer; 1995. p. 305-364.

[81] Mueller EJ, Loida PJ, Sligar SG. Twenty-five years of $p450_{CAM}$ research. Cytochrome P450: Structure, Mechanism, and Biochemistry. Springer; 1995. p. 83-124.

[82] Dawson JH, Sono M. Cytochrome P-450 and chloroperoxidase: thiolate-ligated heme enzymes. Spectroscopic determination of their active-site structures and mechanistic implications of thiolate ligation. Chem Rev 1987;87 (5):1255-1276.

[83] Barton DH, Csuhai E, Doller D, Ozbalik N, Balavoine G. Mechanism of the selective functionalization of saturated hydrocarbons by Gif systems: relationship with methane monooxygenase. Proceedings of the National Academy of Sciences 1990 May 01;87(9):3401-3404.

[84] Barton DH, Beviere SD, Chavasiri W, Csuhai E, Doller D, Liu WG. The functionalization of saturated hydrocarbons. Part 20. Alkyl

hydroperoxides: reaction intermediates in the oxidation of saturated hydrocarbons by GIF-Type reactions and mechanistic studies on their formation. J Am Chem Soc 1992;114(6):2147-2156.

[85] Barton DH, Hu B, Taylor DK, Wahl RUR. The selective functionalization of saturated hydrocarbons. Part 32. Distinction between the Fe II–Fe IV and Fe III–Fe V manifolds in Gif chemistry. The importance of carboxylic acids for alkane activation. Evidence for a dimeric iron species involved in Gif-type chemistry. Journal of the Chemical Society, Perkin Transactions 2 1996(6): 1031-1041.

[86] Barton DHR, Doller D. The selective functionalization of saturated hydrocarbons: Gif chemistry. Acc Chem Res 1992 11/01;25(11):504-512.

[87] Barton DH. Gif chemistry: the present situation. Tetrahedron 1998;54 (22):5805-5817.

[88] Sugimoto H, Spencer L, Sawyer DT. Ferric chloride-catalyzed activation of hydrogen peroxide for the demethylation of N,N-dimethylaniline, the epoxidation of olefins, and the oxidative cleavage of vicinal diols in acetonitrile: a reaction mimic for cytochrome P-450. Proc Natl Acad Sci U S A 1987 Apr;84(7):1731-1733.

[89] Hage JP, Llobet A, Sawyer DT. Aromatic hydroxylation by Fenton reagents {Reactive intermediate $[L_x^{+}Fe^{III}OOH(BH^{+})]$, not free hydroxyl radical (HO·)}. Bioorg Med Chem 1995;3 (10):1383-1388.

[90] Barton DHR, Chabot BM, Delanghe NC, Hu B, Le Gloahec VN, Rojas Wahl RU. Further evidence for the FeII-FeIV and FeIII-FeV manifolds in the substitution of saturated hydrocarbons. Tetrahedron Lett 1995 09/25;36(39):7007-7010.

[91] Hall D. Ocean through time. https://ocean.si.edu/through-time/ocean-through-time. Smithsonian Institute 2019.

[92] Marshall M. Timeline: the evolution of life. https://www.newscientist.com/article/dn17453-timeline-the-evolution-of-life/. NewScientist 2009:1.

[93] Wang W, Gao P. A peptide-mediated and hydroxyl radical HO.-involved oxidative degradation of cellulose by brown-rot fungi. Biodegradation 2002;13(6):383-394.

[94] Xu G, Goodell B. Mechanisms of wood degradation by brown-rot fungi: chelator-mediated cellulose degradation and binding of iron by cellulose. J Biotechnol 2001;87(1): 43-57.

[95] Wang W, Gao PJ. Function and mechanism of a low-molecular-weight peptide produced by *Gloeophyllum trabeum* in biodegradation of cellulose. J Biotechnol 2003;101(2):119-130.

[96] Cameron MD, Aust SD. Cellobiose dehydrogenase-an extracellular fungal flavocytochrome. Enzyme Microb Technol 2001;28(2-3):129-138.

[97] Chen X, Kim J. Callose synthesis in higher plants. Plant signaling & behavior 2009;4(6):489-492.

[98] Progress in understanding how brown-rot fungi degrade cellulose. Biodeterioration Abstracts; 1991.

[99] Kuhn DE, Baker BD, Lafuse WP, Zwilling BS. Differential iron transport into phagosomes isolated from the RAW264.7 macrophage cell lines transfected with Nramp1Gly169 or

Nramp1Asp169. J Leukoc Biol 1999 Jul; 66(1):113-119.

[100] Zwilling BS, Kuhn DE, Wikoff L, Brown D, Lafuse W. Role of iron in Nramp1-mediated inhibition of mycobacterial growth. Infect Immun 1999 Mar;67(3):1386-1392.

[101] Chaturvedi V, Wong B, Newman SL. Oxidative killing of *Cryptococcus neoformans* by human neutrophils. Evidence that fungal mannitol protects by scavenging reactive oxygen intermediates. J Immunol 1996 May 15;156(10):3836-3840.

[102] Seider K, Gerwien F, Kasper L, Allert S, Brunke S, Jablonowski N, et al. Immune evasion, stress resistance, and efficient nutrient acquisition are crucial for intracellular survival of Candida glabrata within macrophages. Eukaryotic Cell 2014;13(1):170-183.

[103] Cleary JA, Kelly GE, Husband AJ. The effect of molecular weight and beta-1,6-linkages on priming of macrophage function in mice by (1,3)-beta-D-glucan. Immunol Cell Biol 1999;77(5):395-403.

[104] Rice PJ, Kelley JL, Kogan G, Ensley HE, Kalbfleisch JH, Browder IW, et al. Human monocyte scavenger receptors are pattern recognition receptors for (1–>3)-beta-D-glucans. J Leukoc Biol 2002;72(1):140-16.

[105] Nicholson S, Bonecini-Almeida M, Lapa eS, Nathan C, Xie QW, Mumford R, et al. Inducible nitric oxide synthase in pulmonary alveolar macrophages from patients with tuberculosis. J Exp Med 1996;183(5):2293-302.

[106] Sveinbjornsson B, Seljelid R. Aminated beta-1,3-D-polyglucose activates salmon pronephros macrophages in vitro. Vet Immunol Immunopathol 1994;41(1-2):113-123.

[107] Kudeken N, Kawakami K, Saito A. Role of superoxide anion in the fungicidal activity of murine peritoneal exudate macrophages against Penicillium marneffei. Microbiol Immunol 1999;43(4):323-330.

[108] Moore CW, Del Valle R, McKoy J, Pramanik A, Gordon RE. Lesions and preferential initial localization of [S-methyl-3H]bleomycin A2 on *Saccharomyces cerevisiae* cell walls and membranes. Antimicrob Agents Chemother 1992 Nov;36(11): 2497-2505.

[109] Moore CW. Internucleosomal cleavage and chromosomal degradation by bleomycin and phleomycin in yeast. Cancer Res 1988 Dec 1;48(23): 6837-6843.

[110] Moore CW. Cleavage of cellular and extracellular *Saccharomyces cerevisiae* DNA by bleomycin and phleomycin. Cancer Res 1989;49(24):6935-640.

[111] Beaudouin R, Lim ST, Steide JA, Powell M, McKoy J, Pramanik AJ, et al. Bleomycin affects cell wall anchorage of mannoproteins in *Saccharomyces cerevisiae*. Antimicrob Agents Chemother 1993;37(6):1264-1269.

[112] Lim ST, Jue CK, Moore CW, Lipke PN. Oxidative cell wall damage mediated by bleomycin-Fe(II) in *Saccharomyces cerevisiae*. J Bacteriol 1995; 177(12):3534-3539.

[113] Ovalle R, Soll CE, Lim F, Flanagan C, Rotunda T, Lipke PN. Systematic analysis of oxidative degradation of polysaccharides using PAGE and HPLC–MS. Carbohydr Res 2001;330(1):131-19.

[114] Ahrgren L, de Belder A. The Action of Fenton's Reagent on Dextran. Starch-Stärke 1975;27(4):121-123.

[115] Klock JC, Starr CM. Polyacrylamide gel electrophoresis of fluorophore-labeled carbohydrates from glycoproteins. Methods Mol Biol 1998;76:115-129.

[116] Klockow AA. The Influence of Buffer Composition on Separation Efficiency and Resolution in Capillary Electrophoresis of 8-Aminonaphthalene-1,3,6-Trisulfonic Acid Labeled Monosaccharides and Complex Carbohydrates. Electrophoresis 1996;17: 110-119.

[117] Ovalle R, Chen L, Soll CE, Moore CW, Lipke PN. Regioselective degradation of [beta] 1, 3 glucan by ferrous ion and hydrogen peroxide (Fenton oxidation). Carbohydr Res 2020;497:108124.

[118] Chizhov AO, Dell A, Morris HR, Reason AJ, Haslam SM, McDowell RA, et al. Structural analysis of laminarans by MALDI and FAB mass spectrometry. Carbohydr Res 1998;310(3):203-210.

[119] Read SM, Currie G, Bacic A. Analysis of the structural heterogeneity of laminarin by electrospray- ionisation-mass spectrometry. Carbohydr Res

[120] Neyra C, Paladino J, Le Borgne M. Oxidation of sialic acid using hydrogen peroxide as a new method to tune the reducing activity. Carbohydr Res 2014; 386:92-98.

[121] Neyra C, Paladino J, Le Borgne M. Mechanisms of depolymerization and activation of a polysialic acid and its tetramer by hydrogen peroxide. Carbohydr Polym 2015;115:494-501.

[122] Sorokin A, Fraisse L, Rabion A, Meunier B. Metallophthalocyanine-catalyzed oxidation of catechols by H2O2 and its surrogates. Journal of Molecular Catalysis A: Chemical 1997;117(1-3): 103-114.

[123] Kachkarova-Sorokina SL, Gallezot P, Sorokin AB. A novel clean catalytic method for waste-free modification of polysaccharides by oxidation. Chemical communications 2004(24):2844-2845.

[124] Sorokin AB, Kachkarova-Sorokina SL, Donzé C, Pinel C, Gallezot P. From native starch to hydrophilic and hydrophobic products: a catalytic approach. Topics in catalysis 2004;27 (1-4):67-76.

[125] Pestovsky O, Bakac A. Reactivity of aqueous Fe (IV) in hydride and hydrogen atom transfer reactions. J Am Chem Soc 2004;126(42): 13757-13764.

[126] Pestovsky O, Stoian S, Bominaar EL, Shan X, Münck E, Que Jr L, et al. Aqueous FeIV O: Spectroscopic Identification and Oxo-Group Exchange. Angewandte Chemie 2005;117(42):7031-7034.

[127] Pestovsky O, Bakac A. Aqueous ferryl (IV) ion: Kinetics of oxygen atom transfer to substrates and oxo exchange with solvent water. Inorg Chem 2006;45 (2):814-820.

[128] Bataineh H, Pestovsky O, Bakac A. pH-induced mechanistic changeover from hydroxyl radicals to iron (IV) in the Fenton reaction. Chemical Science 2012;3(5):1594-1599.

[129] Bataineh H, Pestovsky O, Bakac A. Iron (II) catalysis in oxidation of hydrocarbons with ozone in acetonitrile. ACS Catalysis 2015;5(3):1629-1637.

[130] Bataineh H. Thesis: Solvento iron (IV) oxo complexes in catalytic oxidations and electron transfer reactions. Solvento iron (IV) oxo complexes in catalytic oxidations and electron transfer reactions 2015.

[131] Enami S, Sakamoto Y, Colussi AJ. Fenton chemistry at aqueous interfaces. Proc Natl Acad Sci U S A 2014 Jan 14;111 (2):623-628.

[132] Turan-Ertas T, Gurol MD. Oxidation of diethylene glycol with ozone and modified Fenton processes. Chemosphere 2002;47(3):293-301.

Chapter 2

Photophysical Detection of Singlet Oxygen

Arnab Maity

"Old Man's Advice to Youth: 'Never Lose a Holy Curiosity.'" LIFE Magazine (May 2nd, 1955) p. 64.

Albert Einstein

Abstract

The chemical reactivity of singlet oxygen ($^1O_2$) (SO) derives from its electronically excited state. Being a unique reactive oxygen species SO takes part in many important atmospheric, biological physical, chemical, and therapeutic process and attracted current research interest. To understand the mechanistic pathways in various process the detection and quantification of SO is very important. The direct method of detection is very challenging due to its highly reactive nature. Only direct method of determination of phosphorescence of SO at 1270 nm has been utilised but that also puts some limitation due to very low luminescence quantum yield. Indirect method using UV–Vis spectrophotometric, fluorescent and chemiluminescent probes has been extensively studied for this purpose. Elucidation of various mechanistic processes improvised the use of sophisticated spectroscopic detection probe for SO have been discussed in a simple and lucid manner in this article through citation of literature examples. Four major spectroscopic methods i.e. spectrophotometry, fluorescence, emission and chemiluminescence are elaborately discussed with special emphasis to chemical probes having high selectivity and sensitivity for SO.

Keywords: Recative oxygen species (ROS), Singlet Oxygen (SO), 9,10-Anthracenedipropionic acid (ADPA), 9,10-dimethyl anthracene (DMA), DPA, DPBF, DMAX, ATTA-Eu^{3+}, PATA-Tb^{3+}, MTTA-Eu^{3+}, CLA, MCLA, FCLA

1. Introduction

Reactive Oxygen Species (ROS) is broadly used for those which contain oxygen radical such as superoxide anions ($O_2^{\cdot -}$), hydroxyl radicals ($HO^{\cdot}$), hydrogen peroxide (H_2O_2), nitric oxide (NO), peroxynitrite ($ONOO^-$), hypochlorous acid (HOCl) and singlet oxygen ($^1O_2$) (SO). These are usually metabolites of molecular oxygen formed due to normal or abnormal redox function in human body [1]. Numerous studies suggested that ROS can acts as toxins which can reduce the life span by causing ageing [2], synovial fluid degradation [3], neurodegenerative disease [4] etc. Singlet oxygen is known to be a highly reactive oxygen species that can oxidise a broad range of

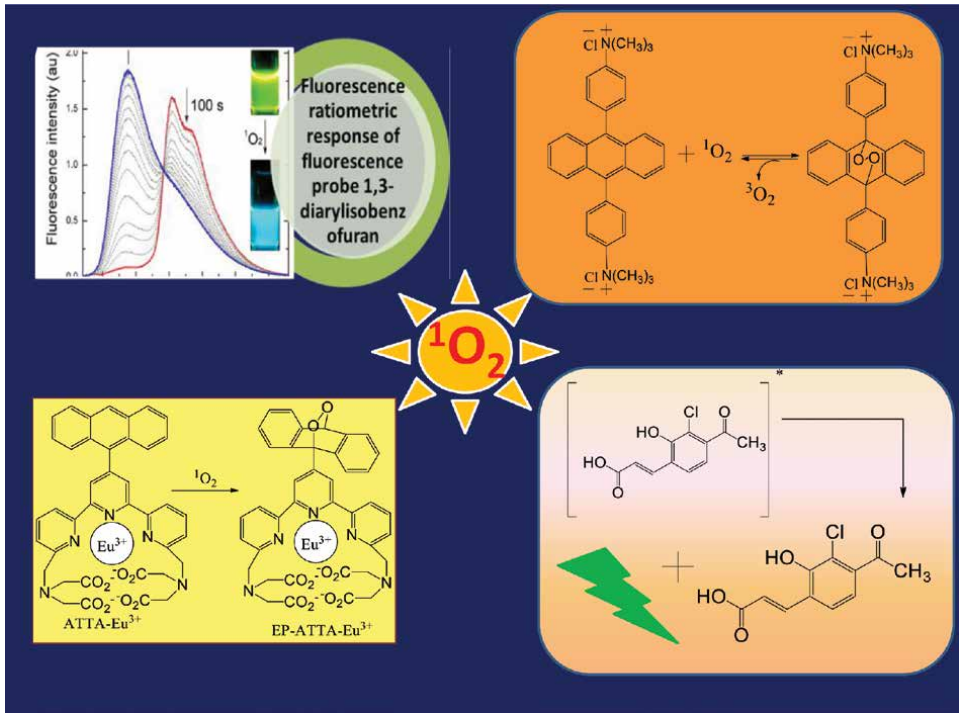


Figure 1.
The various photophysical singlet oxygen detection techniques based on absorbance or luminescence probes have been described pictorially. From top left clockwise (a) fluorescence detection probe (1,3-diarylisobenzofuran, adapted from [13]), (b) UV–Vis absorption based spectrophotometric probe (bis-9,10-anthracene-(4-trimethylphenylammonium) dichloride, adapted from [14]), (c) chemiluminescence probe (SOCL-phenol-dioxetane species, adapted from [15]) and (d) rare earth photoluminescence probe, ([4′-(9-anthryl)-2,2′:6′,2″-terpyridine-6,6″-diyl]bis (methylenenitrilo) tetrakis (acetate)-Eu^{3+} (ATTA-Eu^{3+}) (adapted from [16]).

biomolecules including lipids [5], nucleic acids [6, 7] and proteins [8]. The oxidation chemistry of SO provide key mechanism in the regulation of intracellular signalling pathways [9, 10] and eventually connected to human pathophysiology. As SO is a very short lived species ($\tau_{1/2}$ = 10^{-6} to 10^{-5} s) in the aqueous milieu) [11] studies towards the molecular mechanism of $^1O_2$ in vivo has been significantly retarded. Direct monitoring of SO phosphorescence also lacks practical utility due to very low photoluminescence quantum yields (PLQY≈10^{-6}) [12]. Therefore an enormous demand for SO probe with high sensitivity and large dynamic range is required. In this article various photophysical techniques and related probes usually employed in detecting SO has been delineated in separate paragraph (**Figure 1**).

2. UV–Vis spectrophotometric detection probes

2.1 9,10-Anthracenedipropionic acid (ADPA)

Ground state of molecular oxygen exists as triplet state with a lowest lying excited state being a singlet state. The singlet state of oxygen can be generated in solution via energy transfer from excited photosensitizers (S, e.g., humic substance or Rose Bengal; Eq. (1)) [17].

$$S \rightarrow S^{*} \xrightarrow{O_2} S + {}^{1}O_2 \quad (1)$$

The idea to detect environmentally relevant concentration prompted development of molecular probe that can trap SO and can also be used in studying kinetics. Lindig et.al was the first to introduced 9,10-anthracenedipropionic acid (ADPA) as a quantitative efficient $^1O_2$ detection probe [18]. The benefit of using ADPA is that it is water soluble and reacts rapidly and irreversible with $^1O_2$ to form an endoperoxide with the result of photobleaching of absorbance peak approximately at 378 nm (**Figure 2**) [20]. Craig et.al was the pioneer to demonstrate the use of ADPA for the quantitative measure of $^1O_2$ from the surface of a series of porphyrin-incorporated hydrogels in comparison to the pervious study where ADPA used to ingress into the materials under investigation [19].

2.2 9,10-dimethyl anthracene (DMA)

Another derivative of anthracene i.e. 9,10-dimethyl anthracene (DMA) reacts almost irreversibly with $^1O_2$ in various organic and aqueous medium with a significantly high rate constant (6.8×10^7–5.7×10^{10} $M^{-1}S^{-1}$) with the result of producing non fluorescent 9,10-endoperoxide [21–24]. Elim Albiter et.al reported a facile photosensitized oxidation of 9,10 demethylanthracene with $^1O_2$ in presence of safranin O/silica composite as a heterogeneous photosensitizer [21] in which they reported that oxidation rate does not depend on surface of the composite rather depend only the initial concentration of DMA, light intensity and the amount of composite formed. Their result correlates with the result if the same reaction performed in homogeneous medium. In a similar type experiment Eitan Gross et al. explored DMA inside liposome to study the kinetics of DMA with $^1O_2$ in presence of photosensitizer [22].

2.3 9,10-diphenyl anthracene (DPA)

Addition of two phenyl group in 9 and 10 position of anthacene generates a stable and specific $^1O_2$ trap, 9,10-diphenyl anthracene (DPA) with higher stability of endoperoxide by reaction with $^1O_2$. However, DPA is not a very suitable candidate as the detection method is based on decrease in absorbance at 355 nm band [25]. V. Nardello et al. enhanced the water solubility of 9,10-diphenyl anthracene (DPA) derivative

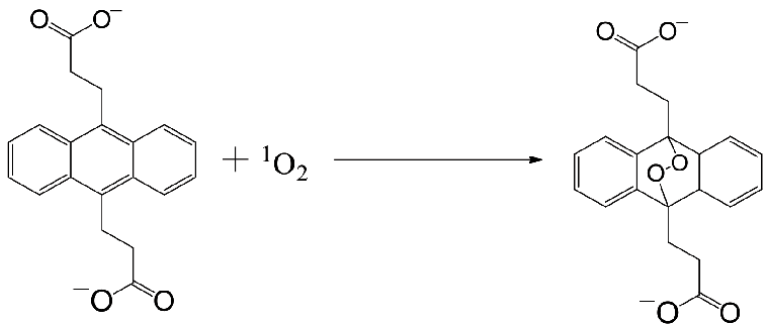


Figure 2.
The formation of endoperoxide upon reaction of ADPA with $^1O_2$ (adapted from [19]).

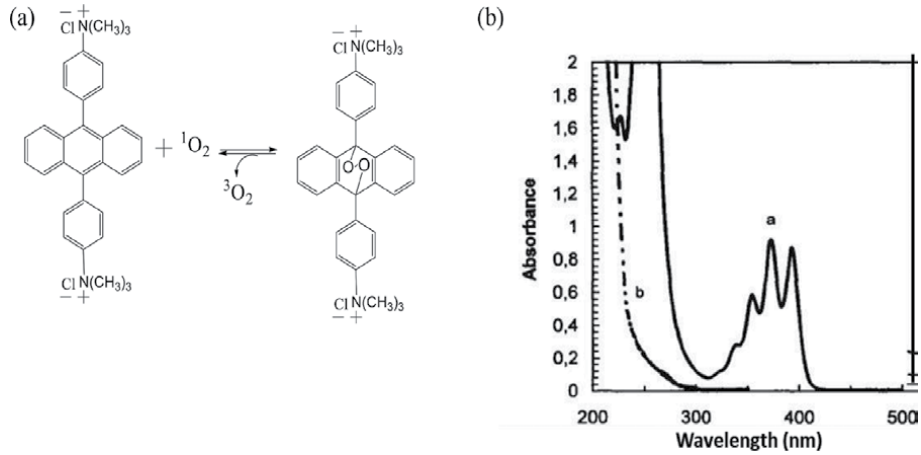


Figure 3.
(a) Interaction of bis-9,10-anthracene-(4-trimethylphenylammonium) dichloride (BPAA) with singlet oxygen resulting into the formation of water soluble endoperoxide (BPAAO$_2$) and (b) UV–Vis absorption spectra of a: BPAA 10^{-4} M in water and b: the corresponding endoperoxide of BPAA, 10^{-4} M in water [adapted from [14]].

by adding two quaternary ammonium functionality with the phenyl ring that do not interfere with $^1O_2$ and also resulting compound [bis-9,10-anthracene-(4-trimethyl-phenylamonium) dichloride] BPAA is very much stable with common oxidising agent (**Figure 3**) [14].

UV–Vis absorption band of BPAA is ranging from 320 to 420 nm which is originated from anthracene core structure and once it binds with $^1O_2$ (**Figure 3**) the absorbance band is quenched totally confirming the formation of endoperoxide.

3. Fluorescent probe for the detection of singlet oxygen species

Among the various available techniques to detect ROS the fluorescence based methodology is an excellent one because of its high sensitivity, high spatial resolution in imaging techniques and also simplicity during data collection [26, 27]. Fluorescent probes are generally non fluorescent before being oxidised by some oxygen species and they are very much specific to some oxidant. Combination of $^1O_2$ trap and a fluorophores like fluorescein [27, 28] reactive dienes [29] including 9,10-disubstituted anthracene [30–33] etc. are the usual method to develop a fluorescent $^1O_2$ probe. In fact $^1O_2$ has huge affinity for biomolecules having cisoid-diene structure and easily undergo [2 + 4] cycloaddition reaction [1, 6, 27]. Thus for most of the probes the fluorescent signals were obtained after [2 + 4] cycloaddition reaction between the probe and $^1O_2$ (**Figure 4**).

3.1 1,3 Diphenylisobenzofuran (DPBF)

D. Song et.al [13] synthesized a series of compounds of 1,3 Diphenylisobenzofuran (DPBF) which can acts as ratiometric fluorescence detection probe having singlet oxygen binding rate constant of 9.6×10^8 $M^{-1}S^{-1}$ in water [13]. Once DPBF reacts with $^1O_2$ forms nonfluorescent endoperoxide or 1,2-dibenzoylbenzene thus fluorescence signal becomes off. To overcome this practical difficulties they synthesised three more derivatives of DPBF namely phenanthrene substituted phenylisobenzofuran

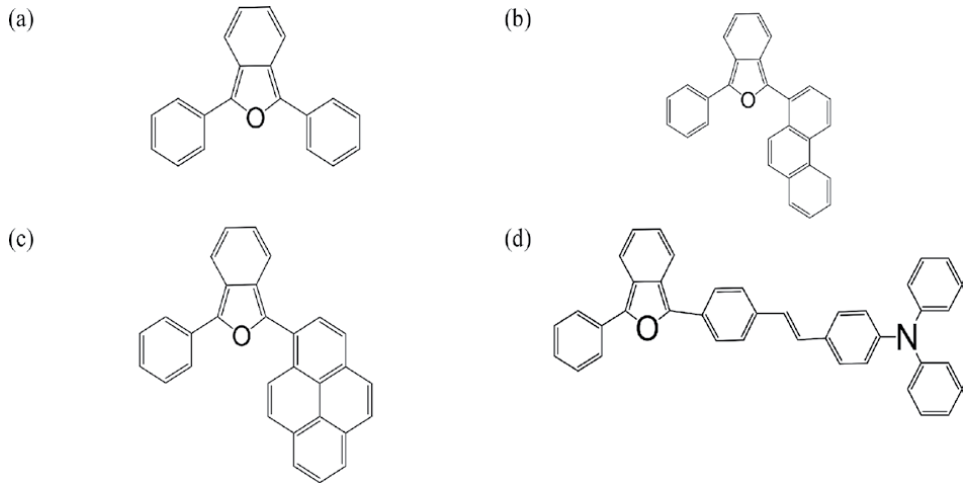


Figure 4.
Chemical structure of fluorescence detection probes for $^1O_2$, (a) DPBF, (b) PPBF, (c) PyPBF and (d) StPBF (adapted from [13]).

(PPBF), pyrene substituted phenylisobenzofuran (PyPBF) and 4-(diphenylamino) stilbene substituted derivative (StPBF) by substituting one phenyl group of DPBF (**Figure 5**) [13].

These $^1O_2$ probes exhibit significant red shift in their emission spectrum as the conjugation increases from DBPF to StPBF.

Upon interaction with $^1O_2$ species the fluorescence signal of DPBF is getting turn off while PPBF, PyPBF and StPBF demonstrate a blue shift of the emission signal with significant ratiometric enhancement of fluorescence as shown in **Table 1**.

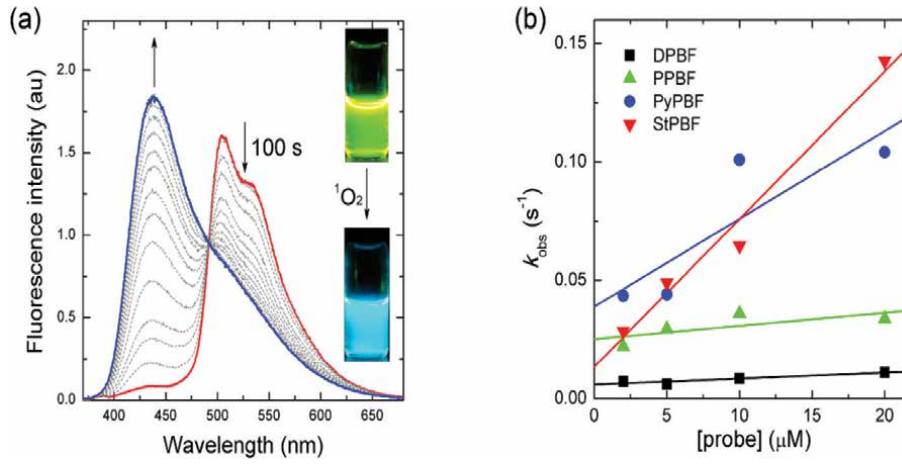


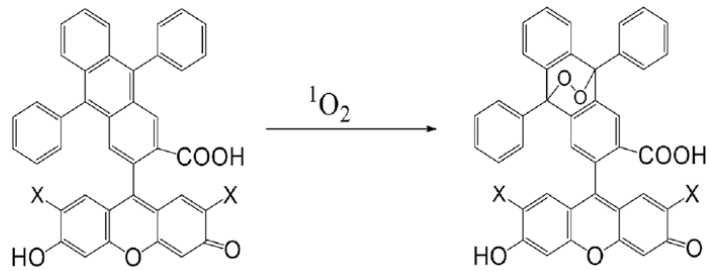
Figure 5.
(a) Fluorescence spectra of StPBF in absence (red in colour) and with gradually increasing concentration of $^1O_2$. The spectra gets blue shifted from 505 nm to 435 nm and fluorescence intensity increases with concentration of $^1O_2$ as directed by arrow in spectra and inset shows the observable colour change from green (in absence) and in presence of $^1O_2$ (blue in colour). (b) Graph of observed rate constant (K_{obs}) vs. concentration of various $^1O_2$ probe (adapted from [13]).

System	λ_{ex} (nm)	λ_{em} nm (probe alone)	λ_{em} (probe + $^1O_2$) nm	Fl ratio
DPBF + $^1O_2$	415	455	Turn off	Not determined
PPBF + $^1O_2$	331	476	370	FI_{476}/FI_{370} = 80
PPBF + $^1O_2$	358	512	398	FI_{512}/FI_{398} = 352
StPBF + $^1O_2$	350	505	435	FI_{505}/FI_{435} = 14

Table 1.
Photophysical data of $^1O_2$ probes [13]

3.2 9-[2-(3-Carboxy-9,10-diphenyl)anthryl]-6-hydroxy-3H-xanthen-3-one (DPAX)

In terms of sensitivity issue a fluorescence probe is always better than probes which work on the basis of absorbance. Umezawa et.al developed a new sensitive and efficient fluorescence probe DPAX namely 9-[2-(3-carboxy-9,10-diphenyl) anthryl]-6-hydroxy-3H-xanthen-3-one, for the detection of $^1O_2$ by fusing a fluorescein moiety, with DPA which serve the characteristics of fluorescence due to fluorescein as well as a good $^1O_2$ trap for the presence of DPA [34]. DPAX and its derivatives show very low fluorescence intensity in aqueous solution but once binding with $^1O_2$ the corresponding endoperoxide (DPAX-ED) shows excellent fluorescence intensity with quantum yield in the range of 0.5–0.7 (**Figure 6**) [35]. The DPAX and its derivatives demonstrate excellent selectivity towards $^1O_2$ as the fluorescence intensity remains unchanged upon reaction with hydrogen peroxide, superoxide and nitric oxide [35]. DPAXs are suitable for application in neutral and basic aqueous solution but the fluorescence intensity is known to be decreased under acidic condition due to the protonation of phenoxide oxygen atom; thus are not suitable for application in acidic conditions [32]. The stability of the fluorescence intensity can be enhanced by incorporating electron withdrawing group like Cl, F at 2 and 7 position of the xanthenes moiety leading to generation of two



X=H, DPAX-1 λ_{ex} =493 nm, λ_{em} =516 nm — DPAX-1-EP λ_{ex} =494 nm, λ_{em} =515 nm

X=Cl, DPAX-2 λ_{ex} =507 nm, λ_{em} =524 nm — DPAX-2-EP λ_{ex} =506 nm, λ_{em} =527 nm

X=F, DPAX-3 λ_{ex} =493 nm, λ_{em} =514 nm — DPAX-3-EP λ_{ex} =494 nm, λ_{em} =515 nm

Figure 6.
Reaction of 9-[2-(3-carboxy-9,10-diphenyl)anthryl]-6-hydroxy-3H-xanthen-3-ones (DPAXs) with $^1O_2$ to produce corresponding DPAX endoperoxides (DPAX-EPs) (adapted from [34]).

derivatives of DPAX namely DPAX-2(Cl derivative) and DPAX-3(F-derivative). This structural change lowered the Pka value of the phenolic oxygen atom [34].

Absorption maxima (A), molar extinction coefficient (ε) and emission maxima (λ_{em}) are more or less same for DPAXs and DPAX-EPs but the quantum efficiencies of fluorescence are altered when DPAXs bind with $^1O_2$. The emission maximum of DPAX-2-EP is shifted to longer wavelength than the corresponding DPAX-2 compound in comparison to other DPAXs compounds (**Table 2**) [34].

DPAX-2 can be utilised as an efficient $^1O_2$ sensor in both basic and neutral medium. Umezawa et al. used two different $^1O_2$ generation system namely, MnO_4^-/H_2O_2 and 3-(4-methyl-1-naphthyl)propionic acid endoperoxide (EP-1) which works in different pH values (10.5 and 7.4 respectively). In both the cases an increase in fluorescence intensity is established when this probe reacts with $^1O_2$ generation system (**Figure 7**).

To confirm the specificity of DPAX-2 towards $^1O_2$, fluorescence experiment was performed in presence of hydrogen peroxide, superoxide and nitric oxide but no appreciable change was observed for those species. These observations suggest the specificity of this probe for $^1O_2$ [34].

Compounds	Absorption maxima(nm)	ε (×10^4 M^{-1} cm^{-1})	λ_{max} (emission)	Quantum yield (Φf)
DPAX-1	493	6.1	516	0.007
DPAX-1-EP	494	7.9	515	0.53
DPAX-2	507	5.7	524	0.006
DPAX-2-EP	506	8.9	527	0.66
DPAX-3	493	7.6	514	0.006
DPAX-3-EP	494	6.7	515	0.70

Table 2.
Absorbance and fluorescence properties of DPAXs and DPAX-EPs (adopted from [36]).

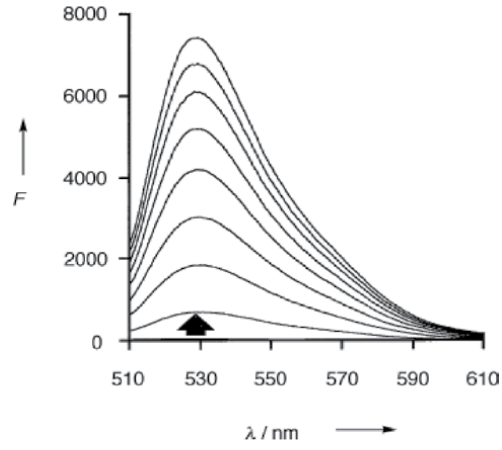


Figure 7.
Emission spectra of DPAX-2 at 505 nm in reaction with $^1O_2$, generated from MnO_4^-/H_2O_2 system. The reaction was performed in 0.1 M sodium phosphate buffer medium of pH 10.5 containing 0.1 mM EDTA at 25°C (adapted from [34]).

3.3 9-[2-(3-Carboxy-9,10-dimethyl)anthryl]-6-hydroxy-3H-xanthen-3-one (DMAX)

Following the same approach of Umezawa group, Tanaka et al. synthesised another fluorescence probe molecule for the faster and efficient detection of $^1O_2$, 9-[2-(3-carboxy-9,10-dimethyl)anthryl]-6-hydroxy-3H-xanthen-3-one (DMAX) (**Figure 8**), targeting to achieve great sensitivity and rapid rate of formation of endoperoxide compare to already reported one i.e. DPAX (**Figure 8**) [27]. The crucial point of DMA compound is that it reacts rapidly with $^1O_2$ to give the 9,10 endoperoxide, DMA-EP with rate constant k = 9.1 × 10^8 M^{-1} s^{-1} in water. This observation clearly indicates that DMAX shows much greater sensitivity for $^1O_2$ than DPAX. Comparing to this reaction, the classical singlet oxygen trap 1,3-diphenylisobenzophuran (DPBF) reacts with $^1O_2$ with a comparable rate constant k = 9.6 × 10^8 M^{-1} s^{-1} but DPBF reacts with other reactive oxygen species like hypochlorite, hydroxyl radical to generate the same products.

Both DMAX and its endoperoxide DMAX-EP have similar excitation and emission wavelength (λ_{ex} = 492 nm and λ_{em} = 515 nm) but DMAX-EP is highly fluorescent whereas DMAX itself is practically non-fluorescent (**Figure 8**). From their study Tanaka et al. confirmed that fluorescence intensity of DMAX increases with

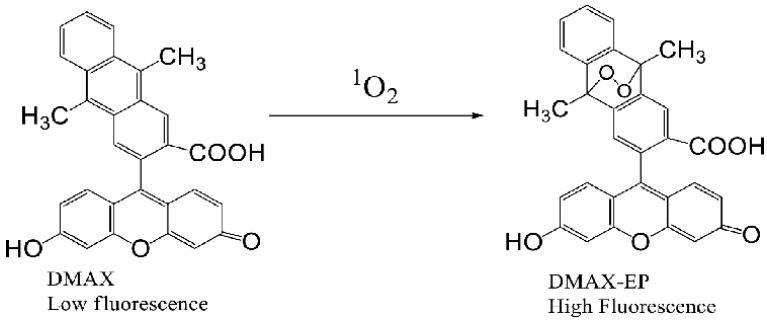


Figure 8.
Reaction of 9-[2-(3-carboxy-9,10-dimethyl)anthryl]-6-hydroxy-3H-xanthen-3-one (DMAX) with $^1O_2$ to produce DMAX-EP (adapted from [27]). λ_{ex} = 492 nm and λ_{em} = 515 nm.

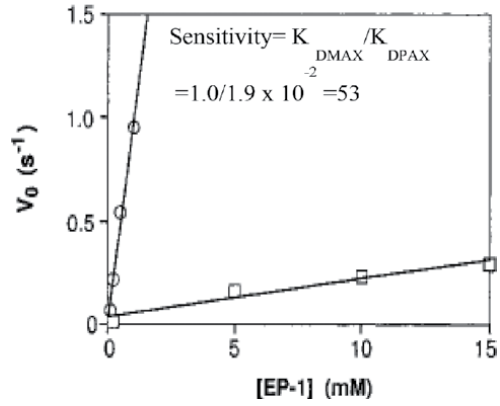


Figure 9.
Initial rate of fluorescence increase of DMAX (in hollow sphere) and DPAX-1(in hollow square) with increasing concentration of EP-1 [adapted from [27]].

concentration dependent manner of singlet oxygen generator, 3-(1,4-Dihydro-1,4-epidioxy-4-methyl-1-naphthyl)propionic acid (EP-1) and a good linear relationship has been observed for fluorescence intensity and concentration of EP-1. This enables DMAX to use as a quantitative detection probe for $^1O_2$. **Figure 9** demonstrate the change of gradient of fluorescence intensity of DMAX and DPAX-1 with increasing concentration of EP-1 having gradient 1.0 and 1.9×10^{-2} (arbitrary unit)s^{-1}[EP-1 (mM)$]^{-1}$ for DMAX and DPAX respectively. This result suggests that DMAX reacts with $^1O_2$ more rapidly and sensitivity is 53 times more than that of DPAX [27].

Tanaka et al. further confirmed that DMAX did not show any change in fluorescence intensity upon reaction with 1.0 mM H_2O_2, 0.1 mM nitric oxide and 0.2 mM superoxide suggesting the specificity towards $^1O_2$. Further the hydrophobicity of DMAX is less than that of DPAXs making it suitable to use for assays in biological sample [27].

4. Rare earth chelate based luminescence probes

Rare-earth based chelate luminescence probe can also serve significant role in the detection of singlet oxygen. In comparison to organic fluorescence probe rare earth based chelate probes exhibits markedly different advantages including long luminescence lifetime, sharp emission profile, large Stokes shift, which makes them suitable to use in time-resolved luminescence detection of singlet oxygen [36]. Large Stokes shift helps to reduce the influence caused by excitation light effectively. Time resolved luminescence measurement using rare-earth-chelated probe can efficiently reduce the interference originated from the background noise associated with biological sample, scattering lights (Tyndall, Rayleigh and Raman scattering) and the optical components [16]. Rare earth chelate probes which are successfully applied for the detection of singlet oxygen including Eu, Tb and Re based chelate complexes are well-known [16, 31, 37].

4.1 [4′-(9-Anthryl)-2,2′:6′,2″-terpyridine-6,6″-diyl]bis (methylenenitrilo) tetrakis (acetate)-Eu^{3+} (ATTA-Eu^{3+})

Bo Song et al. first synthesised the Eu^{3+} cheleate based phosphorescence probe, [4′ -(9-anthryl)-2,2′:6′,2″-terpyridine-6,6″-diyl] bis (methylenenitrilo) tetrakis (acetate)-Eu^{3+} (ATTA-Eu^{3+}) (shown in **Figure 9**) for the time resolved luminescence study. The probe is highly sensitive, selective for $^1O_2$ and water soluble for time resolved luminescence detection of $^1O_2$ with a detection limit of 2.8 nM/L [16]. The almost non luminescent ATTA-Eu^{3+} specifically reacts with $^1O_2$ to yield its endoperoxide (**Figure 10**) (EP- ATTA-Eu^{3+}) resulting a great enhancement of luminescence intensity (luminescence quantum yield of EP- ATTA-Eu^{3+} is 17 fold greater than that of ATTA-Eu^{3+}) as the population of the excited state of Eu^{3+} was increased enormously after formation of endoperoxide [16, 27]. The endoperoxide compound EP-ATTA-Eu^{3+} exhibit favourable chemical stability with a conditional stability constant was measured to be 10^{20} level. Apart from that no decrease in phosphorescence intensity was observed even after storage of EP-ATTA-Eu^{3+} for several days at room temperature.

Another advantage of EP-ATTA-Eu^{3+} is that the phosphorescence intensity of EP-ATTA-Eu^{3+} is stable even at very low pH of 3 whereas in case of fluorescein based probe (DPAXs and DMAXs) the rapid decrease of fluorescence intensity is reported below pH 7 [16, 27].

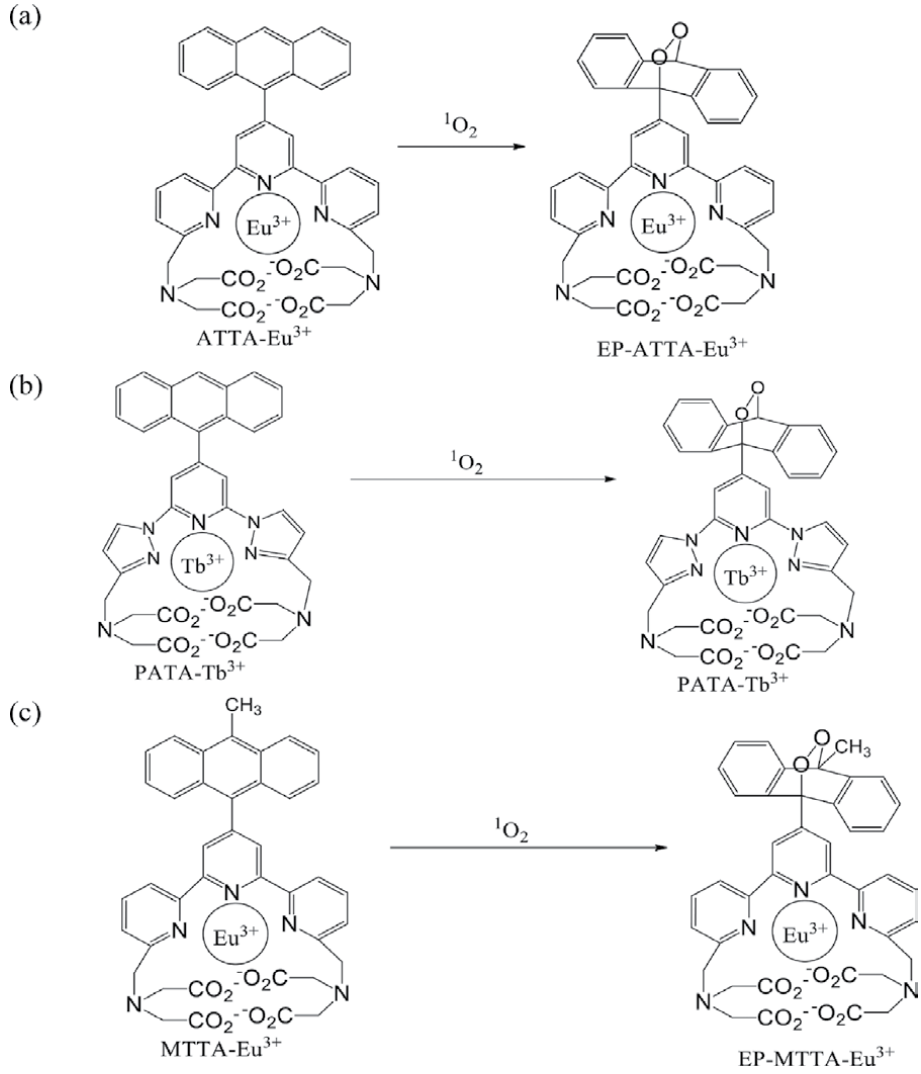


Figure 10.
The chemical structure and the reaction scheme of (a) ATTA-Eu^{3+}, (b) PATA-Tb^{3+} and (c) MTTA-Eu^{3+} with singlet oxygen (adapted from [16]).

4.2 N,N,N′,N′-[2,6-Bis-(3′-aminomethyl-1′-pyrazolyl)-4-(9″-anthryl)pyridine] tetrakis(acetate)-Tb^{3+} (PATA-Tb^{3+})

Mingqian Tan et al. developed another chelate complex of Tb^{3+}, N,N,N′,N′-[2,6-bis-(3′-aminomethyl-1′-pyrazolyl)-4-(9″-anthryl)pyridine] tetrakis(acetate) Tb^{3+} (PATA-Tb^{3+}), (shown in **Figure 10b**) is known to be effective fluorescent probe for the detection of $^1O_2$ [38]. Because of the presence of 9-anthryl moiety within the ligand the compound, PATA-Tb^{3+} is almost non fluorescent but once it reacts with $^1O_2$ the corresponding endoperoxide, EP-PATA-Tb^{3+} becomes strongly fluorescent with almost 23 fold enhancement of fluorescent quantum yield [39]. PATA- Tb^{3+} is known to be an excellent fluorescent probe for $^1O_2$ due to the characteristics, including high water solubility, wide applicable pH range and long fluorescence lifetime of

endoperoxide (2.76 ms) which makes it suitable for time resolved fluorescent detection with a detection limit as low as 10.8 nM/L [27, 38]. The specificity of PATA- Tb^{3+} towards $^1O_2$ is also confirmed upon reaction with some other reactive oxygen species, including hydroxyl radical, superoxide ion, peroxynitrite and hydrogen peroxide, the no significant change of fluorescence intensity support its specificity for $^1O_2$.

4.3 [4′-(10-Methyl-9-anthryl)-2,2′:6′,2′′-terpyridine-6,6′′-diyl] bis(methylenenitrilo)tetrakis(acetate)-Eu^{3+}, abbreviated as MTTA-Eu^{3+}

To improve the selectivity, sensitivity and rapid reaction with singlet oxygen species, Bo Song et al. introduced a new chelate complex of Eu^{3+}, [4′-(10-methyl-9-anthryl)-2,2′:6′,2′′-terpyridine-6,6′′-diyl]bis(methylenenitrilo) tetrakis(acetate)-Eu^{3+}, abbreviated as MTTA- Eu^{3+} as a $^1O_2$ probe [39]. MTTA- Eu^{3+} complex is highly water soluble and the reaction rate with $^1O_2$ is much faster in comparison to previously mentioned ATTA-Eu^{3+} (**Figure 10a** and **c**) with reaction rate constant of 10^{10} $M^{-1}S^{-1}$. Apart from that this complex can also be applied for a wide pH range from 3 to 10 accompanied by the large enhancement of luminescence quantum yield from 0.90% to 13.8%. The complex is almost non luminescent and when specifically reacts with $^1O_2$ to form the endoperoxide it becomes highly fluorescent with luminescent lifetime changes from 0.8 to 1.29 ms which makes the complex suitable for time gated luminescent measurement. The quantitative detection limit of $^1O_2$ using MTTA- Eu^{3+} complex is found to be 3.8 nM/L which is very much comparable to the detection limit of ATTA- Eu^{3+} complex [31, 39].

5. Transition metal based singlet oxygen probe

Though lanthanide based fluorescent probes possess many advantages including low background interference, widely applicable pH range, and excellent water solubility; these probes can be expected to utilise for visualising spatial and temporal distribution of $^1O_2$ in aqueous system but a major drawback of these probes is that they need ultraviolet light for excitation which might cause cell damage, thus limiting their application in biological system. Liu et al. demonstrated a Re^I complex, $Re(CO)_3Cl(aeip)$ {aeip = 2-(anthracen-9-yl)-1-ethyl-imidazo[4,5-f] [1, 10], phenanthroline, which can be excited via visible light of 410 nm wavelength in aqueous medium thus minimising the effect of cell damage (shown in **Figure 11**) [31]. The Re^I complex is non-luminescent in the native state probably due to quenching of luminescence of Re → aeip (M → L) charge transfer transition in the excited state through exchange triplet-triplet intramolecular energy transfer by anthryl moiety

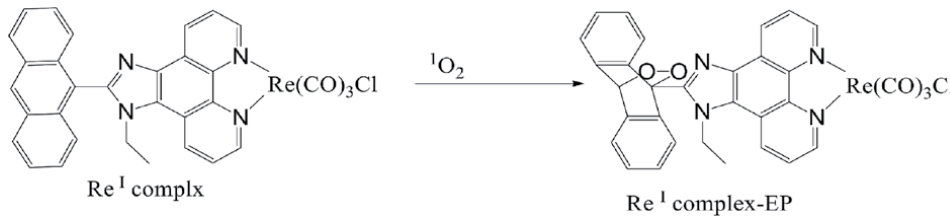


Figure 11.
The Re^I complex and the formation of endoperoxide via reaction with $^1O_2$ (adapted from [37]).

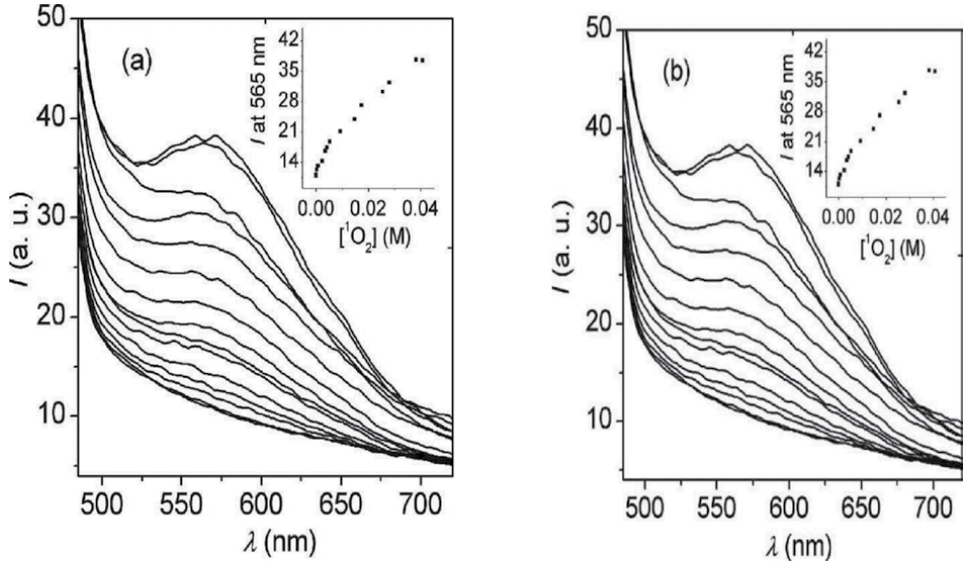


Figure 12.
Changes in the luminescence intensity of Re^I complex (λ_{ex} = 410 nm) with increasing concentration of $^1O_2$ in (a) in neutral and (b) in alkaline medium. The inset of these figures show that the sharp change in luminescence intensity at 565 nm with gradually increasing concentration of $^1O_2$ (adapted from [40]).

as mentioned in similar type Re^I complexes [37]. The strong enhancement of luminescence of Re^I complex due to the formation of endoperoxide in both neutral and alkaline medium is perceived, that due to the termination of electronic coupling between the anthryl and the parent Re^I complex (**Figure 11**). In comparison to other fluorescent probes of Eu^{3+} and Tb^{3+} complexes, Re^I complex exhibits higher molar absorption at the visible wavelength of 410 nm and fluorescence can also be initiated with this wavelength of light [31].

With increasing concentration of $^1O_2$ the luminescence intensity increases as shown in **Figure 12** and the luminescence quantum yield changes from 8.9×10^{-5} to 7.1×10^{-4} and 4.7×10^{-5} to 8.7×10^{-4} in neutral and alkaline media [37]. The limit of detection obtained using Re^I complex is found to be 4.9 nM/L and 10.5 nM/L in neutral and alkaline medium respectively, which are very much comparable with that obtained for Eu^{3+} and Tb^{3+} complex [31, 37, 39].

The additional benefit of visible light excitation and long lifetime enable Re^I complex to be used in biological systems.

6. Chemiluminescence singlet oxygen probe

In the previous discussion, the detection of $^1O_2$ was based upon either decrease in the absorbance of singlet oxygen probe or enhancement of luminescence signal of fluorescein based probe in presence of $^1O_2$. In this respect Chemiluminescence is supposed to be one of the most suitable methods for singlet oxygen detection as it does not require any excitation light source, so background fluorescence and interference caused by background light can be eliminated. At the same time the signal to noise ratio can be improved and the possible damage of living cell caused by UV irradiation during fluorescence measurement can be eliminated.

6.1 TTF substituted singlet oxygen probe: 2-methyl-6-phenyl-3,7-dihydroimidazo[1,2-α]pyrazine-3-one (CLA), MCLA and FCLA

A number of Chemiluminescence probes have been developed in recent years for $^1O_2$ detection. Among the mostly used Chemiluminescence probes for $^1O_2$ includes 2-methyl-6-phenyl-3,7-dihydroimidazo[1,2-α] pyrazine-3-one (CLA), and its derivatives MCLA and FCLA [41–43] (shown in **Figure 13**). These compounds are very good in detecting $^1O_2$ and spontaneously emit light but the major drawback of these compound is that they not only reacts with singlet oxygen but also with superoxide anion; thus lacks the selectivity for $^1O_2$.

Chemiluminescence probe with a strong electron donor tetra thiafulvalenem (TTF) and anthracene as a luminophore possess excellent selectivity and sensitivity for $^1O_2$ detection. As TTF moiety is a strong electron donor, it enhances the reaction between anthracene skeleton to react specifically with $^1O_2$ to form highly luminescent endoperoxide.

Guanxin Zhang et al. reported 4,4′(5′)-bis[2-(9-anthryloxy)ethylthio] tetrathiafulvalene (as shown in **Figure 14**) as selective and sensitive probe for $^1O_2$ with a much better response for $^1O_2$, representing better selectivity than CLA [40, 44]. 4,5-dimethylthio-4′-[2-(9-anthryloxy) ethylthio] tetrathiafulvalene was another Chemiluminescence probe with similar functionality [40, 45]. A linear relationships between the Chemiluminescence intensity and the amount of $^1O_2$ was found for H_2O_2/ClO^- system and the LOD of 76 nM/L was reported for $^1O_2$. In mixed solvent of

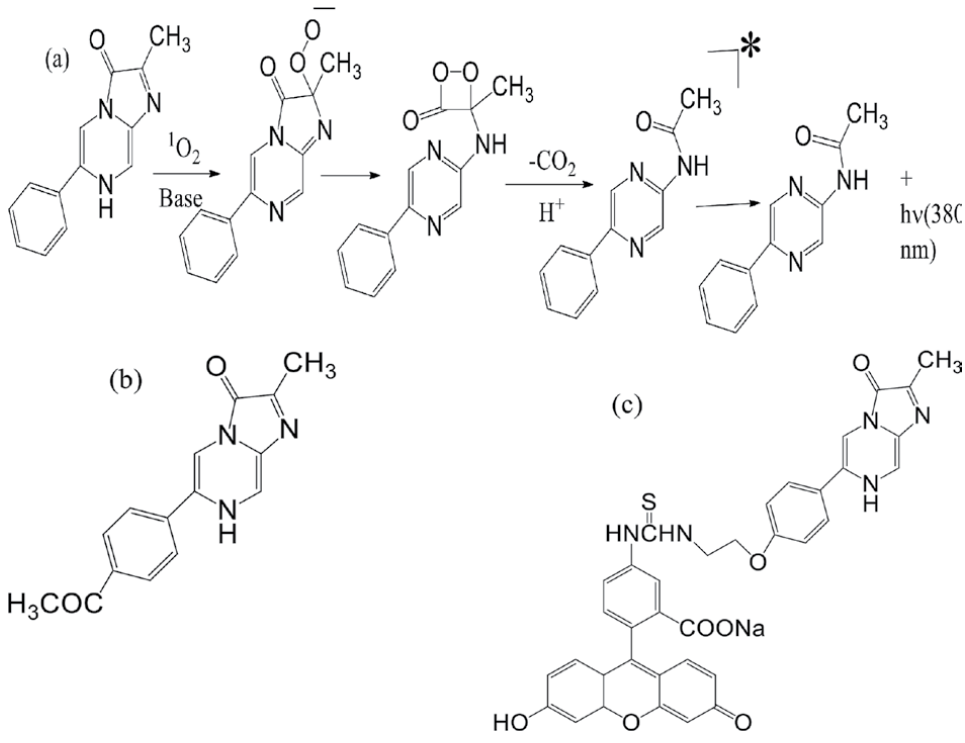


Figure 13.
(a) The chemical structure of CLA and its reaction with singlet oxygen; (b) the chemical structure of MCLA and (c) the chemical structure of FCLA (adapted from [41–43]).

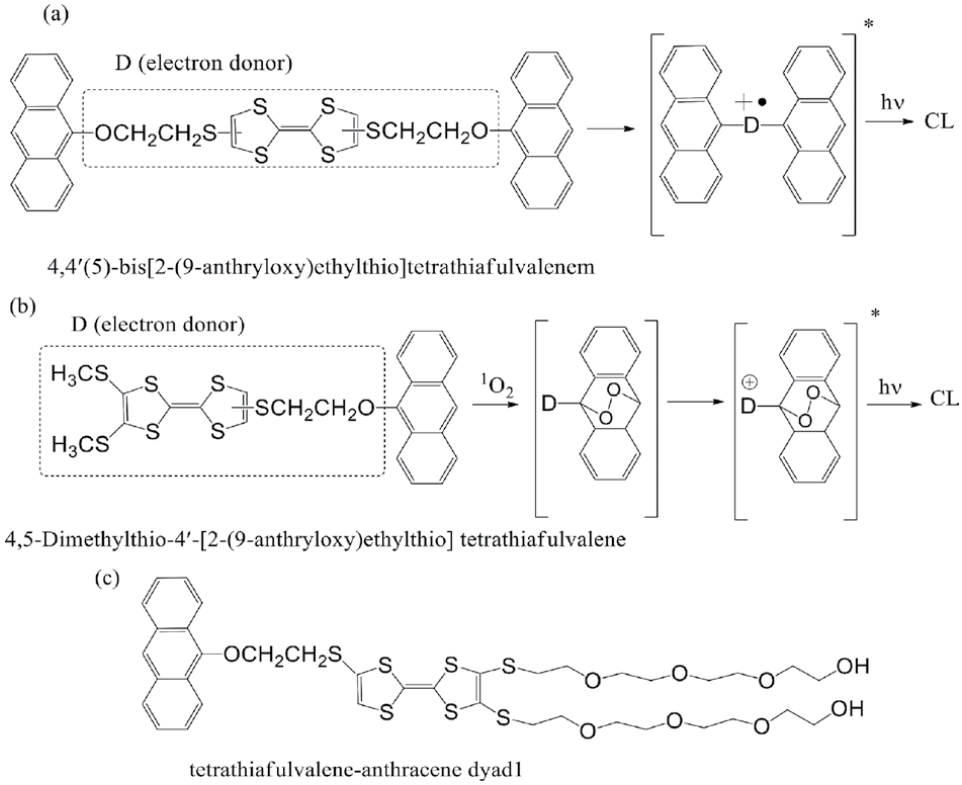


Figure 14.
The chemical structure and reaction scheme of (a) 4,4′(5′)-bis[2-(9-anthryloxy)ethylthio] tetrathiafulvalene, (b) 4,5-dimethylthio-4′-[2-(9-anthryloxy)ethylthio] tetrathiafulvalene with with $^1O_2$ and (c) the chemical structure of tetrathiafulvalene-anthracence dyad1 (adapted from [40, 44, 45]).

tetrahydrofuran and H_2O, both the probes cannot be applied. However, tetrathiafulvalene-anthracence dyad1 (as shown in **Figure 13c**) dissolve easily in methanol and ethanol and permits detection of $^1O_2$ under relatively low polarity solvent.

6.2 Stable dioxetene chemiluminescence probe

Dioxetene chemistry offers potential for selective and sensitive detection of $^1O_2$. McNeill and co-workers reported "trap and trigger" Chemiluminescence probe for $^1O_2$ [46] using Schaap's dioxetene [47] enol ether precursor to trap the $^1O_2$ in the first step and in the second step Chemiluminescence was triggered by adding fluoride ion. This probe was limited application in organic solvents due to the quenching mechanism of the emitting species in water [48]. Nir Hananya et al. developed a new Chemiluminescence probe by incorporating an electron withdrawing substituent at the ortho position of phenol group of Schaap's dioxetene, namely SOCL-CPP [15] (**Figure 15**). SOCL-CPP reacts with $^1O_2$ to generate a phenol-dioxetene species that spontaneously decompose in aqueous medium to generate corresponding electronically excited benzoate ester [15].

A green light emission is obtained from excited benzoate ester. The incorporation of acrylic acid substituent at the ortho position of phenol is to generate a donor-acceptor

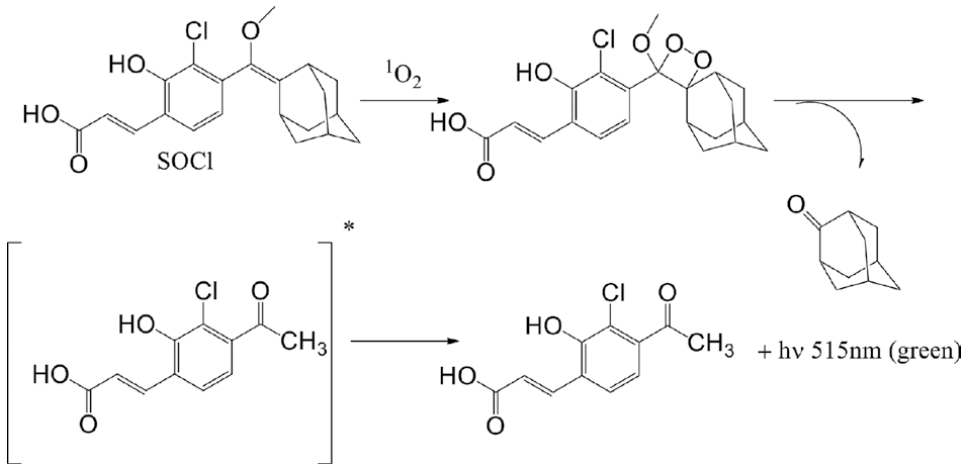


Figure 15.
Structure of SO chemiluminescent probe **SOCL** *and its chemiexcitation pathway upon reaction with* $^1O_2$ *(adapted from [15]).*

pair that enhances the emissive nature of benzoate intermediate [15]. Addition of a chlorine substituents at the ortho position reduces the pKa of the phenol and thus enriches the percentage of phenoxy ion that eventually accelerate the chemiexcitation kinetics of the phenol-dioxitene species to monitor in real time.

7. Conclusions

Singlet oxygen as a highly reactive form of molecular oxygen plays a vital role in many environmental and biomedical processes. Selective and sensitive detection and quantification of singlet oxygen species provides crucial information for understanding its involvement and mechanism in various processes. EPR method for the detection of $^1O_2$ has major disadvantage of requiring an expensive instrument and complicated operating procedures. Direct photoluminescence measurement from $^1O_2$ at about 1270 nm is useful for singlet oxygen detection but that also suffers drawback due to very low quantum efficiency. UV–Vis absorbance probes for selective detection of $^1O_2$ is significant but low sensitivity put some limitation for spectrophotometric method. Molecular fluorescence or lanthanide based fluorescence probe and Chemiluminescence probe provides high sensitivity and desirable selectivity, therefore ensure great potential for singlet oxygen detection. Additional benefits of fluorescence probe including the capability of detection of $^1O_2$ among various other reactive oxygen species. The temporal and spatial resolution of these probes can provide detailed information on site and the kinetics of singlet oxygen production or decay. In comparison to organic fluorescence probe, lanthanide complex based time gated luminescence probe possess many advantages such as long luminescence lifetime, large Stokes shift and sharp emission profile that makes them suitable for time gated detection mode for minimising background luminescence interference. Chemiluminescence probes does not require any excitation light sources, can be applied in certain cases where background fluorescence and various light scattering phenomena lower the signal to noise ratio. Furthermore, due to high sensitivity

of Chemiluminescence detection, only low concentration of probe is necessary eventually decreasing the occurrence of artifactual interference of secondary reactions. Although, much has been conferred in this concise chapter, perhaps these are not all, but the future will invoke more questions and thus newer and emerging methods, would help expand the level of our understanding.

Author details

Arnab Maity
Department of Chemistry, Akal University, Bathinda, Punjab, India

*Address all correspondence to: arnab_chm@auts.ac.in

IntechOpen

References

[1] You, Y.; Nam, W., Designing photoluminescent molecular probes for singlet oxygen, hydroxyl radical, and iron–oxygen species. *Chemical Science* **2014,** *5* (11), 4123-4135.

[2] Finkel, T.; Holbrook, N. J., Oxidants, oxidative stress and the biology of ageing. *Nature* **2000,** *408* (6809), 239-47.

[3] McCord, J. M., Free radicals and inflammation: protection of synovial fluid by superoxide dismutase. *Science* **1974,** *185* (4150), 529-31.

[4] Barnham, K. J.; Masters, C. L.; Bush, A. I., Neurodegenerative diseases and oxidative stress. *Nat Rev Drug Discov* **2004,** *3* (3), 205-14.

[5] Francis Wilkinson, W. P. H. a. A. B. R., Rate Constants for the Decay and Reactions of the Lowest Electronically Excited Singlet State of Molecular Oxygen in Solution. An Expanded and Revised Computation. *Journal of Physical and Chemical Reference Data* **1995,** *24* (2), 664-672.

[6] Kang, P.; Foote, C. S., Formation of Transient Intermediates in Low-Temperature Photosensitized Oxidation of an 8-13C-Guanosine Derivative. *Journal of the American Chemical Society* **2002,** *124* (17), 4865-4873.

[7] Cadet, J.; Bellon, S.; Berger, M.; Bourdat, A. G.; Douki, T.; Duarte, V.; Frelon, S.; Gasparutto, D.; Muller, E.; Ravanat, J. L.; Sauvaigo, S., Recent aspects of oxidative DNA damage: guanine lesions, measurement and substrate specificity of DNA repair glycosylases. *Biol Chem* **2002,** *383* (6), 933-43.

[8] Davies, M. J., Singlet oxygen-mediated damage to proteins and its consequences. *Biochem Biophys Res Commun* **2003,** *305* (3), 761-70.

[9] Klotz, L. O.; Kröncke, K. D.; Sies, H., Singlet oxygen-induced signaling effects in mammalian cells. *Photochem Photobiol Sci* **2003,** *2* (2), 88-94.

[10] Kröncke, K.-D.; Klotz, L.-O.; Suschek, C. V.; Sies, H., Comparing Nitrosative *Versus* Oxidative Stress toward Zinc Finger-dependent Transcription: UNIQUE ROLE FOR NO *. *Journal of Biological Chemistry* **2002,** *277* (15), 13294-13301.

[11] Lindig, B. A.; Rodgers, M. A. J., Laser photolysis studies of singlet molecular oxygen in aqueous micellar dispersions. *The Journal of Physical Chemistry* **1979,** *83* (13), 1683-1688.

[12] Schweitzer, C.; Schmidt, R., Physical Mechanisms of Generation and Deactivation of Singlet Oxygen. *Chemical Reviews* **2003,** *103* (5), 1685-1758.

[13] Song, D.; Cho, S.; Han, Y.; You, Y.; Nam, W., Ratiometric Fluorescent Probes for Detection of Intracellular Singlet Oxygen. *Organic Letters* **2013,** *15* (14), 3582-3585.

[14] Nardello, V.; Aubry, J.-M., Synthesis and properties of a new cationic water-soluble trap of singlet molecular oxygen. *Tetrahedron Letters* **1997,** *38* (42), 7361-7364.

[15] Hananya, N.; Green, O.; Blau, R.; Satchi-Fainaro, R.; Shabat, D., A Highly Efficient Chemiluminescence Probe for the Detection of Singlet Oxygen in Living Cells. *Angewandte Chemie International Edition* **2017,** *56* (39), 11793-11796.

[16] Song, B.; Wang, G.; Yuan, J., A new europium chelate-based

phosphorescence probe specific for singlet oxygen. *Chemical Communications* **2005,** (28), 3553-3555.

[17] Zafiriou, O. C., Marine organic photochemistry previewed. *Marine Chemistry* **1977,** *5* (4), 497-522.

[18] Lindig, B. A.; Rodgers, M. A. J.; Schaap, A. P., Determination of the lifetime of singlet oxygen in water-d2 using 9,10-anthracenedipropionic acid, a water-soluble probe. *Journal of the American Chemical Society* **1980,** *102* (17), 5590-5593.

[19] Craig, R. A.; McCoy, C. P.; De Baróid, Á. T.; Andrews, G. P.; Gorman, S. P.; Jones, D. S., Quantification of singlet oxygen generation from photodynamic hydrogels. *Reactive and Functional Polymers* **2015,** *87*, 1-6.

[20] Moreno, M. J.; Monson, E.; Reddy, R. G.; Rehemtulla, A.; Ross, B. D.; Philbert, M.; Schneider, R. J.; Kopelman, R., Production of singlet oxygen by Ru(dpp(SO3)2)3 incorporated in polyacrylamide PEBBLES. *Sensors and Actuators B: Chemical* **2003,** *90* (1), 82-89.

[21] Albiter, E.; Alfaro, S.; Valenzuela, M. A., Photosensitized oxidation of 9,10-dimethylanthracene with singlet oxygen by using a safranin O/silica composite under visible light. *Photochemical & Photobiological Sciences* **2015,** *14* (3), 597-602.

[22] Gross, E.; Ehrenberg, B.; Johnson, F. M., SINGLET OXYGEN GENERATION BY PORPHYRINS AND THE KINETICS OF 9,10-DIMETHYLANTHRACENE PHOTOSENSITIZATION IN LIPOSOMES. *Photochemistry and Photobiology* **1993,** *57* (5), 808-813.

[23] Günther S., G.; Lemp M., E.; Zanocco, A. L., ON THE USE OF 9,10-DIMETHYLANTHRACENE AS CHEMICAL RATE CONSTANT ACTINOMETER IN SINGLET MOLECULAR OXYGEN REACTIONS. *Boletín de la Sociedad Chilena de Química* **2000,** *45*, 637-644.

[24] Okamoto, M.; Wada, O.; Tanaka, F.; Hirayama, S., Fluorescence Quenching by Oxygen of 9,10-Dimethylanthracene in Liquid and Supercritical Carbon Dioxide. *The Journal of Physical Chemistry A* **2001,** *105* (3), 566-572.

[25] Gomes, A.; Fernandes, E.; Lima, J. L., Fluorescence probes used for detection of reactive oxygen species. *J Biochem Biophys Methods* **2005,** *65* (2-3), 45-80.

[26] Soh, N.; Katayama, Y.; Maeda, M., A fluorescent probe for monitoring nitric oxide production using a novel detection concept. *Analyst* **2001,** *126* (5), 564-566.

[27] Tanaka, K.; Miura, T.; Umezawa, N.; Urano, Y.; Kikuchi, K.; Higuchi, T.; Nagano, T., Rational design of fluorescein-based fluorescence probes. Mechanism-based design of a maximum fluorescence probe for singlet oxygen. *J Am Chem Soc* **2001,** *123* (11), 2530-6.

[28] Gollmer, A.; Arnbjerg, J.; Blaikie, F. H.; Pedersen, B. W.; Breitenbach, T.; Daasbjerg, K.; Glasius, M.; Ogilby, P. R., Singlet Oxygen Sensor Green®: photochemical behavior in solution and in a mammalian cell. *Photochem Photobiol* **2011,** *87* (3), 671-9.

[29] Evans, D. F.; Upton, M. W., Studies on singlet oxygen in aqueous solution. Part 1. Formation of singlet oxygen from hydrogen peroxide with two-electron oxidants. *Journal of the Chemical Society, Dalton Transactions* **1985,** (6), 1141-1145.

[30] Schmitz, C.; Aubry, J. M.; Rigaudy, J., A new access to the anthracene core:

Synthesis of two water soluble singlet oxygen traps derived from 1,3-diphenylisobenzofuran and 9,10-diphenylanthracene. *Tetrahedron* **1982,** *38* (10), 1425-1430.

[31] Liu, Y.-J.; Wang, K.-Z., Visible-Light-Excited Singlet-Oxygen Luminescence Probe Based on Re(CO)3Cl(aeip). *European Journal of Inorganic Chemistry* **2008,** *2008* (33), 5214-5219.

[32] Baier, J.; Fuß, T.; Pöllmann, C.; Wiesmann, C.; Pindl, K.; Engl, R.; Baumer, D.; Maier, M.; Landthaler, M.; Bäumler, W., Theoretical and experimental analysis of the luminescence signal of singlet oxygen for different photosensitizers. *Journal of Photochemistry and Photobiology B: Biology* **2007,** *87* (3), 163-173.

[33] Baier, J.; Maier, M.; Engl, R.; Landthaler, M.; Bäumler, W., Time-Resolved Investigations of Singlet Oxygen Luminescence in Water, in Phosphatidylcholine, and in Aqueous Suspensions of Phosphatidylcholine or HT29 Cells. *The Journal of Physical Chemistry B* **2005,** *109* (7), 3041-3046.

[34] Umezawa, N.; Tanaka, K.; Urano, Y.; Kikuchi, K.; Higuchi, T.; Nagano, T., Novel Fluorescent Probes for Singlet Oxygen. *Angew Chem Int Ed Engl* **1999,** *38* (19), 2899-2901.

[35] Wu, H.; Song, Q.; Ran, G.; Lu, X.; Xu, B., Recent developments in the detection of singlet oxygen with molecular spectroscopic methods. *TrAC Trends in Analytical Chemistry* **2011,** *30*, 133-141.

[36] Dai, Z.; Tian, L.; Xiao, Y.; Ye, Z.; Zhang, R.; Yuan, J., A cell-membrane-permeable europium complex as an efficient luminescent probe for singlet oxygen. *J Mater Chem B* **2013,** *1* (7), 924-927.

[37] Schanze, K. S.; Brent MacQueen, D.; Perkins, T. A.; Cabana, L. A., Studies of intramolecular electron and energy transfer using the fac-(diimine)ReI(CO)3 chromophore. *Coordination Chemistry Reviews* **1993,** *122* (1), 63-89.

[38] Tan, M.; Song, B.; Wang, G.; Yuan, J., A new terbium(III) chelate as an efficient singlet oxygen fluorescence probe. *Free Radical Biology and Medicine* **2006,** *40* (9), 1644-1653.

[39] Song, B.; Wang, G.; Tan, M.; Yuan, J., A Europium(III) Complex as an Efficient Singlet Oxygen Luminescence Probe. *Journal of the American Chemical Society* **2006,** *128* (41), 13442-13450.

[40] Sun, S.; Li, X.; Zhang, G.; Ma, H.; Zhang, D.; Bao, Z., Determination of H2O2-dependent generation of singlet oxygen from human saliva with a novel chemiluminescence probe. *Biochimica et Biophysica Acta (BBA) - General Subjects* **2006,** *1760* (3), 440-444.

[41] Norihiko, Y.; Nobuo, T.; Takeshi, T., Mechanism of Photosensitized Chemiluminescence of 2-Methyl-6-phenylimidazo[1,2-a]pyrazin-3(7H)-one (CLA) in Aqueous Solution. *Chemistry Letters* **1999,** *28* (6), 451-452.

[42] Zhu, D.; Xing, D.; Wei, Y.; Li, X.; Gao, B., Evaluation of the degree of medical radiation damage with a highly sensitive chemiluminescence method. *Luminescence* **2004,** *19* (5), 278-282.

[43] He, Y.; Xing, D.; Yan, G.; Ueda, K.-i., FCLA chemiluminescence from sonodynamic action in vitro and in vivo. *Cancer Letters* **2002,** *182* (2), 141-145.

[44] Zhang, G.; Li, X.; Ma, H.; Zhang, D.; Li, J.; Zhu, D., A selective and sensitive chemiluminescence reaction of 4,4′(5′)-bis[2-(9-anthryloxy)ethylthio] tetrathiafulvalene with singlet oxygen.

Chemical Communications **2004,** (18), 2072-2073.

[45] Li, X.; Zhang, G.; Ma, H.; Zhang, D.; Li, J.; Zhu, D., 4,5-Dimethylthio-4′-[2-(9-anthryloxy)ethylthio] tetrathiafulvalene, a Highly Selective and Sensitive Chemiluminescence Probe for Singlet Oxygen. *Journal of the American Chemical Society* **2004,** *126* (37), 11543-11548.

[46] MacManus-Spencer, L. A.; Latch, D. E.; Kroncke, K. M.; McNeill, K., Stable Dioxetane Precursors as Selective Trap-and-Trigger Chemiluminescent Probes for Singlet Oxygen. *Analytical Chemistry* **2005,** *77* (4), 1200-1205.

[47] Schaap, A. P.; Chen, T.-S.; Handley, R. S.; DeSilva, R.; Giri, B. P., Chemical and enzymatic triggering of 1,2-dioxetanes. 2: fluoride-induced chemiluminescence from tert-butyldimethylsilyloxy-substituted dioxetanes. *Tetrahedron Letters* **1987,** *28* (11), 1155-1158.

[48] Matsumoto, M., Advanced chemistry of dioxetane-based chemiluminescent substrates originating from bioluminescence. *Journal of Photochemistry and Photobiology C: Photochemistry Reviews* **2004,** *5* (1), 27-53.

Chapter 3

Superoxide Dismutase: A Key Enzyme for the Survival of Intracellular Pathogens in Host

Radheshyam Maurya and Madhulika Namdeo

Abstract

Superoxide dismutase (SOD) is a crucial enzyme required to maintain the redox potential of the cells. It plays a vital role in protecting normal cells from reactive oxygen species (ROS) produced during many intracellular pathogens infections. SOD removes excess superoxide radicals (O^{2-}) by converting them to hydrogen peroxide (H_2O_2) and molecular oxygen (O_2). Several superoxide dismutase enzymes have been identified based on the metal ion as a cofactor. Human SOD differs from the intracellular pathogens in having Cu/Zn and Mn as metal cofactors. However, SOD of intracellular pathogens such as *Trypanosoma, Leishmania, Plasmodium*, and *Mycobacterium* have iron (Fe) as metal cofactors. Iron Superoxide Dismutase (FeSOD) is an essential enzyme in these pathogens that neutralizes the free radical of oxygen (O^{-}) and prevents the formation of Peroxynitrite anion ($ONOO^{-}$), helping the pathogens escape from redox-based cytotoxic killing. Moreover, most intracellular bacteria hold MnSOD or FeSOD in their cytoplasm such as *Salmonella* and *Staphylococcus*, whereas periplasm of some pathogenic bacteria and fungi are also cofactors with Cu/Zn and identified as CuZnSOD. This chapter will review the various types SOD present in intracellular pathogens and their role in the survival of these pathogens inside their host niche.

Keywords: Superoxide dismutase, Intracellular Pathogen, Reactive oxygen species, Antioxidant enzyme

1. Introduction

Reactive oxygen species are primarily the result of the by-product of the redox process and may also be produced to initiate intracellular signaling and antimicrobial activity. The general phenomenon is to maintain the ROS level in the cell by antioxidant enzymes and antioxidants molecules present in cells [1]. One of the prime sources of ROS in mammalian cells is the respiratory chain in mitochondria. It's well established that ROS generation is an essential modulator of inflammatory reactions in mammals. The enzyme NADPH oxidase induced the oxidative burst, leading to a dramatic increase in oxygen consumption and increasing the phagocytosis process. Activated macrophage induced the expression of IFN-γ and TNF-α cytokines,

improving NADPH oxidase activity resulting in ROS production, such as oxygen-free radicals ($O_2^{\bullet -}$) superoxide. The $O_2^{\bullet -}$ species are converted into hydroxyl radical ($HO^{\bullet}$), hydrogen peroxide (H_2O_2), and peroxynitrite ($ONOO^-$) by spontaneously or enzymatic reaction [2, 3]. Activation of nitric oxide synthase (iNOS) or (NOS_2) protein in macrophage stimulates the increased secretion of nitric oxide (NO) and $^{\bullet}$NO-metabolite levels within the cell. ROS is the first superoxide radicals produced by mitochondria. ROS is a highly reactive oxygen species and does not diffuse quickly from cells since the leading site of ROS production is in the inner mitochondrial membrane. H_2O_2 is derived from mitochondrial ROS and detoxified by superoxide dismutase. ROS detoxification has been assigned in ROS-generating sites in the cell, such as mitochondria, glycosome, endoplasmic reticulum, and cytosol. Hydrogen peroxide (H_2O_2) is not considered a free radical by definition since it lacks free electrons. Still, NO is deemed to be free radicals, has also been involved in ROS-mediated damage. However, NO has a dual nature, like as beneficial as well as vicious [4–6].

Aerobic organisms exhibited two major antioxidant defense systems to minimize the ROS-mediated damage occurring due to oxygen-free radicals. The first one is enzymatic defense, and the second is low molecular weight antioxidants such as vitamins and phytochemicals. In general, cells control oxidative stress by three essential antioxidant enzymes which are present in it; (i) Superoxide dismutase is a class of oxidoreductase enzymes that contain metal ions in their active site (Fe or Mn and/or Cu/Zn) and is responsible for converting superoxide anion into H_2O_2. (ii) glutathione peroxidase is responsible for the reduction of H_2O_2 into hydroperoxides using glutathione as hydrogen donor, and (iii) catalase is responsible for the breakdown of H_2O_2 into O_2 and H_2O [7]. Since the activity glutathione peroxidase is required glutathione as hydrogen donor. Thus, the NADPH-dependent reduction of oxidized glutathione to maintain a steady state of glutathione is needed for GSH activity [1].

Superoxide dismutase catalyzes the dismutation of oxygen free radical to O_2 and H_2O_2 in the cell. SOD enzymes also participate in signaling pathways by controlling ROS action and protecting the cells from the toxic effects of superoxide radicals. Intracellular SODs mainly restrict superoxide action, which harms the cells by damaging the Fe-S cluster-containing enzymes. Extracellular SODs also guard the cells from superoxide released by the host or pathogens. For example, extracellular SODs of microbial pathogens are protected by ROS-mediated killing of host cells. The host cells antioxidant system includes enzymes such as SOD, catalases, and peroxidases [8, 9].

2. Superoxide dismutase

The evolutionary history of metalloenzyme superoxide dismutase (SOD) is aged and has been there before the differentiation of eubacteria from archaea bacteria. It is ubiquitous protein present in all living organisms and plays a vital role in the extreme pressure defense against superoxide radicals in the cell. The SOD catalyzes the conversion of the two molecules of virulent oxygen free radical (O^-) into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2) by using two equivalents of H^+ ions [10]. SOD is marked as a strong free radical scavenger that can eliminate the toxic effects of superoxide produced during the reduction of molecular oxygen. SODs enzyme family have been classified based on several factors, and one is on the metal ion. In general, SODs contain the metal cofactor at their catalytic core and are classified into three major groups: copper/zinc (Cu/Zn-SOD) [11, 12], manganese (Mn-SOD) [13], and iron (Fe-SOD) [14–16]. SOD containing MnSOD, FeSOD and CuZnSOD are encrypted

by the gene sodA, sodB, and sodC, respectively. Nickel (Ni)- and iron-zinc (Fe/Zn) containing isozymes have also been identified in several bacteria [17, 18]. FeSOD has mainly reported in prokaryotes except few protozoan parasites, whereas MnSOD and CuZnSOD are found in both prokaryotes and eukaryotes. All these isoforms were identified based on their diverse sensitivities to cyanide (CN) and H_2O_2. The Cu, Zn-SOD is extremely sensitive to CN and H_2O_2 [19]. Mn-SOD is insensitive to CN and H_2O_2 [20], while Fe-SOD is not sensitive to CN but sensitive to H_2O_2 [21]. In addition, Mn-SOD and Fe-SOD, both were inhibited by chloroform–ethanol, but Cu, Zn-SOD is insensitive [22].

Moreover, SODs of intracellular bacteria are further classified into three groups based on their localization; Mn- and Fe-cofactor SODs are found in the cytosol. In contrast, the third one of SOD cofactor by Cu-Zn and is attached with periplasm or anchored with the lipid of the outer envelope [23, 24]. Cu/Zn-SOD of bacteria dismutase superoxide produced by host cell during phagocytosis contributes to helping bacterial virulence [25, 26]. Additionally, few families of SODs also use a Ni ion as cofactor at their catalytic core to initiate its functions [27]. A study has shown that superoxide dismutase from *Streptococcus* is capable of making a cofactor substitution with Fe in place of Mn [28]. On the other hand, *Leishmania tropica*, *Trypanosoma brueci*, and *Crithidia fasciculate* have superoxide dismutase, which is insensitivity to cyanide but sensitive to azide and peroxide [29]. SODs of Trypanosomatids are having Fe as a metal cofactor at their catalytic core and are categorized as iron superoxide dismutase (Fe-SOD). Other protozoan parasites also have the same Fe-SOD, such as *Plasmodium falciparum* and *Entamoeba histolytica*, where enzyme-mediated free radical catabolism is fully Fe-SOD dependent [30]. Fe-SOD isoform was first discovered in *Escherichia coli* in 1973 by Yost and Fridovich. Subsequently, the same isoform was characterized in *T. cruzi* in 1977. Like Trypanoredoxin (TR), SODs of *T. cruzi* differ from the mammalian host. Trypanosomatids, other protozoan parasites (*P. falciparum and E. histolytica*), some plants, and Archaea possess only Fe-SOD. However, humans and other mammalian hosts contain Cu/Zn-SOD and Mn-SOD as core metal **Figure 1** [31].

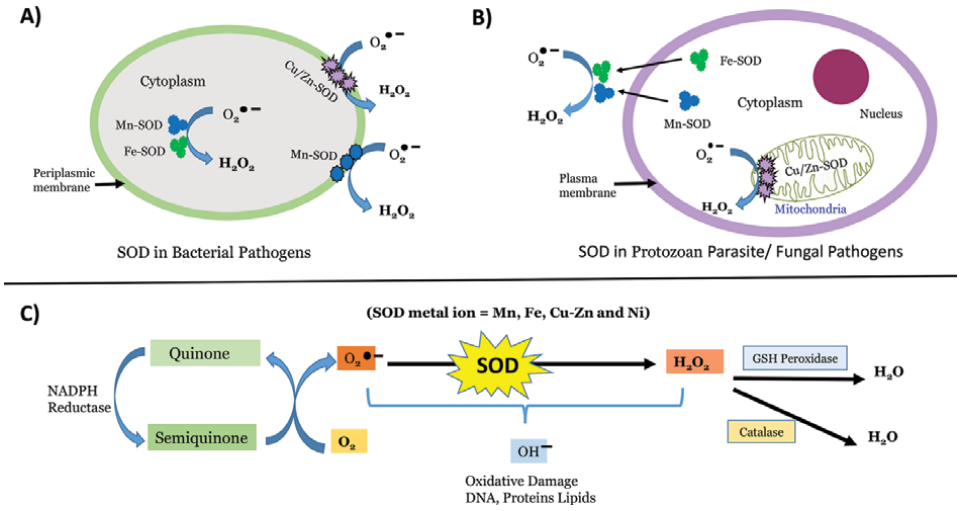


Figure 1.
Schematic representation of SOD localization in intracellular pathogens and SOD chemical reaction. A) Localization of SOD in bacterial pathogens, B) localization of SOD in protozoan and fungal pathogens and C) SOD reaction in mitochondria of protozoan and fungal pathogens.

This chapter will discuss role of superoxide dismutase in various intracellular pathogens that are belong to protozoan parasites genus *Trypanosoma, Leishmania, Plasmodium* and *Toxoplasma*, bacterial intracellular pathogens belongs to genus *Mycobacterium, Salmonella, Francisella* and *Staphylococcus* and fungal intracellular pathogens belongs to genus *Cryptococcus and Histoplasma* etc.

3. Role of SOD in intracellular parasites

There are several intracellular protozoan parasites which are causing severe illness in human's beings and if left untreated 100% mortality. These intracellular parasites belonging to the genus *Plasmodium*, *Leishmania,* and *Trypanosoma*, causing a spectrum of diseases like malaria, Leishmaniasis, African sleeping sickness, and Chagas disease in humans [1]. Antioxidant defense of pathogenic protozoan parasites is significantly distinct from each other as well as compared to their mammalian host. Trypanosomatids, as well as *Plasmodium* species have an Fe-containing SOD isoform, which is typically found in bacteria but absent in other eukaryotic cells [32, 33]. The main function of Fe-SOD is to neutralizing the $O^{[-]}$ that are formed during the generation of the superoxide radical [34]. Parasite persistence is determined by a balance between the ability of the immune response and resistance against free radicals produced by host cells. *Leishmania*-infected macrophages are able to produce inflammatory cytokines, ROS, and $^{\bullet}$NO derivatives, which usually lead to the killing of the phagocytosed microorganism. However, *Leishmania* and *Trypanosoma spp*. are few protozoa that can survive and resist cytotoxic environments within the macrophage, and further, they can able to replicate in such a hostile condition **Table 1** [4, 5].

3.1 Trypanosomiasis

Chagas is a parasitic disease caused by intracellular parasites *Trypanosoma cruzi*. The prevalence of the disease is around 6–7 million worldwide, mainly in Latin America and listed in 17 neglected tropical diseases (NTD) classified by the WHO (WHO-2021). The present chemotherapy is relay on two available drugs 5-nitrofuran-nifurtimox (NFX) and 2-nitroimidazole benznidazole [65]. *T. cruzi* contains only Fe-dependent superoxide dismutase (Fe-SOD). Parasites have two dimeric Fe-SOD isoforms, one mitochondrial and one cytosolic isoform. However, Mateo et al. [35] investigated and characterized 4 Fe-SODs in *T. cruzi* epimastigotes, mainly cytosolic. The level of Fe-SOD increases during the differentiation of short stumpy forms of the parasite into dividing procyclic forms [66]. Therefore, Fe-SODs could be a promising drug target for the development of anti-chagasic drugs because of their exclusivity in *T. cruzi*. Furthermore, the crystal structures of the cytosolic Fe-SOD and the mitochondrial Fe-SOD from *T. cruzi* suggest that each enzyme has two polypeptide chains and two active sites composed of a Fe2+/Fe3+ ion, respectively. In Chagas disease, phagocytosis of parasites by macrophages is the first line of defense against the parasites by the host. Macrophage produces superoxide radical ($O2^{\bullet-}$), which diffuses into parasitophorous vacuoles, causing toxic environments to the parasites. However, *T. cruzi* is also equipped with an antioxidant network to counter the host-derived ROS activity. During infections, parasites are internalized into the phagolysosomal compartment and activate the NADPH oxidase 2 complex (Nox2) of the host macrophage [67]. Nox2 activity in macrophages results in intraphagosomal formation of oxygen free radicals ($O2^{\bullet-}$) and $O2^{\bullet-}$ derived ROS, which is required to

Infectious Group	Disease	Agent	Major metal ions	Sub-class of SOD	Role of SOD in Pathogenesis	Reference
Parasitic disease	Trypanosomiasis	*Trypanosoma cruzi, T. bruci*	Fe-SOD	SOD-B1 & B2, SOD-A & SOD-C	Increase the resistance of parasite and decrease ROS mediated phagocytic killing	[30, 35]
	Leishmaniasis	*Leishmania major, L. donovani, L. tropica, L. major, L. chagasi*	Fe-SOD	SOD-A, SOD-B1 & B2	Increase the virulence of the parasites and decrease ROS-mediated phagocytic killing	[36–39]
	Malaria	*Plasmodium falciparum, P. ovale, P. malariae, P. vivax*	Fe-SOD	SOD-1 & 2	Limit the toxicity of ROS produced during hemoglobin degradation	[40–43]
	Toxoplasmosis	*Toxoplasma gondii*	Fe-SOD	SOD-B1, SOD2 & SOD3	Increased the intracellular growth of parasites. Triggered the humoral and cellular immune responses	[44–46]
Bacterial disease	Tuberculosis	*Mycobacterium tuberculosis, M. leprae*	Fe-SOD, Cu/Zn-SOD	SOD-B SOD-C	Inhibits the iNOS activity, IFN-γ expression & control apoptosis and TLR2 expression and signaling	[25, 47, 48]
	Salmonellosis	*Salmonella typhimurium,*	Mn-SOD, Cu/Zn-SOD	SOD-A SOD-C	Neutralizing the ROS mediated activity and inducible nitric oxide synthase activities and increase the virulence	[49–52]
	Tularemia	*Francisella tularensis*	Fe-SOD, Cu/Zn-SOD	SOB-B, SOD-C	Limits the iron requirement to produce the highly lethal OH· free radicals. Increase the virulence of bacteria	[53–56]
	Boils and Toxic shock syndrome	*Staphylococcus aureus*	Mn-SOD Fe-SOD	SOD-A SOD-M	Increase the resistance to oxidative stress, and induced virulence and infection	[57–59]
Fungal Disease	Cryptococcosis	*Cryptococcus neoformans*	Zn-SOD, Mn-SOD	SOD1 SOD-2	Increase the virulence factor and menadione resistance	[60–63]
	Histoplasmosis	*Histplasma capsulatum*	Cu/Zn-SOD	SOD-1 SOD-3	Decrease the ROS-mediated oxidative killing.	[64]

Table 1.
Distribution of superoxide dismutase (SOD) & their sub-class in various intracellular pathogens and their role in pathogenesis of respective diseases.

neutralize parasite proliferation and disrupt its differentiation in the early stage of infection. Macrophages derived from Nox2-deficient (gp91phox−/−) mice produced marginal amounts of superoxide radical and are more susceptible to parasite infection than those macrophages derived from wild-type mice. Nox2-derived superoxide radical plays a crucial role in controlling *T. cruzi* infection in the early phase of a murine model of Chagas disease [68]. Inhibition or ablation of the Nox2 enzyme has shown to be detrimental for controlling the infection of a number of pathogens *in vitro* and *in vivo* [69, 70].

Trypanosoma brucei is an obligate intracellular protozoan parasite that causes sleeping sickness in humans in many countries of sub-Saharan Africa. Various sub-species of parasites cause the disease and responsible for more than 90% of all trypanosomal diseases in humans [71]. Overexpression of SOD-B1 in *T. brucei* has shown hypersensitivity to a trypanocidal agent such as benznidazole and gentian violet. A similar study in *L. chagasi* revealed that an increase in SOD-B1 protein leads to resistance toward paraquat and nitroprusside [72]. Deleting one copy of Sod-B1 gene in the *L. chagasi* increased the sensitivity to the drug and a significantly decreased the parasites survival within the host macrophage. *T. brucei* serves four SOD isoforms, of which three are iron-dependent, which is typically very much similar to prokaryotic SODs. Localization studies reveal that out of four SOD, two are predominantly found in the glycosome (*Tb*SOD-B1 and *Tb*SOD-B2), and the other two are found in mitochondria (*Tb*SOD-A and *Tb*SOD-C) [30]. Overexpression of cytosolic Fe-SOD-B of *T cruzi* showed more resistance to the phagocytic killing of macrophages and increased intracellular proliferation than wild-type (WT) parasites. Fe-SOD-B overexpressed mutant parasites showed higher infectivity than WT but lost in gp91-phox−/− macrophages, emphasizing the role of $O2^{\bullet-}$ in parasite killing [67]. *Tc*FeSOD-A gene amplification increases the *Tc*FeSOD protein expression and enzyme activity in a *T. Cruzi* induced resistance to benznidazole and gentian violet treatments [73]. The reduced expression of *Tb*SOD-B leads to rapid accumulation of superoxide anion within the trypanosome responsible for detoxifying highly toxic radical in the parasite [74].

3.2 Leishmaniasis

Leishmaniasis is an intracellular protozoan disease caused by *Leishmania* parasites. Leishmaniasis is usually prevalent in tropical and subtropical regions of the world [36, 75]. *Leishmania* parasite infects host macrophages, survives in parasitophorous vacuoles of the macrophage, and escapes from the oxidative killing of the parasite by neutralizing the ROS activity. *Leishmania* Fe-SOD can be classified into two types based on their localization: FeSOD-A isoform is localized in mitochondria, and is related to cellular respiration; FeSOD-B1 and FeSOD-B2 are localized in glycosomes and reduce the oxidative stress generated from cellular reactions [37]. *L. major* contains Sod-B1, Sod-B2, and Sod-C genes on chromosome 32 and sod-A gene on chromosome 8. Sod-B1 and Sod-B2 genes are organized in tandem in both *L. chagasi* and *L. donovani*. Metacyclic promastigote of *L. amazonensis*, when lacking one allele of the Sod-A gene, failed to replicate in macrophages and severely attenuated their ability to established the cutaneous lesions in mice. In addition, the reduction of SOD-A expression in parasites resulting in increased susceptibility to oxidative damage. The failure of SODA/sod-A functions in promastigotes compromised their differentiation

into axenic amastigotes. Hence, SOD-A promotes *Leishmania* virulence by protecting the parasites against oxidative stress and initiating ROS-mediated signaling mechanisms, which are required to determine infective forms [37]. *L. chagasi* SOD-B1 null mutant parasites are not viable inside host macrophages. Furthermore, parasites lacking one SOD-B1 allele have markedly reduced their viability [38]. Moreover, WT and SOD-B1/Δsodb1 *L. major* promastigotes have equal capacity to establish infection in murine bone marrow macrophages. However, in contrast to WT parasites, *L. major* SOD-B1/Δsodb1 deficient parasites are declined in number over time in macrophages. The results suggesting its normal level of SOD-B1 is required for *L. major* endurance in macrophages and virulence in mice [76]. The Fe-SOD transcript level and enzyme activity are higher in the amastigote than in the promastigote stage of the parasite when treated with nitroprusside and parquet in *L. chagasi* [72]. In *Leishmania*, FeSOD-A appears to be the first line of defense against ROS and is crucial for parasite survival inside macrophages. Antimony (SbIII) resistant *L. (Viannia) brazilensis* (LbSbR) and *L.* (Leishmania) *infantum* (LiSbR) lines express higher FeSOD-A specific enzyme activity compared to wild type control and showed more resistance toward Antimony (SbIII) [77, 78]. Moreover, miltefosine resistant *L. donovani* are able induce the overexpression of *Ld*FeSODA to protects from drug-induced cytotoxicity, reduces superoxide generation, and involves in suppression of oxidative stress-induced programmed cell death by reducing the phosphatidylserine exposure, DNA damage [79, 80]. Increased exposure of *L. donovani* to miltefosine makes resistance due to the release of *Ld*FeSOD-A into the cytosol from mitochondria. This release of *Ld*FeSOD-A into the cytosol or the inhibition of *Ld*FeSOD-A import into the mitochondria makes the mitochondria even more susceptible to oxidative stress due to the accumulation of ROS. Mitochondria of the parasite are more vulnerable to ROS, leading to programmed cell death, emphasizing its role in keeping healthy mitochondria [39].

3.3 Malaria

Malaria is caused by an intracellular protozoan parasite belongs to the genus Plasmodium. Malaria is endemic in most of tropical countries and subtropical regions of Asia, Africa, South, and Central America. Plasmodium can differentiate and replicate inside hepatocytes, and then released as merozoites into the bloodstream, which subsequently invades red blood cells (RBCs) [81]. Plasmodium parasite uses SOD to reduce the toxicity of ROS throughout the intra-erythrocytic stage of parasite survival. The SOD activity in *Plasmodium falciparum* and rodent malaria species is characterized as iron-dependent and the first level of the antioxidant defense system of the parasite [40, 81, 82]. *P. falciparum* consists two distinct genes coding for different SOD such as *Pf*FeSOD-1 and *Pf*FeSOD-2 [40]. *Pf*FeSOD-1 is a cytosolic protein and expressed during the intra-erythrocytic cycle of the parasite [41, 83]. FeSOD-1 is also reported in *P. ovale*, *P. malariae*, and *P. vivax* and very close apicomplexan parasites such as *Toxoplasma gondii* [42]. Since FeSOD-1 is a cytosolic protein, it is unlikely to act on a superoxide anion in the parasite food vacuole during hemoglobin digestion. Thus, it is plausible that parasites might be taking a large amount of Cu/Zn-SOD from the host erythrocyte to detoxify the superoxide anions in their organelles [84]. Plasmodium parasite utilizes SODs enzymes to limit the toxicity of ROS produced during hemoglobin degradation in the erythrocytic cycle. These enzymes play a crucial role in parasite persistence and their intracellular survival during the

intra-erythrocytic stage of the life cycle. FeSOD1 of *Plasmodium vinckei* (PvSOD1) also plays a central role in the oxidative defense of these parasites. However, PvSOD1 is inhibited by H_2O_2 and peroxynitrite, but not by cyanide and azide [85]. The FeSOD-2 of *P. falciparum* is a mitochondrial SOD with an elongated N-terminal protein extension, reminiscent of a bipartite apicoplast-localized protein [43, 86]. An inhibition study of recombinant *P. falciparum* FeSOD suggested that SOD is a highly selective drug target to designed antimalarial drugs. The study further identified many antimalarial drugs which have shown antimalarial activities against *P. falciparum and even* a strain moderately resistant to chloroquine [87].

3.4 Toxoplasmosis

Toxoplasma gondii is an obligate intracellular protozoan pathogen that infects nearly all warm-blooded animals. Toxoplasmosis is one of the most prevalent parasitic diseases, an estimated one-third of the global population are at risk. Still, it is considered a neglected parasitic disease [88]. *T. gondii* causes life-threatening illnesses in developing fetuses and in persons with immunocompromised [89]. In chronic infection, *T. gondii* spreads in various organs such as the heart and brain through the circulatory system [90]. *T. gondii* RH tachyzoites treated with resveratrol and pyrimethamine significantly increased SOD activity to restrain ROS action for their survival [44]. Interestingly, human macrophages failed to produced ROS during *T. gondii*-infection [91], possibly due to an immune evasion mechanism of parasites. *T. gondii* targets the host NADPH oxidase enzyme by reducing the expression of Nox4 transcript and protein, resulting in diminished the release of intracellular ROS. In infected cells, Nox4 gene expression was associated with activation of PI3K/AKT signaling [92]. However, superoxide dismutase and catalase enzymes might be playing a role in intracellular survival but, it does not have a basis for differences in virulence to mice [93]. *In T. gondii,* SODs are found in nearly all developmental stages of parasites, suggesting their importance in detoxifying superoxide radicals to protect the parasite. *T. gondii* contains three types of SOD; SOD-B1 (Fe-SOD), different from the Mn-binding SOD of humans. SOD-B1 is a cytoplasmic and essential enzyme, and SOD-B1 gene knock-outs lead to be lethal for parasites [94, 95]. SOD2 and SOD3 are found in the mitochondria of parasites and have conserved residues to bind iron. However, they are very similar in the primary sequence to SODs from *P. falciparum* [45] *T. gondii* superoxide dismutase (*Tg*SOD) also affects the intracellular multiplication of both bradyzoite and tachyzoite forms of parasites. A recombinant DNA vaccine containing the antigen gene of *T. gondii* were elicited high levels of antibodies, a Th1 type of immune response with significant production of IFN-γ, and low levels of IL-4 or IL-10 in BALB/c mice [96]. Moreover, a DNA vaccine containing the TgSOD gene triggered potent humoral and cellular immune responses, and it stimulates biased protective immunity against acute *T. gondii* infection in BALB/c mice [46]. SOD-DNA vaccines of L. amazonensis immunized mice were partially protected from parasites once challenged. Mice showed a mixed immune response, including the production of IFN-γ and IL-4 from CD4+ and CD8+ T lymphocytes [69]. In addition, the SOD vaccine of *Brugia malayi* was also shown to trigger a typical Th1 response against infective larvae and microfilariae in jirds with filarial infection [97]. The above finding reveals that SOD-dependent vaccines have potential vaccine efficacy, either by protein or DNA-based vaccines, to control intracellular pathogen by activating the protective Th1 type of immune responses in animals.

4. Role of SOD in intracellular bacteria

There are several intracellular bacteria which are causing severe illness in human's beings and if left untreated 100% mortality. Most pathogenic bacteria contain MnSOD or FeSOD in their cytoplasm, while CuZnSOD has been found on the periplasm of pathogenic bacteria and played an essential role during phagocytosis [11, 23]. In addition to their ability to detoxify free radicals during aerobic growth, bacterial SODs are also critical in determining the virulence factors. In several intracellular bacterial infections, SOD-C acts as a critical virulence factor, and its localization to the periplasmic membrane protects bacteria from ROS derived from host cells [49, 98–100]. Moreover, many virulent bacteria maintain two copies of the sodC gene [101]. The evolutionary maintenance of an extra sodC gene copy indicates that SOD is essential for pathogenic bacteria for their survival inside the host niche [101]. These pathogens belong to the categories of genus *Mycobacterium, Salmonella, Staphylocccus* and *Francisella*, causing spectrum of disease like tuberculosis, leprosy, typhoid, boils, furuncles, cellulitis and tularemia etc. **Table 1**.

4.1 Tuberculosis and leprosy

Mycobacterium is an intracellular bacterium, which is causing two distinct disease manifestations in humans, such as Tuberculosis and Leprosy. Tuberculosis (TB) is caused by *M. tuberculosis*, a leading infectious agent that claims millions of deaths worldwide/year [102]. *M. tuberculosis* is encountered several exogenous and endogenous redox pressures throughout its pathogenic life cycle. Therefore, they use various in-house enzymes to detoxify and neutralize the redox potential produced by host cells. Catalase–Peroxidase, Superoxide dismutase, and Alkyl Hydroperoxidase are the enzymes involved in the clearance of oxidative stress [47].

M. Tuberculosis is a highly pathogenic bacterium contains Fe-SOD and expresses 93-fold more superoxide dismutase. In contrast, non-pathogenic mycobacterium *M. Smegmatis* has Mn-SOD, and *M. Tuberculosis* export more enzyme than *M. smegmatis* [48]. Superoxide dismutase (SOD) of *M. tuberculosis* is a 207-residue enzyme with molecular mass of 23 kDa [103]. Treatment with diethyldithiocarbamate, a potent inhibitor of SOD, increased *M. lepraemurium* survival in murine splenic macrophages [104], suggesting that SOD protein is required for the long-term survival of mycobacterium in vivo [104] *M. tuberculosis* has two distinct SOD proteins, SOD-A and SOD-C. SOD-A is one of the main extracellular proteins contains Mn, Fe-SOD. SOD-C is much lower protein contains Cu, Zn SOD, and present in the outer membrane of the bacteria. SOD-C was upregulated during phagocytosis by macrophage, suggesting its importance in protecting the *M. tuberculosis* membrane against damage from superoxide radicals [25]. SOD of *M. tuberculosis* scavenge oxygen free radicals and inhibits the release of NO by inhibiting iNOS activity. It impairs acquired by down-regulating the IFN-γ expression as well as control the caspase-dependent apoptosis. SOD also inhibits innate immunity by down-regulating TLR2 expression as well as control the TLR2 dependent signaling in the cells [104].

Mycobacterium leprae is the causative agent of leprosy or Hansen's disease. *M. leprae* is the single known bacterial pathogen that infects superficial peripheral nerves. It is an intracellular pathogen that infects both myelinated and nonmyelinated Schwann cells of the nerve and proliferates within the monocyte/macrophage series cells. Peripheral nerves are not protected from the immune response of host due to the blood–brain barrier [105]. Hence, the advantage of *M. leprae* is to escape from the

phagocytosis actions of the macrophage may be a critical factor in its pathogenicity [106]. The SOD activity of *M. leprae* is lower than the other mycobacteria species such as *M. lepraemurium, M. phlei* [107]. Therefore, the ability to clear the *M. leprae* infection via SOD pathway appeared to be a distinct mannerism and is not dependent on macrophage activation and differentiation.

4.2 Salmonellosis

Salmonella typhimurium is a facultative intracellular bacterium that resides within modified phagosomes in macrophage promotes replication and escape from killing by ROS [108]. *S. typhimurium* infects a wide range of hosts, including animals, humans, and poultry. *S. typhimurium* causes acute gastroenteritis in humans and typhoid-like disease in mice. If left untreated, 100% fatal [50]. *Salmonella* infects the epithelial wall of the intestine and escapes from the innate immunity and ROS activity of the host. The SOD of *S. typhimurium* protects the bacterium from excessive ROS activity produced outside or inside of the host cell [109, 110]. Thus, SOD was considered a critical factor for bacterial survival by neutralizing the ROS activity [111]. The sod-A gene inactivation in *Salmonella* species is connected with limited protection from ROS and decreased virulence during mice infection [26, 109]. sod-A-deficient bacterium displayed a slightly lower growth rate compared to the wild-type strain. The loss of the sod-A gene in mutant bacteria harms the ability to infect the host cell. Consequently, the sod-A mutant bacterium is highly susceptible to the bactericidal action of host cells and has also shown attenuated virulence properties. More specifically, SOD-A plays a vital role in biofilm formation, increased resistance against oxidative stress, and overcome from bactericidal complement system of serum [51]. *Salmonella* combats phagocytic free radicals by producing the periplasmic superoxide dismutase. Periplasmic Cu, Zn-cofactor superoxide dismutase (SOD-C) protects *S. typhimurium* from extracellular phagocyte-derived oxidative damage by host cells. *Salmonella* deficient sod-C gene has shown abated survival inside the macrophage, increased ROS susceptibility, and attenuated virulence factor during in-vivo infection. Conclusively, SOD protects periplasmic or inner membrane targets by controlling the phagocytosis-dependent oxidative burst or inducible nitric oxide synthase activities during in vivo infection [49]. The evolutionary acquisition of the sod-C gene in *Salmonella* species extends an increased virulence trait of bacterium [52].

However, cytosolic Mn-SOD enzyme is essential for detoxifying intracellular superoxide radicals but not involved virulence [112]. SOD of *Streptococcus suis* resistant to anti-oxidative stress and ROS-generating herbicides, which is known to cause a severe damage to DNA, RNA, and proteins molecules that might contribute to its virulence in mice [53].

4.3 Tularemia

Francisella. tularensis is an intracellular pathogen that causes a disease called Tularemia. The disease is considered a potential biological threat for humans due to its extreme infectivity and substantial capacity to cause severe illness and death. The hallmark of the bacterium is their capability to survive and replicate within macrophages [113] and other cell types [114, 115]. The bacterium's survival depends on its ability to combat the microbicidal activity of macrophages such as ROS and reactive nitrogen species. *F. tularensis* require oxygen for their growth and possess ROS-scavenging enzymes such as super oxide dismutases, peroxidases, and catalases [116, 117].

Like other bacterial pathogens, *F. tularensis* contains two types of SOD gene: FeSOD (sod-B) and CuZnSOD (sod-C). SOD-B plays a dual role in protecting *F. tularensis* from the oxidative stress of the host. SOD-B binds to the iron with high affinity and limits the availability of iron requirement to produce the highly lethal OH·. Secondly, detoxification of superoxide prevents cellular damage of DNA, proteins, and lipids associated with O^{2-} toxicity [53, 54]. SOD-B dismutation decreasing the reaction of O2 with NO to form peroxynitrite (ONOO) and protect bacteria from ONOO- toxicity [55]. ONOO- has been shown to have a significant role in the IFN-γ -induced killing of *F. tularensis* (live vaccine strain) LVS by murine macrophages [99, 118]. However, the genome sequence of *F. tulrensis* LVS has possessed a single functional copy of the sod-B gene [117]. Hence, sod-B gene alteration leads to reduced SOD-B enzyme expression might be associated with high sensitivity to oxidative stress suggesting that sod-B is essential for bacterial survival under oxidative stress conditions. Therefore, increased survival of mice infected with sod-B mutant *F. tularensis* suggesting that SOD-B plays a role in virulence [56].

A recent study suggests SOD-C (CuZnSOD) of *F. tularensis* also plays a vital role in virulence factors. SOD-C is localized in the periplasm to protect from superoxide radicals (O^{2-}) derived from host cells. *F. tularensis* depleted sod-C (ΔsodC) mutant and *F. tularensis* ΔsodC mutant with attenuated sod-B gene expression (sodB ΔsodC) exhibited attenuated intracellular survival in IFN-γ-activated macrophages compared to the wild-type *F. tularensis* LVS. Transcomplementation of the sod-C gene in ΔsodC mutant bacteria or checking the IFN-γ-dependent production of O^{2-} or NO enhanced the survival of the sod mutant's bacteria in macrophage. The virulence capacity of the sodB ΔsodC mutant bacteria was significantly more attenuated as compared to ΔsodC mutant. Furthermore, lack of IFN-γ, iNOS, or PHOX restored the virulence of ΔsodC mutant strains, suggesting that the CuZnSOD of the bacterium is playing a critical role in restricting the bactericidal activities of ROS and RNS. The ΔsodC and sodB ΔsodC mutants were also significantly attenuated for virulence in intranasally challenged C57BL/6 mice compared to the wild-type *F. tularensis* LVS, indicating that SOD-C is required for resisting host-generated ROS and contribute to survival and virulence of *F. tularensis* in mice [119].

4.4 Staphylococcus (boils and toxic shock syndrome)

Staphylococcus aureus is a gram-positive bacterium, which causes a broad spectrum of diseases in humans. It is a facultative intracellular bacterium that invades and replicates within many types of phagocytic and non-professional phagocytes cells, such as endothelial cells, mammary cells, fibroblasts, and osteoclasts [120]. Bacterium commonly symptomatically colonizes in one-third of the population of the globe and is a leading cause of antibiotic-resistant [121]. Methicillin-resistant *S. aureus* (MRSA) strains are one of the utmost dangerous species and have shown resistance to all β-lactam antibiotics as well as other antimicrobials [122]. *S. aureus* is capable of subverting xenophagy and escaping from the cytosol of the host cell during intracellular infection [118, 122, 123]. During intracellular survival, *S. aureus* is capable to protects itself from the oxidative burst by numerous mechanisms, including enzymes such as SODs that detoxify the action of ROS activity [124, 125]. *S. aureus* serves two distinct SODs, SOD-A and SOD-M, both of which are cytoplasmic and reported as Mn-dependent [57, 126]. All Staphylococci species are contained SOD-A protein, while *S. aureus* also has a unique protein SOD-M [58]. The loss of either SOD-A or SOD-M in a skin model of infection or loss of both SODs in a systemic mouse model

of infection diminishes the ability of *S. aureus* to cause disease, highlighting the importance of SOD in the virulence [127, 128].

The lack of both SODs in *S. aureus* shown bacterium is more sensitive to host cells during manganese starvation, suggesting the importance of SOD in overcoming nutritional immunity. Mn starvation in host-mediated protein calprotectin reduces staphylococcal SOD activity during in vitro and in-vivo infection. Hence, Mn deficiency renders *S. aureus* more sensitive to oxidative stress and neutrophil-mediated killing [127, 129, 130]. SOD-A protein is essential for countering oxidative stress and disease progression when manganese is abundant. At the same time, SOD-M is important under manganese-deplete conditions. However, SOD-A is strictly manganese-dependent, whereas SOD-M contains either of two or more different metal atoms, having similar enzymatic activity when filled with manganese or iron. During host-dependent Mn starvation, *S. aureus* enables the ability of SOD-M to utilize Fe to retain its SOD activity. Subsequently, *S. aureus* enhances the ability to overcome nutritional immunity, resistance to oxidative stress, and ultimately induced virulence and infection [59].

5. Role of SOD in other fungal infection

Superoxide of pathogenic fungus are cofactors with Cu/Zn or Mn metals. The enzymes are localized in the cytosol as well as in mitochondria and involved in cell differentiation and multi-stress conditions. Mitochondrial Mn-SODs prevent the damages of oxidative stress, osmotic and thermal stresses in yeast cells. SODs protein has been shown to contribute to the virulence of many intracellular pathogenic fungi, such as *C. neoformans* [60], and *H. capsulatum*, both are capable to some degree of neutralizing the lethal levels of ROS produced by the host cells [64]. *C. neoformans* have Zn-SOD and Mn-SOD, while *H. capsulatum* has Cu/Zn-SOD. However, some fungal pathogens and fungal-like oomycetes have a unique SOD, such as Cu-SODs (SOD5). SOD5 are closely associated with the ubiquitous class of Cu/Zn-SODs but lack a Zn cofactor [34] and are believed to act on substrate level [131–133]. Unlike Cu/ZnSODs, which is found in both intra- and extracellularly, Cu-SODs are found exclusively in extracellular, and they appear primarily appended to the GPI anchors protein of cell surface [134, 135]. Cu-SODs have been proved to protect pathogens from the oxidative burst of the host regulated by immune cells [9] **Table 1**.

5.1 Cryptococcosis

Cryptococcus neoformans (*Cn*) is a facultative intracellular fungal pathogen and can propagate inside the host macrophages during many stages of experimental and human infections [136, 137]. *Cryptococcus* is a soil fungus that causes life-threatening meningitis in immunocompromised patients [138, 139]. *Cryptococcus* is an encapsulated pathogenic yeast composed primarily of glucuronoxylomannan (GXM). This polysaccharide helps the fungus play a defensive and offensive role during pathogenesis. It protects the fungus against phagocytosis and promoting intracellular pathogenesis through the cytotoxic release of polysaccharides into macrophage vacuoles [136]. *Cryptococcus* rarely causes clinically visible infections in healthy hosts, but it can be present in latency and persistence inside macrophages

[61, 62]. *C. neoformans var. gattii* predominantly infects individuals having a normal immune response, whereas var. grubii and neoformans are common in immunocompromised individuals. *C. neoformans var. gattii* hinders macrophage phagocytic response, whereas the other two varieties are readily killed by ROS released by phagocytic cells [140, 141].

C. neoformans is resistance to ROS mediated oxidative killing of macrophage by inducing the SOD activity and might be playing an important role in virulence of this fungus. Exogenous supplementation of SOD significantly controlled the bacterial growth by inducing human neutrophil function, suggesting that SOD plays a protective role during *C. neoformans* infection [63]. *Cryptococcus neoformans* var. *gattii* contains two types of SODs such as copper, zinc-depend SOD (SOD1) and Mn-dependent (SOD2) isoenzymes [142]. Both SOD1 and SOD2 are intracellular SODs, and deletion of their encoding genes reduces the fungal virulence in vivo model of infection. Furthermore, the mutant fungus also increases sensitivity to pharmacologically-induced intracellular oxidative stress [143]. The sod1 mutant *C. neoformans* was shown three characteristic features 1) highly sensitivity toward oxidative killing by human polymorphonuclear (PMN) cells and by the redox cycling agent menadione. 2) The sod1 mutant was markedly attenuated in virulence when raising the infection in mice, and it also showed significantly susceptibility to in vitro killing by human neutrophils. 3) SOD1 deletion also appeared to be defects in the expression of a number of virulence factors such as laccase, urease, and phospholipase. Complementation of the sod1 gene mutant *C. neoformans* with SOD1 protein regained the virulence factor and menadione resistance. Hence, the antioxidant function of SOD1 is critical for the pathogenesis of the fungus during intracellular survival [60, 141, 144].

5.2 Histoplasmosis

Histoplasma capsulatum is an intracellular fungal pathogen structurally similar to yeast cells. *H. capsulatum* successfully infect host cells like neutrophils and macrophages. *H. capsulatum* is prevalent in the Midwestern United States and Latin America. Macrophages efficiently phagocytize the *Histoplasma* cells, but they failed to kill the fungus despite having ample ROS production. *Histoplasma* cells counter the ROS-mediated oxidative stress of the host by three proteins that are possibly involved in defending *Histoplasma* from ROS. sod1 and sod3 gene deficient *Histoplasma* strains shown the spatial specificity of the SOD1 and SOD3 superoxide dismutases for internal and external (i.e., host-derived) superoxide, respectively. SOD-3 is the primary source of extracellular SODs, and its expression is significantly enriched in the pathogenic phase of fungus cells. *Histoplasma* SOD-3 offers higher resistance of fungus against the phagocytic killing of host cells leading to increased capacity to cause disease in immunocompetent hosts. In *in vivo* studies, sod-3 gene deficient *Histoplasma* strains were shown the attenuation in virulence in mice. Furthermore, restoration of Δsod3 mutant *Histoplasma* virulence in mice unable to produce superoxide radicals conclusively proves that SOD3 functions in the detoxification of superoxide generated by the host. SOD-3 also prevents the superoxide-dependent killing of *Histoplasma* yeast cells. The host to control the infection of *Histoplasma* requires ROS production. Hence, SOD-3 is a central virulence factor of *Histoplasma* and help to fungus survives under oxidative stress produced by host phagocytic cells during infection [64].

6. Conclusion

Superoxide's are the critical molecules produced by host cells to counter intracellular pathogens during infection. ROS is mainly produced within mitochondria of cells as byproducts of normal cell respiration. Defects in oxidative phosphorylation in cells could lead to an increase or decrease in ROS production by host cells. ROS-mediated destruction can directly affect the components of the electron transport system of host cells. Therefore, to reduce the ROS activity, host cells are evolved with three types of SODs such as NiSOD, Fe or MnSOD, and CuZnSOD to control the ROS activity produced by itself. More importantly, the immune cells of the host used ROS as defense molecules against various kinds of human pathogens during their infection.

Intracellular pathogens are also furnished with all types of SODs such as NiSOD, Fe or MnSOD, and CuZnSOD. Pathogens are using these SODs in neutralizing the free radicals produced by host cells during infection. SODs of intracellular pathogens can modulate the interaction with phagocytic cells at the onset of phagocytosis by altering the local concentrations of superoxide anion in parasitophorous vacuoles of host cells. SODs of these pathogens are also required to neutralized O2- generated by IFN-γ-activated macrophages, but not necessary for survival in quiescent macrophages. However, the role of SOD in combating other infection does not solely depend on the phagocytic ability of macrophages. In conclusion, SODs of intracellular pathogens are the key determinants of their survival inside the host niche. Furthermore, it also plays a vital role in the severity of disease and virulence of these pathogens by protecting them from extracellular host-derived ROS activity.

Author details

Radheshyam Maurya* and Madhulika Namdeo
Department of Animal Biology, School of Life Sciences, University of Hyderabad, Hyderabad, India

*Address all correspondence to: rmusl@uohyd.ac.in; rmusl@uohyd.ernet.in

IntechOpen

References

[1] Turrens JF. Oxidative stress and antioxidant defenses: A target for the treatment of diseases caused by parasitic protozoa. Mol Aspects Med 2004; 25: 211-220. DOI:10.1016/j.mam.2004.02.021.

[2] Babior BM, Kipnes RS, Curnutte JT. Biological defense mechanisms. The production by leukocytes of superoxide, a potential bactericidal agent. J Clin Invest 1973; 52: 741-744. DOI:10.1172/JCI107236.

[3] Babior BM. Phagocytes and oxidative stress. Am J Med 2000; 109: 33-44. DOI:10.1016/s0002-9343(00)00481-2.

[4] Assche T Van, Deschacht M, Inocêncio RA, et al. Free Radical Biology & Medicine Leishmania – macrophage interactions : Insights into the redox biology. Free Radic Biol Med 2020; 51: 337-351. DOI:10.1016/j.freeradbiomed.2011.05.011.

[5] Stafford JL, Neumann NF, Belosevic M. Macrophage-mediated innate host defense against protozoan parasites. Crit Rev Microbiol 2002; 28: 187-248. DOI:10.1080/1040-840291046731.

[6] Kirkinezos IG, Moraes CT. Reactive oxygen species and mitochondrial diseases. 2001; 12: 449-457. DOI:10.1006/scdb.2001.0282.

[7] Chance B, Sies H, Boveris A. Hydroperoxide metabolism in mammalian organs. Physiol Rev 1979; 59: 527-605. DOI:10.1152/physrev.1979.59.3.527.

[8] Tamayo D, Mu JF, Lopez Á, et al. Identification and Analysis of the Role of Superoxide Dismutases Isoforms in the Pathogenesis of Paracoccidioides spp. 2016; 1-23. DOI:10.1371/journal.pntd.0004481.

[9] Press P, Ra DOI, Schatzman XSS, et al. cro Copper-only superoxide dismutase enzymes and iron starvation stress in Candida fungal pathogens. 2020; 295: 570-583. DOI:10.1074/jbc.RA119.011084.

[10] McCord JM, Fridovich I. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J Biol Chem 1969; 244: 6049-6055.

[11] Battistoni A, Pacello F, Folcarelli S, et al. Increased expression of periplasmic Cu,Zn superoxide dismutase enhances survival of *Escherichia coli* invasive strains within nonphagocytic cells. Infect Immun 2000; 68: 30-37. DOI:10.1128/IAI.68.1.30-37.2000.

[12] Gee JM, Valderas MW, Kovach ME, et al. The *Brucella abortus* Cu,Zn superoxide dismutase is required for optimal resistance to oxidative killing by murine macrophages and wild-type virulence in experimentally infected mice. Infect Immun 2005; 73: 2873-2880. DOI:10.1128/IAI.73.5.2873-2880.2005.

[13] Poyart C, Pellegrini E, Gaillot O, et al. Contribution of Mn-cofactored superoxide dismutase (SodA) to the virulence of Streptococcus agalactiae. Infect Immun 2001; 69: 5098-5106. DOI:10.1128/IAI.69.8.5098-5106.2001.

[14] Bowler C, Camp W Van, Montagu M Van, et al. Superoxide Dismutase in Plants. CRC Crit Rev Plant Sci 1994; 13: 199-218. DOI:10.1080/07352689409701914.

[15] Seyler RWJ, Olson JW, Maier RJ. Superoxide dismutase-deficient mutants of Helicobacter pylori are hypersensitive

to oxidative stress and defective in host colonization. Infect Immun 2001; 69: 4034-4040. DOI:10.1128/IAI.69.6.4034-4040.2001.

[16] Khelef N, DeShazer D, Friedman RL, et al. In vivo and in vitro analysis of Bordetella pertussis catalase and Fe-superoxide dismutase mutants. FEMS Microbiol Lett 1996; 142: 231-235. DOI:10.1111/j.1574-6968.1996.tb08435.x.

[17] Kim EJ, Chung HJ, Suh B, et al. Transcriptional and post-transcriptional regulation by nickel of sodN gene encoding nickel-containing superoxide dismutase from Streptomyces coelicolor Muller. Mol Microbiol 1998; 27: 187-195. DOI:10.1046/j.1365-2958.1998.00674.x.

[18] Kim EJ, Chung HJ, Suh B, et al. Expression and regulation of the sodF gene encoding iron- and zinc- containing superoxide dismutase in Streptomyces coelicolor Muller. J Bacteriol 1998; 180: 2014-2020. DOI:10.1128/jb.180.8.2014-2020.1998.

[19] Kwiatowski J, Kaniuga Z. Isolation and characterization of cytosolic and chloroplast isoenzymes of Cu,Zn-superoxide dismutase from tomato leaves and their relationships to other Cu,Zn-superoxide dismutases. Biochim Biophys Acta - Protein Struct Mol Enzymol 1986; 874: 99-115. DOI:https://doi.org/10.1016/0167-4838(86)90107-X.

[20] Kawaguchi T, Noji S, Uda T, et al. A monoclonal antibody against COOH-terminal peptide of human liver manganese superoxide dismutase. J Biol Chem 1989; 264: 5762-5767. DOI:10.1016/s0021-9258(18)83615-8.

[21] Asada K, Kanematsu S, Okada S, et al. Chemical and biochemical aspects of superoxide and superoxide dismutase. *by JV Bannister HAO Hill, Elsevier/ North-Holland,* New York 1980; 136-153.

[22] Hartz JW, Deutsch HF. Subunit Structure of Human Superoxide Dismutase. J Biol Chem 1972; 247: 7043-7050. DOI:https://doi.org/10.1016/S0021-9258(19)44691-7.

[23] Wu CH, Tsai-Wu JJ, Huang YT, et al. Identification and subcellular localization of a novel Cu,Zn superoxide dismutase of *Mycobacterium tuberculosis*. FEBS Lett 1998; 439: 192-196. DOI:10.1016/s0014-5793(98)01373-8.

[24] D'orazio M, Folcarelli S, Mariani F, et al. Lipid modification of the Cu,Zn superoxide dismutase from *Mycobacterium tuberculosis*. Biochem J 2001; 359: 17-22. DOI:10.1042/0264-6021:3590017.

[25] Piddington DL, Fang FC, Laessig T, et al. Cu,Zn superoxide dismutase of *Mycobacterium tuberculosis* contributes to survival in activated macrophages that are generating an oxidative burst. Infect Immun 2001; 69: 4980-4987. DOI:10.1128/IAI.69.8.4980-4987.2001.

[26] Sansone A, Watson PR, Wallis TS, et al. The role of two periplasmic copper- and zinc-cofactored superoxide dismutases in the virulence of Salmonella choleraesuis. Microbiology 2002; 148: 719-726. DOI:10.1099/00221287-148-3-719.

[27] Miller A-F. Superoxide dismutases: ancient enzymes and new insights. FEBS Lett 2012; 586: 585-595. DOI:10.1016/j.febslet.2011.10.048.

[28] Martin ME, Byers BR, Olson MO, et al. A Streptococcus mutans superoxide dismutase that is active with either manganese or iron as a cofactor. J Biol Chem 1986; 261: 9361-9367.

[29] Le Trant N, Meshnick SR, Kitchener K, et al. Iron-containing superoxide dismutase from Crithidia

fasciculata. Purification, characterization, and similarity to Leishmanial and trypanosomal enzymes. J Biol Chem 1983; 258: 125-130. DOI:10.1016/s0021-9258(18)33229-0.

[30] Wilkinson SR, Prathalingam SR, Taylor MC, et al. Functional characterisation of the iron superoxide dismutase gene repertoire in Trypanosoma brucei. Free Radic Biol Med 2006; 40: 198-209. DOI:10.1016/j.freeradbiomed.2005.06.022.

[31] Beltran-Hortelano I, Perez-Silanes S, Galiano S. Trypanothione Reductase and Superoxide Dismutase as Current Drug Targets for Trypanosoma cruzi: An Overview of Compounds with Activity against Chagas Disease. Curr Med Chem 2017; 24: 1066-1138. DOI:10.2174/0929867324666161227094049.

[32] Meshnick SR, Trang NL, Kitchener K, et al. Iron-containing superoxide dismutase in trypanosomatids. In: *Oxy radicals and their scavenger systems: molecular aspects*. Elsevier New York, 1983, pp. 348-352.

[33] Bécuwe P, Gratepanche S, Fourmaux M-N, et al. Characterization of iron-dependent endogenous superoxide dismutase of Plasmodium falciparum. Mol Biochem Parasitol 1996; 76: 125-134. DOI:https://doi.org/10.1016/0166-6851(95)02552-9.

[34] Fontecave M, Gräslund A, Reichard P. The function of superoxide dismutase during the enzymatic formation of the free radical of ribonucleotide reductase. J Biol Chem 1987; 262: 12332-12336. DOI:https://doi.org/10.1016/S0021-9258(18)45357-4.

[35] Mateo H, Marín C, Pérez-Cordón G, et al. Purification and biochemical characterization of four iron superoxide dismutases in Trypanosoma cruzi. Mem Inst Oswaldo Cruz 2008; 103: 271-276. DOI:10.1590/S0074-02762008000300008.

[36] Ghosh S, Goswami S, Adhya S. Role of superoxide dismutase in survival of Leishmania within the macrophage. Biochem J 2003; 369: 447-452. DOI:10.1042/BJ20021684.

[37] Mittra B, Laranjeira-Silva MF, Miguel DC, et al. The iron-dependent mitochondrial superoxide dismutase SODA promotes Leishmania virulence. J Biol Chem 2017; 292: 12324-12338. DOI:10.1074/jbc.M116.772624.

[38] Plewes KA, Barr SD, Gedamu L. Iron superoxide dismutases targeted to the glycosomes of Leishmania chagasi are important for survival. Infect Immun 2003; 71: 5910-5920. DOI:10.1128/IAI.71.10.5910-5920.2003.

[39] Getachew F, Gedamu L. Molecular & Biochemical Parasitology Leishmania donovani mitochondrial iron superoxide dismutase A is released into the cytosol during miltefosine induced programmed cell death. Mol Biochem Parasitol 2012; 183: 42-51. DOI:10.1016/j.molbiopara.2012.01.005.

[40] Dive D, Gratepanche S, Yera H, et al. Communications in Free Radical Research Superoxide dismutase in Plasmodium : a current survey Short refereed paper Superoxide dismutase in Plasmodium : a current survey. 0002. Epub ahead of print 2013. DOI: 10.1179/135100003225002871. DOI:10.1179/135100003225002871.

[41] Sienkiewicz N, Daher W, Dive D, et al. Identification of a mitochondrial superoxide dismutase with an unusual targeting sequence in Plasmodium falciparum. Mol Biochem Parasitol 2004; 137: 121-132. DOI:10.1016/j.molbiopara.2004.05.005.

[42] Dive D, Gratepanche S, Yera H, et al. Superoxide dismutase in Plasmodium: a current survey. Redox Rep 2003; 8: 265-267. DOI:10.1179/13510000322 5002871.

[43] Foth BJ, Ralph SA, Tonkin CJ, et al. Dissecting apicoplast targeting in the malaria parasite Plasmodium falciparum. Science 2003; 299: 705-708. DOI:10.1126/ science.1078599.

[44] Chen Q-W, Dong K, Qin H-X, et al. Direct and Indirect Inhibition Effects of Resveratrol against Toxoplasma gondii Tachyzoites In Vitro. *Antimicrob Agents Chemother*; 63. Epub ahead of print March 2019. DOI: 10.1128/AAC.01233-18. DOI:10.1128/AAC.01233-18.

[45] Ding M, Kwok LY, Schlüter D, et al. The antioxidant systems in Toxoplasma gondii and the role of cytosolic catalase in defence against oxidative injury. Mol Microbiol 2004; 51: 47-61. DOI:10.1046/ j.1365-2958.2003.03823.x.

[46] Liu Y, Cao A, Li Y, et al. Immunization with a DNA vaccine encoding Toxoplasma gondii Superoxide dismutase (TgSOD) induces partial immune protection against acute toxoplasmosis in BALB/c mice. BMC Infect Dis 2017; 17: 403. DOI:10.1186/ s12879-017-2507-5.

[47] Trivedi A, Singh N, Bhat SA, et al. Chapter 4 - Redox Biology of Tuberculosis Pathogenesis. In: Poole RK (ed). Academic Press, pp. 263-324. DOI:https://doi.org/10.1016/ B978-0-12-398264-3.00004-8.

[48] Harth G, Horwitz MA. Export of recombinant Mycobacterium tuberculosis superoxide dismutase is dependent upon both information in the protein and mycobacterial export machinery: A model for studying export of leaderless proteins by pathogenic mycobacteria. J Biol Chem 1999; 274: 4281-4292. DOI:10.1074/jbc.274.7.4281.

[49] De Groote MA, Ochsner UA, Shiloh MU, et al. Periplasmic superoxide dismutase protects Salmonella from products of phagocyte NADPH-oxidase and nitric oxide synthase. Proc Natl Acad Sci U S A 1997; 94: 13997-14001. DOI:10.1073/pnas.94.25.13997.

[50] Wang Y, Liu B, Zhang J, et al. Infection with sodA mutant of S. Typhimurium leads to up-regulation of autophagy in Raw264·7 macrophages. Lett Appl Microbiol 2019; 69: 11-15. DOI:10.1111/lam.13164.

[51] Wang Y, Branicky R, Noë A, et al. Superoxide dismutases: Dual roles in controlling ROS damage and regulating ROS signaling. J Cell Biol 2018; 217: 1915-1928. DOI:10.1083/jcb.201708007.

[52] Fang FC, Degroote MA, Foster JW, et al. Virulent Salmonella typhimurium has two periplasmic Cu, Zn-superoxide dismutases. Proc Natl Acad Sci U S A 1999; 96: 7502-7507. DOI:10.1073/ pnas.96.13.7502.

[53] Miller RA, Britigan BE. Role of oxidants in microbial pathophysiology. Clin Microbiol Rev 1997; 10: 1-18. DOI:10.1128/CMR.10.1.1.

[54] Halliwell B, Gutteridge JM. Lipid peroxidation in brain homogenates: the role of iron and hydroxyl radicals. Journal of neurochemistry 1997; 69: 1330-1331. DOI:10.1046/j.1471-4159.1997.69031330.x.

[55] Sadosky AB, Wilson JW, Steinman HM, et al. The iron superoxide dismutase of Legionella pneumophila is essential for viability. J Bacteriol 1994;

176: 3790-3799. DOI:10.1128/jb.176.12.3790-3799.1994.

[56] Bakshi CS, Malik M, Regan K, et al. Superoxide dismutase B gene (sodB)-deficient mutants of Francisella tularensis demonstrate hypersensitivity to oxidative stress and attenuated virulence. J Bacteriol 2006; 188: 6443-6448. DOI:10.1128/JB.00266-06.

[57] Karavolos MH, Horsburgh MJ, Ingham E, et al. Role and regulation of the superoxide dismutases of Staphylococcus aureus. Microbiology 2003; 149: 2749-2758. DOI:10.1099/mic.0.26353-0.

[58] Valderas MW, Gatson JW, Wreyford N, et al. The Superoxide Dismutase Gene *sodM* Is Unique to *Staphylococcus aureus*: Absence of *sodM* in Coagulase-Negative Staphylococci. J Bacteriol 2002; 184: 2465-2472. DOI:10.1128/JB.184.9.2465-2472.2002.

[59] Garcia YM, Barwinska-Sendra A, Tarrant E, et al. A Superoxide Dismutase Capable of Functioning with Iron or Manganese Promotes the Resistance of Staphylococcus aureus to Calprotectin and Nutritional Immunity. PLoS Pathog 2017; 13: 1-19. DOI:10.1371/journal.ppat.1006125.

[60] Cox GM, Harrison TS, McDade HC, et al. Superoxide dismutase influences the virulence of Cryptococcus neoformans by affecting growth within macrophages. Infect Immun 2003; 71: 173-180. DOI:10.1128/IAI.71.1.173-180.2003.

[61] Narasipura SD, Chaturvedi V, Chaturvedi S. Characterization of Cryptococcus neoformans variety gattii SOD2 reveals distinct roles of the two superoxide dismutases in fungal biology and virulence. Mol Microbiol 2005; 55: 1782-1800. DOI:10.1111/j.1365-2958.2005.04503.x.

[62] Kozel TR, Goldman DL, Lee SC, et al. Persistent *Cryptococcus neoformans*Pulmonary Infection in the Rat Is Associated with Intracellular Parasitism, Decreased Inducible Nitric Oxide Synthase Expression, and Altered Antibody Responsiveness to Cryptococcal Polysaccharide. Infect Immun 2000; 68: 832-838. DOI:10.1128/IAI.68.2.832-838.2000.

[63] Chaturvedi S, Hamilton AJ, Hobby P, et al. Molecular cloning, phylogenetic analysis and three-dimensional modeling of Cu,Zn superoxide dismutase (CnSOD1) from three varieties of *Cryptococcus neoformans*. Gene 2001; 268: 41-51. DOI:https://doi.org/10.1016/S0378-1119(01)00408-5.

[64] Youseff BH, Holbrook ED, Smolnycki KA, et al. Extracellular Superoxide Dismutase Protects Histoplasma Yeast Cells from Host-Derived Oxidative Stress. 8. Epub ahead of print 2012. DOI: 10.1371/journal.ppat.1002713. DOI:10.1371/journal.ppat.1002713.

[65] Bern C, Montgomery SP, Herwaldt BL, et al. Evaluation and treatment of chagas disease in the United States: A systematic review. J Am Med Assoc 2007; 298: 2171-2181. DOI:10.1001/jama.298.18.2171.

[66] Kabiri M, Steverding D. Identification of a developmentally regulated iron superoxide dismutase of Trypanosoma brucei. Biochem J 2001; 360: 173-177. DOI:10.1042/0264-6021:3600173.

[67] Martínez A, Prolo C, Estrada D, et al. Cytosolic Fe-superoxide dismutase safeguards Trypanosoma cruzi from

macrophage-derived superoxide radical. Proc Natl Acad Sci U S A 2019; 116: 8879-8888. DOI:10.1073/pnas.1821487116.

[68] Prolo C, Estrada D, Piacenza L, et al. NOX2-derived superoxide radical is crucial to control acute Trypanosoma cruzi infection. Redox Biology 2021; 46: 102085. DOI:10.1016/j.redox.2021.102085.

[69] Campos BLS, Silva TN, Ribeiro SP, et al. Analysis of iron superoxide dismutase-encoding DNA vaccine on the evolution of the Leishmania amazonensis experimental infection. Parasite Immunol 2015; 37: 407-416. DOI:10.1111/pim.12206.

[70] Dhiman M, Garg NJ. P47phox−/− Mice Are Compromised in Expansion and Activation of CD8+ T Cells and Susceptible to Trypanosoma cruzi Infection. *PLoS Pathog*; 10. Epub ahead of print 2014. DOI: 10.1371/journal.ppat.1004516. DOI:10.1371/journal.ppat.1004516.

[71] Cooper A, Tait A, Sweeney L, et al. Genetic analysis of the human infective trypanosome Trypanosoma brucei gambiense: Chromosomal segregation, crossing over, and the construction of a genetic map. *Genome Biol*; 9. Epub ahead of print 2008. DOI: 10.1186/gb-2008-9-6-r103. DOI:10.1186/gb-2008-9-6-r103.

[72] Paramchuk WJ, Ismail SO, Bhatia A, et al. Cloning, characterization and overexpression of two iron superoxide dismutase cDNAs from Leishmania chagasi: role in pathogenesis. Mol Biochem Parasitol 1997; 90: 203-221. DOI:10.1016/s0166-6851(97)00141-2.

[73] Nogueira FB, Krieger MA, Nirdé P, et al. Increased expression of iron-containing superoxide dismutase-A (TcFeSOD-A) enzyme in Trypanosoma cruzi population with in vitro-induced resistance to benznidazole. Acta Trop 2006; 100: 119-132. DOI:10.1016/j.actatropica.2006.10.004.

[74] He S, Dayton A, Kuppusamy P, et al. Induction of oxidative stress in Trypanosoma brucei by the antitrypanosomal dihydroquinoline OSU-40. Antimicrob Agents Chemother 2012; 56: 2428-2434. DOI:10.1128/AAC.06386-11.

[75] Getachew F, Gedamu L. Leishmania donovani iron superoxide dismutase A is targeted to the mitochondria by its N-terminal positively charged amino acids. Mol Biochem Parasitol 2007; 154: 62-69. DOI:10.1016/j.molbiopara.2007.04.007.

[76] Davenport BJ, Martin CG, Beverley SM, et al. SODB1 is essential for Leishmania major infection of macrophages and pathogenesis in mice. PLoS Negl Trop Dis 2018; 12: 1-20. DOI:10.1371/journal.pntd.0006921.

[77] Matrangolo FSV, Liarte DB, Andrade LC, et al. Comparative proteomic analysis of antimony-resistant and-susceptible Leishmania braziliensis and Leishmania infantum chagasi lines. Mol Biochem Parasitol 2013; 190: 63-75. DOI:10.1016/j.molbiopara.2013.06.006.

[78] Tessarollo NG, Andrade JM, Moreira DS, et al. Parasitology International Functional analysis of iron superoxide dismutase-A in wild-type and antimony-resistant Leishmania braziliensis and Leishmania infantum lines. Parasitol Int 2015; 64: 125-129. DOI:10.1016/j.parint.2014.11.001.

[79] Mishra J, Singh S. Miltefosine resistance in Leishmania donovani involves suppression of oxidative stress-induced programmed cell death. Exp Parasitol 2013; 135: 397-406. DOI:10.1016/j.exppara.2013.08.004.

[80] Veronica J, Chandrasekaran S, Dayakar A, et al. Iron superoxide dismutase contributes to miltefosine resistance in Leishmania donovani. 2019; 286: 3488-3503. DOI:10.1111/febs.14923.

[81] Bécuwe P, Slomianny C, Camus D, et al. Presence of an endogenous superoxide dismutase activity in three rodent malaria species. Parasitol Res 1993; 79: 349-352. DOI:10.1007/BF00931821.

[82] Breidbach T, Krauth-Siegel RL, Steverding D. Ribonucleotide reductase is regulated via the R2 subunit during the life cycle of Trypanosoma brucei. FEBS Lett 2000; 473: 212-216. DOI:10.1016/S0014-5793(00)01533-7.

[83] Gratepanche S, Ménage S, Touati D, et al. Biochemical and electron paramagnetic resonance study of the iron superoxide dismutase from Plasmodium falciparum. Mol Biochem Parasitol 2002; 120: 237-246. DOI:10.1016/s0166-6851(02)00004-x.

[84] Fairfield AS, Meshnick SR, Eaton JW. Malaria parasites adopt host cell superoxide dismutase. Science 1983; 221: 764-766. DOI:10.1126/science.6348944.

[85] Prakash K, Goyal M, Soni A, et al. Molecular cloning and biochemical characterization of iron superoxide dismutase from the rodent malaria parasite Plasmodium vinckei. Parasitol Int 2014; 63: 817-825. DOI:10.1016/j.parint.2014.07.004.

[86] Müller S. MicroReview Redox and antioxidant systems of the malaria parasite Plasmodium falciparum. 2004; 53: 1291-1305. DOI:10.1111/j.1365-2958.2004.04257.x.

[87] Soulère L, Delplace P, Davioud-Charvet E, et al. Screening of Plasmodium falciparum iron superoxide dismutase inhibitors and accuracy of the SOD-assays. Bioorg Med Chem 2003; 11: 4941-4944. DOI:10.1016/j.bmc.2003.09.011.

[88] Lima TS, Lodoen MB. Mechanisms of human innate immune evasion by Toxoplasma gondii. Front Cell Infect Microbiol 2019; 9: 1-8. DOI:10.3389/fcimb.2019.00103.

[89] Montoya JG, Liesenfeld O. Toxoplasmosis. Lancet 2004; 363: 1965-1976. DOI:https://doi.org/10.1016/S0140-6736(04)16412-X.

[90] Harker KS, Ueno N, Lodoen MB. Toxoplasma gondii dissemination: a parasite's journey through the infected host. Parasite Immunol 2015; 37: 141-149. DOI:10.1111/pim.12163.

[91] Wilson CB, Tsai V, Remington JS. Failure to trigger the oxidative metabolic burst by normal macrophages: possible mechanism for survival of intracellular pathogens. J Exp Med 1980; 151: 328-346. DOI:10.1084/jem.151.2.328.

[92] Zhou W, Quan J-H, Lee Y-H, et al. Toxoplasma gondii Proliferation Require Down-Regulation of Host Nox4 Expression via Activation of PI3 Kinase/Akt Signaling Pathway. PLoS One 2013; 8: e66306. DOI:10.1371/journal.pone.0066306.

[93] Sibley LD, Lawson R, Weidner E. Superoxide dismutase and catalase in Toxoplasma gondii. Mol Biochem Parasitol 1986; 19: 83-87. DOI:10.1016/0166-6851(86)90069-1.

[94] Odberg-Ferragut C, Renault JP, Viscogliosi E, et al. Molecular cloning, expression analysis and iron metal cofactor characterisation of a superoxide dismutase from Toxoplasma gondii. Mol Biochem Parasitol 2000; 106: 121-129. DOI:10.1016/s0166-6851(99)00211-x.

[95] Bosch SS, Kronenberger T, Meissner KA, et al. Oxidative stress control by apicomplexan parasites. Biomed Res Int 2015; 2015: 351289. DOI:10.1155/2015/351289.

[96] Meng M, He S, Zhao G, et al. Evaluation of protective immune responses induced by DNA vaccines encoding Toxoplasma gondii surface antigen 1 (SAG1) and 14-3-3 protein in BALB/c mice. Parasit Vectors 2012; 5: 273. DOI:10.1186/1756-3305-5-273.

[97] Dabir S, Dabir P, Goswamy K, et al. Prophylactic evaluation of recombinant extracellular superoxide dismutase of Brugia malayi in jird model. Vaccine 2008; 26: 3705-3710. DOI:https://doi.org/10.1016/j.vaccine.2008.04.061.

[98] Fang FC. Antimicrobial reactive oxygen and nitrogen species: concepts and controversies. Nat Rev Microbiol 2004; 2: 820-832. DOI:10.1038/nrmicro1004.

[99] Lindgren H, Stenman L, Tärnvik A, et al. The contribution of reactive nitrogen and oxygen species to the killing of Francisella tularensis LVS by murine macrophages. Microbes Infect 2005; 7: 467-475. DOI:https://doi.org/10.1016/j.micinf.2004.11.020.

[100] Vazquez-Torres A, Fang FC. Oxygen-dependent anti-Salmonella activity of macrophages. Trends Microbiol 2001; 9: 29-33. DOI:https://doi.org/10.1016/S0966-842X(00)01897-7.

[101] Battistoni A. Role of prokaryotic Cu,Zn superoxide dismutase in pathogenesis. Biochem Soc Trans 2003; 31: 1326-1329. DOI:10.1042/bst0311326.

[102] Koch A, Mizrahi V. Mycobacterium tuberculosis. Trends Microbiol 2018; 26: 555-556. DOI:10.1016/j.tim.2018.02.012.

[103] Zhang Y, Lathigra R, Garbe T, et al. Genetic analysis of superoxide dismutase, the 23 kilodalton antigen of Mycobacterium tuberculosis. Mol Microbiol 1991; 5: 381-391. DOI:10.1111/j.1365-2958.1991.tb02120.x.

[104] Liao D, Fan Q, Bao L. The role of superoxide dismutase in the survival of Mycobacterium tuberculosis in macrophages. Jpn J Infect Dis 2013; 66: 480-488. DOI:10.7883/yoken.66.480.

[105] Chavarro-Portillo B, Soto CY, Guerrero MI. Mycobacterium leprae's evolution and environmental adaptation. Acta Trop 2019; 197: 105041. DOI:10.1016/j.actatropica.2019.105041.

[106] Holzer TJ, Nelson KE, Schauf V, et al. Mycobacterium leprae fails to stimulate phagocytic cell superoxide anion generation. Infect Immun 1986; 51: 514-520. DOI:10.1128/iai.51.2.514-520.1986.

[107] Wheeler PR, Gregory D. Superoxide dismutase, peroxidatic activity and catalase in Mycobacterium leprae purified from armadillo liver. J Gen Microbiol 1980; 121: 457-464. DOI:10.1099/00221287-121-2-457.

[108] Leon-Sicairos N, Reyes-Cortes R, Guadrón-Llanos AM, et al. Strategies of intracellular pathogens for obtaining iron from the environment. *Biomed Res Int*; 2015. Epub ahead of print 2015. DOI:10.1155/2015/476534. DOI:10.1155/2015/476534.

[109] Pacello F, Ceci P, Ammendola S, et al. Periplasmic Cu,Zn superoxide dismutase and cytoplasmic Dps concur in protecting *Salmonella enterica* serovar Typhimurium from extracellular reactive oxygen species. *Biochim Biophys Acta* 2008; 1780: 226-232. DOI:10.1016/j.bbagen.2007.12.001.

[110] Heindorf M, Kadari M, Heider C, et al. Impact of Acinetobacter baumannii superoxide dismutase on motility, virulence, oxidative stress resistance and susceptibility to antibiotics. PLoS One 2014; 9: e101033. DOI:10.1371/journal.pone.0101033.

[111] Herman A, Serfecz J, Kinnally A, et al. The Bacterial iprA Gene Is Conserved across Enterobacteriaceae, Is Involved in Oxidative Stress Resistance, and Influences Gene Expression in Salmonella enterica Serovar Typhimurium. J Bacteriol 2016; 198: 2166-2179. DOI:10.1128/JB.00144-16.

[112] Jolla L. Periplasmic superoxide dismutase protects. Microbiology 1997; 94: 13997-14001.

[113] Lauriano CM, Barker JR, Yoon S-S, et al. MglA regulates transcription of virulence factors necessary for *Francisella tularensis* intraamoebae and intramacrophage survival. *Proc Natl Acad Sci* 2004; 101: 4246 LP – 4249. DOI:10.1073/pnas.0307690101.

[114] McCaffrey RL, Allen L-AH. Francisella tularensis LVS evades killing by human neutrophils via inhibition of the respiratory burst and phagosome escape. J Leukoc Biol 2006; 80: 1224-1230. DOI:10.1189/jlb.0406287.

[115] Forestal CA, Benach JL, Carbonara C, et al. *Francisella tularensis* Selectively Induces Proinflammatory Changes in Endothelial Cells. *J Immunol* 2003; 171: 2563 LP – 2570. DOI:10.4049/jimmunol.171.5.2563.

[116] Shimaniuk NI, Pavlovich N V, Mishan'kin BN. [Superoxide dismutase activity in representatives of the genus Francisella]. Zhurnal Mikrobiol Epidemiol i Immunobiol 1992; 7-9.

[117] Larsson P, Oyston PCF, Chain P, et al. The complete genome sequence of Francisella tularensis, the causative agent of tularemia. Nat Genet 2005; 37: 153-159. DOI:10.1038/ng1499.

[118] Sendi P, Proctor RA. Staphylococcus aureus as an intracellular pathogen: the role of small colony variants. Trends Microbiol 2009; 17: 54-58. DOI:https://doi.org/10.1016/j.tim.2008.11.004.

[119] Melillo AA, Mahawar M, Sellati TJ, et al. Identification of Francisella tularensis live vaccine strain CuZn superoxide dismutase as critical for resistance to extracellularly generated reactive oxygen species. J Bacteriol 2009; 191: 6447-6456. DOI:10.1128/JB.00534-09.

[120] Bravo-Santano N, Ellis JK, Mateos LM, et al. Intracellular *Staphylococcus aureus* Modulates Host Central Carbon Metabolism To Activate Autophagy. *mSphere* 2018; 3: e00374-e00318. DOI:10.1128/mSphere.00374-18.

[121] Klevens RM, Morrison MA, Nadle J, et al. Invasive Methicillin-Resistant Staphylococcus aureus Infections in the United States. JAMA 2007; 298: 1763-1771. DOI:10.1001/jama.298.15.1763.

[122] Nakonieczna J, Michta E, Rybicka M, et al. Superoxide dismutase is upregulated in Staphylococcus aureus following protoporphyrin-mediated photodynamic inactivation and does not directly influence the response to photodynamic treatment. BMC Microbiol 2010; 10: 323. DOI:10.1186/1471-2180-10-323.

[123] Lowy FD. Intracellular Pathogen ? In Vitro 2000; 341-343.

[124] Lynch M, Kuramitsu H. Expression and role of superoxide dismutases (SOD) in pathogenic bacteria. Microbes Infect 2000; 2: 1245-1255. DOI:10.1016/s1286-4579(00)01278-8.

[125] Imlay JA. Pathways of oxidative damage. Annu Rev Microbiol 2003; 57: 395-418. DOI:10.1146/annurev.micro.57.030502.090938.

[126] Valderas MW, Hart ME. Identification and characterization of a second superoxide dismutase gene (sodM) from Staphylococcus aureus. J Bacteriol 2001; 183: 3399-3407. DOI:10.1128/JB.183.11.3399-3407.2001.

[127] Kehl-Fie TE, Chitayat S, Hood MI, et al. Nutrient Metal Sequestration by Calprotectin Inhibits Bacterial Superoxide Defense, Enhancing Neutrophil Killing of Staphylococcus aureus. Cell Host Microbe 2011; 10: 158-164. DOI:https://doi.org/10.1016/j.chom.2011.07.004.

[128] Clements MO, Watson SP, Foster SJ. Characterization of the major superoxide dismutase of Staphylococcus aureus and its role in starvation survival, stress resistance, and pathogenicity. J Bacteriol 1999; 181: 3898-3903. DOI:10.1128/JB.181.13.3898-3903.1999.

[129] Kehl-Fie TE, Zhang Y, Moore JL, et al. MntABC and MntH Contribute to Systemic Staphylococcus aureus Infection by Competing with Calprotectin for Nutrient Manganese. Infect Immun 2013; 81: 3395-3405. DOI:10.1128/IAI.00420-13.

[130] Damo SM, Kehl-Fie TE, Sugitani N, et al. Molecular basis for manganese sequestration by calprotectin and roles in the innate immune response to invading bacterial pathogens. Proc Natl Acad Sci U S A 2013; 110: 3841-3846. DOI:10.1073/pnas.1220341110.

[131] Seetharaman S V, Winkler DD, Taylor AB, et al. NIH Public Access. 2011; 49: 5714-5725. DOI:10.1021/bi100314n. Disrupted.

[132] Fisher CL, Cabelli DE, Hallewell RA, et al. Investigations of Lysine-136 and Its Role in the Electrostatic Triad of Human Cu , Zn Superoxide Dismutase. 1997; 112: 103-112.

[133] Hayward LJ, Rodriguez JA, Kim JW, et al. Decreased Metallation and Activity in Subsets of Mutant Superoxide Dismutases Associated with Familial Amyotrophic Lateral Sclerosis. J Biol Chem 2002; 277: 15923-15931. DOI:10.1074/jbc.M112087200.

[134] Fradin C, Groot P De, Maccallum D, et al. Granulocytes govern the transcriptional response , morphology and proliferation of *Candida albicans* in human blood. 2005; 56: 397-415. DOI:10.1111/j.1365-2958.2005.04557.x.

[135] Richard ML. MINIREVIEW Comprehensive Analysis of Glycosylphosphatidylinositol-Anchored Proteins in *Candida albicans*. 2007; 6: 119-133. DOI:10.1128/EC.00297-06.

[136] Feldmesser M, Tucker S, Casadevall A. Intracellular parasitism of macrophages by Cryptococcus neoformans. Trends Microbiol 2001; 9: 273-278. DOI:10.1016/s0966-842x(01)02035-2.

[137] Feldmesser M, Kress Y, Novikoff P, et al. Cryptococcus neoformans is a facultative intracellular pathogen in murine pulmonary infection. Infect Immun 2000; 68: 4225-4237. DOI:10.1128/IAI.68.7.4225-4237.2000.

[138] Mitchell TG, Perfect JR. Cryptococcosis in the era of AIDS--100 years after the discovery of Cryptococcus

neoformans. Clin Microbiol Rev 1995; 8: 515-548. DOI:10.1128/CMR.8.4.515.

[139] Steenbergen JN, Shuman HA, Casadevall A. *Cryptococcus neoformans* interactions with amoebae suggest an explanation for its virulence and intracellular pathogenic strategy in macrophages. *Proc Natl Acad Sci* 2001; 98: 15245 LP – 15250. DOI:10.1073/pnas.261418798.

[140] Garcia-Hermoso D, Janbon G, Dromer F. Epidemiological Evidence for Dormant *Cryptococcus neoformans* Infection. J Clin Microbiol 1999; 37: 3204-3209. DOI:10.1128/JCM.37.10.3204-3209.1999.

[141] da Silva EG, Baroni F de A, Viani FC, et al. Virulence profile of strains of Cryptococcus neoformans var. grubii evaluated by experimental infection in BALB/c mice and correlation with exoenzyme activity. J Med Microbiol 2006; 55: 139-142. DOI:10.1099/jmm.0.46206-0.

[142] Chaturvedi V, Wong B, Newman SL. Oxidative killing of *Cryptococcus neoformans* by human neutrophils. Evidence that fungal mannitol protects by scavenging reactive oxygen intermediates. *J Immunol* 1996; 156: 3836 LP – 3840.

[143] Trinh J V, Steinbach WJ, Schell WA, et al. Cerebral phaeohyphomycosis in an immunodeficient child treated medically with combination antifungal therapy. Med Mycol 2003; 41: 339-345. DOI: 10.1080/369378031000137369.

[144] Narasipura SD, Ren P, Dyavaiah M, et al. An efficient method for homologous gene reconstitution in Cryptococcus gattii using URA5 auxotrophic marker. Mycopathologia 2006; 162: 401-409. DOI:10.1007/s11046-006-0076-z.

Chapter 4

Superoxide Dismutase in Psychiatric Diseases

Vladimir Djordjević

Abstract

As with many other human diseases, oxidative stress is implicated in many neuropsychiatric disorders, including schizophrenia, bipolar disorder, depression and Alzheimer's disease. Due to high oxygen consumption and a lipid-rich environment, the brain is highly susceptible to oxidative stress or redox imbalance. Both increased production of reactive oxygen species and antioxidant defense disorders have been demonstrated in psychiatric patients. Superoxide dismutase (SOD) is the primary, critical enzyme in the detoxification of superoxide radicals, because they are the main ROS, primarily generated in the most biological reactions of free radical formation. There are inconsistent data on this enzyme activity in patients with different psychoses. Since psychotic disorders are complex and heterogeneous disorders, it is not surprising that different authors have found that SOD activity is increased, decreased, or unchanged in the same type of psychosis. This review examines and discusses some recent findings linking SOD activity to schizophrenia, bipolar disorder, depression and Alzheimer's disease.

Keywords: superoxide dismutase, schizophrenia, bipolar disorder, depression, Alzheimer's disease

1. Introduction

More than 90% of molecular oxygen (which is essential for aerobic lifestyle) intaken in the human body is reduced into water by receiving four electrons from the electron-transport system in the respiratory chain of mitochondria. A small amount of oxygen is incorporated in biological substrates, and rest of oxygen is transformed into reactive oxygen species (ROS) that include potentially toxic oxygen free radicals [1] and very reactive non-radical species. The reduction of oxygen by one electron at a time produces superoxide anion radical ($O_2^{\cdot -}$), the precursor of most ROS and a mediator in oxidative chain reactions. Superoxide is then dismutated either spontaneously or by superoxide dismutase into hydrogen peroxide (H_2O_2). H_2O_2 can be fully reduced to water or partially reduced (in a reaction catalyzed by reduced transition metals) to hydroxyl radical ($OH^{\cdot}$) which is one of the strongest oxidants in nature. Except the respiratory chain of mitochondria, enzymatic sources of superoxide production include phagocyte NADPH (nicotinamide adenine dinucleotide phosphate) oxidase, cytochrome P_{450}-dependent oxygenases and xanthine oxidase (XO). Non-enzymatic production occurs when a single electron is directly transferred to oxygen by reduced

coenzymes or prosthetic groups (flavins or iron sulfur clusters), by xenobiotics previously reduced by certain enzymes (adriamycin or paraquat), or by mitochondrial redox centres that may leak electrons to oxygen [2].

Basal cellular metabolism continuously produces ROS that occurs in endogenous sources such as mitochondria, peroxisomes, cytochrome $P_{450,}$ inflammatory cell activation and other cellular elements [3], but *in vivo* the mainly ROS production occurs within the mitochondria [4]. In physiological conditions, when the redox status is balanced, ROS are produced in appropriate levels because they are necessary and beneficial for normal physiological functions: they can protect the cell from infections [5, 6]; they play a role in the regulation of cardiac and vascular cell functioning [6]; they regulate intracellular processes such as calcium concentration, protein phosphorylation/dephosphorylation and transcription factor activation. ROS directly interact with critical signaling molecules to initiate signaling in a broad variety of cellular processes, such as proliferation and survival (MAP kinases and PI3 kinase), apoptosis, ROS homeostasis, and antioxidant gene regulation (Ref-1 and Nrf-2) [7].

Maintenance of ROS at the physiological level is enabled by the antioxidant system which consists of antioxidative enzymes (superoxide dismutase, peroxidase, catalase, glutathione reductase and thioredoxin) and non-enzymatic antioxidants including reduced glutathione (GSH), vitamins (A, C, E), thiols, zinc, selenium, uric acid, albumin, bilirubin, N-acetylcysteine, and melatonin). Any disturbance of the balance between radical production and antioxidant defense (the overproduction of ROS and/or insufficiency of the antioxidant defense mechanisms) [8, 9], leads to oxidative stress and the manifestation of toxic effects of reactive species. The brain is especially sensitive to oxidative damage because it has high capacity to consume large amounts of oxygen (more than 20% of totally inhaled oxygen) that directly enhances the production of free radicals [5]; it has scarce antioxidant system; low expression of SOD, GPx and catalase; a significantly lower concentration of reduced GSH in comparison with other tisses; in some regions, the brain contains high concentrations of vitamin C and metals (e.g. iron, zinc, copper and manganese) which makes favorable conditions for the production of free radicals through the Fenton reaction; it is rich in polyunsaturated fatty acids that make it susceptible to oxidative attack. This situation is exacerbated by many factors including oxidative potential of monoamines, secondary oxidative cell damage induced by neurotoxic effects of excitotoxic amino acids (glutamate), and secondary inflammatory response. Due to the inability of neurons to produce glutathione which plays the main role in the protection of neuronal tissue from ROS [10], and in the modulation of redox-sensitive sites including NMDA receptors [11], the brain has the limited capacity to scavenge ROS. Besides, neurons are the first cells that can be affected if the concentration of ROS enhances or the concentration of antioxidants declines. Increasing body of evidence shows that partially reduced oxygen species are involved in the pathogenesis of more than hundred human diseases including psychiatric diseases such as schizophrenia, bipolar disorder, depression and Alzheimer's disease.

2. Superoxide dismutase

Superoxide dismutase (SOD; EC 1.15.1.1) is an enzyme that catalyzes the dismutation of the toxic superoxide radical, into either molecular oxygen or hydrogen peroxide, thus preventing peroxynitrite production and further damage [12].

Superoxide anion radical ($O_2^{\cdot -}$) can be formed by one-electron reduction of molecular oxygen or by one-electron oxidation of hydrogen peroxide. It is highly effective in the inactivation of some enzymes, but it cannot directly oxidize unsaturated faty acids. In biological systems, it is generated accedentally via the electron transport systems in either the endoplasmic reticulum or mitochondria via electron leakage from intermediate electron carriers onto oxygen; via autooxidation of redox-active chemicals [13]; via glycation of proteins [14]; and via thiol oxidation. By various mechanisms superoxide is generated by oxidases, in particular, xanthine oxidase and NADPH oxidase of the phagocytic cells. During phagocytosis, neutrophils produce 16 times more superoxide (4.7 nmol/10^6 cells per minute) than that produced in resting cells [15]. The superoxide produced in this manner allows phagocytes to kill the microorganisms in the invading host. Vascular endothelial cells, fibroblasts, lymphocytes and many other human cells release superoxide involved in intracellular signaling in physiological conditions. Xanthine oxidoreductase (XOR), which has a key role in purine catabolism, may exists in two forms, xanthine oxidase (XO) and xanthine dehydrogenase (XDH). The enzyme originally exists in its XDH form, but is readily converted to XO either irreversibly by proteolysis or reversibly by oxidation of Cys residues to form disulfide bridges [16, 17]. The reoxidation of fully reduced XO yields two H2O2 and two superoxide radicals [18], which may lead to the formation more toxic reactive species. However, low concentrations of superoxide and hydrogen peroxide are initially used by the cell for the mobilization of the antioxidative system.

Since superoxide is the primary ROS produced from a variety of sources, its dismutation by SOD is of primary importance for each cell. Three forms of superoxide dismutase are present in humans: a copper- and zinc-containing superoxide dismutase (CuZnSOD/SOD1) localized predominantly in cytoplasmic and nuclear compartments as well as peroxisomes of all mammalian cells [19], a manganese superoxide dismutase (MnSOD/SOD2) localized within the mitochondrial matrix, and a copper- and zinc containing SOD predominantly found in extracellular compartments (EC SOD/SOD3). CuZnSOD present in eukaryotic cell is found sensitive to cyanide and located in the form of dimer. It may be inactivated by hydrogen peroxide, leading to the generation of either Cu (II)-OH. or its ionized form Cu (II)-O.- [20]. This enzyme can further catalyze the peroxidation of a wide vaiety of compounds.

EC SOD is homotetrameric glycoprotein whose each subunit contains a copper and zinc atom, has a high affinity for heparin sulfate and presumably scavenges superoxide that is released from the cell surface. Besides EC SOD important role in the regulation of extracellular superoxide levels, it is also important as a modulator of NO activity. EC SOD is highly expressed in blood vessels constituting up to 70% of the SOD activity in both pulmonary and systemic arteries. Its expression is mainly regulated by cytokines which increase (IFN_γ) or decrease (TNFα and TGFß) EC SOD expression [21].

In eukaryotic cells MnSOD is a homotetramer located in the matrix of mitochondria. It is produced constitutively but can also be induced by cytokines (IL-1, TNF) or endotoxin [22]. In addition to cytokines, a wide range of reactive oxygen metabolites, both inducible and basal levels, may induce Mn SOD expression in distinct cell types [23] that may play a decisive role in the pathogenesis of tissue injury following oxidative stress. There are indices that transcriptional upregulation of MnSOD is mediated through the activation of nuclear transcriptional factor κB (NF- κB) by oxidants.

3. Superoxide dismutase and psychiatric diseases

3.1 Superoxide dismutase and schizophrenia

A number of studies noted increases in free radicals, alterations in antioxidant defense mechanism, increases in lipid peroxidation products and higher levels of pro-apoptotic markers in patients with neuropsychiatric disorders [24–27].

One of the critical scavenging enzymes that have been reported most commonly in schizophrenia is SOD. In 1986 Abdalla et al. [28] noted that both neuroleptic-treated and untreated schizophrenic patients showed about 60% higher SOD activity than those found in control individuals. Later, elevated SOD activity in chronic schizophrenic patients has been reported in a number of studies [29–32]. Djordjevic et al. [32] also showed that SOD activity was a significantly higher in patients who were younger than 34 years. These results showed that erythrocyte SOD activity is increased in the early phase of schizophrenia and may be induced in response to oxidant stress and that oxidant stress is a primary event and that SOD activity abnormalities are its consequence. That is recently confirmed by the results [33] which have shown that juvenile antioxidant treatment prevented adult deficits in a developmental model of schizophrenia. Higher conversion of superoxide might elevate hydrogen peroxide level which, in turn, could inactivate SOD [34] leading to the inhibition of the enzyme activity in the later stage of the disease. Djordjevic et al. [32] also showed that SOD activity depends on the onset of the disease, the number of psychotic episodes, the duration of the disease, and medical treatment. The authors suggested the use of antioxidants as the adjuvant therapy in the prodromal and early phase of schizophrenia. Activities of SOD and levels of malondialdehyde (MDA – commonly known as a marker of oxidative stress) in erythrocyte were significantly higher in patients with acute and chronic schizophrenia. Further, SOD activity positively correlated with scales and duration of disease while erythrocyte MDA concentration, GPx activity and GSH level were lower in patients than in controls [35]. In another study, activities of erythrocyte SOD, catalase and MDA were all greater in schizophrenics than in controls [36]. Wu et al. [31] found that both never-medicated first-episode and chronic patients had significantly increased plasma SOD activities compared to controls, and that chronic schizophrenic patients on antipsychotic medication had significantly higher SOD activities than first-episode schizophrenics. They also showed that SOD activity negatively correlated with positive symptoms of schizophrenia in first-episode patients. Never-treated first-episode patients had significantly higher MnSOD and total SOD activities than healthy controls, and bot enzymes showed positive correlation with depresivity and general psychopathology [37]. Increased SOD activity found in chronic medicated schizophrenic patients at baseline, decreased significantly after 45 days and 90 days of supplementation with alpha-lipoic acid [38]. Contrary to these results, decreased SOD activity has been observed in neuroleptic-naïve first-episode schizophreniform and schizophrenic patients [39]. In the study of Dadheech et al. [40] significantly lower SOD and GPx activities in schizophrenics were associated with high blood MDA levels. The condition worsened with advancing age, smoking, among literate masses and in chronic schizophrenics; whereas gender did not show any effect. However, age-dependent changes were demonstrated because it was noted a significant negative correlation between SOD activity and age [41]. A significant negative correlation was obtained between SOD activity and the duration of the schizophrenic disease [32]. In an animal model it was shown (Wistar rats) that mitochondrial SOD and GPx activities remaind unaltered between 12 and 24 months

of age, with no difference between two genders, while the gender and age-differences were observed in MnSOD expression [42]. Schizophrenic patients with tardive dyskinesia (TD) showed lower plasma MnSOD activity [43], CuZnSOD activity and total antioxidant status levels, but higher MDA levels than those without TD. An increase in CuZnSOD and MnSOD in frontal cortex and substantia innominata areas of schizophrenia subjects was also observed [44]. Significantly lower SOD and catalase activities were associated with decreased GSH levels in patients with schizophrenia [45]. Among the schizophrenic patients, antioxidative enzyme activities were significantly lower in untreated patients than in the treated ones. This finding suggest that the efficacy of neuroleptics may in part be mediated by promoting an endogenous antioxidative mechanism [46]. Significantly lower SOD and GPx activities [47] did not show any difference between the three patient subgroups treated with closapine, risperidone or typical antipsychotics. Contrary, 12-week treatment with risperidone significantly decreased the initially high blood SOD levels as well as symptoms in schizophrenia [48]. Significantly lower SOD and catalase activities were also found in schizophrenic patients and their unaffected siblings compared to the control group, while GPx activity was also lower in the patient group but it was significantly higher in their unaffected siblings than in controls [49]. Moreover, a significant increase in MDA level has been found associated with significantly decreased level of vitamin C and SOD activity [50]. Meta analysis for markers of oxidative stress showed that SOD activity was significantly decreased in the disorganized type of schizophrenia patients compared to healthy controls [51]. In attempt to explain the divergence of findings related to SOD in schizophrenia, some authors have studied SOD polymorphism. However, the investigation of the functional polymorphism (Ala-9Val) in the MnSOD gene did not show any significant difference in either genotype or allele frequency between the schizophrenic and control group, nor between the polymorphism and symptom severity [52]. Another study [53] found a decrease in -9Ala (mutant) alele among patients with tardive dyskinesia, suggesting that the -9Ala (high activity) MnSOD allele may play a role in protecting against susceptibility to tardive dyskinesia in patients with schizophrenia.

The above mentioned studies showed some inconsistent results related to SOD activity which might be a consequence of several possible reasons: schizophrenia is highly heterogeneous disease; redox dysfunction may be, at least in part, state dependent; tissue-specific changes may underlie the pathophysiology [54]. Finally, there are suggesstion that the decreased level of SOD activity may have a direct implication to the oxidative stress, and that the increased enzyme levels may reflect a compensatory effect or a preceding oxidative stress in the cell [55].

3.2 Superoxide dismutase and bipolar disorder (BD)

Bipolar disorder is a highly heritable mental disorder clinically presented as unusual shifts in mood, energy and cognitive levels. It is characterized by intermittent episodes of mania or hypomania, usually interlaced with depressive episodes and these symptoms may seriously damage relationships, job or school performance, and even cause suicide. Similarly to schizophrenia, there is an increase in the number of studies related to BD and oxidative stress.

Some evidences support the role of a subtle mitochondrial compromise in BD [56]. Post-mortem studies using brain tissue of BD patients demonstrated decreased expression of mitochondrial electron transport chain genes [57] and increased levels of oxidative stress parameters [58]. The second source of oxidative stress might be

hyperactivation of the glutamatergic and dopaminergic systems in BD. Glutamatergic hyperactivity leads to increased calcium influx which increases oxidative stress [59] which in turn increases glutamate [60]. The excessive dopamine production also increases oxidative stress due to the production of reactive oxygen species in dopamin metabolism [61]. Oxidative stress further induces dopamine uptake thus increasing dopamine activity in a vicious cycle. It is also showed that oxidative stress is associated with decreased γ-Aminobutyric acid release as well as with decreased serotonergic function [62].

Up now, evidence suggests elevated oxidative stress among BD patients. Several studies have reported that patients with BD have significant alterations in antioxidant enzymes, lipid peroxidation, and nitric oxide levels [63]. There is opinion that individuals who have bipolar disorder typically have lower levels of SOD and higher levels of oxidative stress as measured by nitrc oxide. After 30 day treatment SOD activity significantly increased but did not reach the control levels on the 30th day. Persistent low SOD activity might point out an oxidative imbalance in BD depressive patients and may be associated with incapacity of coping with oxidative stress [64]. However, previous studies have shown an increase in SOD activity in BD patients compared to controls [65, 66], but the phase of the disease was not taken into account in these studies. However, both SOD activity and NO level were found elevated in euthymic bipolar patients compared to controls, and NO levels significantly correlated with the total number of the manic episodes and the total number of any kind of mood episodes [67]. Increased antioxidant activity may reflect a preceding cellular oxidative stress or serve as a compensatory mechanism, and SOD may increase as a defense mechanism against increased NO levels in BD. Later, Gergerlioglu et al. [68] showed that patients in manic episode of bipolar disorder had significantly higher NO at first and 30th days after treatment, whilst at the same time SOD activities were lower than controls. At first day SOD activity was higher than at 30th day. SOD activity at 30th day is negatively correlated with the number of previous manic attacks. Contrary to these results, serum SOD activity was found significantly increased in manic and depressed BD patients as well as in schizophrenics when compared to either controls or euthymic ones. Oxidative products were also significantly higher in bipolar euthymic, bipolar manic, bipolar depressed patients and schizophrenics compared to control [69]. The results from meta-analysis recently published [70] showed a significant increase in SOD and a significant decrease in GPX in medication-free BD-mania patients compared to controls. After treatment there was no any differences in enzyme activities between patients and controls. Increased SOD+Catalase activity was observed in patients with a symptomatic affective phase of the disease but not in those with euthymia. Thus, the combination of SOD+catalase could be suggested as a state marker of affective disease, which is different in euthymia. BD patients also showed lower GSH levels and elevated oxidative stress markers. One of the reasons for the discrepancy of the results related to SOD in BD patients may be an involvement of patients in different phases of the disease. The second major drawback of the most studies was that the patients were using some drugs, such as mood stabilizers, antipsychotics and antidepressants, because it would not be appropriate to stop the treatment in these patients. Risperidone and olanzapine that are used for the treatment of schizophrenia and BD, have a secondary antioxidant benefit. In patients given these drugs SOD levels decreased [71], and levels of GSH and vitamines E and C significantly inreased [72]. Another study noted that risperidone, olanzapine and clozapine have no effect on SOD activity [73] while Li et al. [74] showed that olanzapine increases SOD activity. Later, de Sousa et al. [75] presented a reactive increase in

antioxidant enzyme levels during depressive episodes in early stage BD with minimal prior treatment. Patients with BD depression showed a significant increase in catalase and GPX and no changes in SOD activity and lipid peroxidation levels. Lithium only induced a decrease in lipid peroxides and SOD levels. Both, lithium and valproate have antioxidant and antiinflammatory effects thus protecting brain cells from dysfunction and apoptosis and enhancing brain-derived neurotrophic factor [70]. Some antidepressants, like amytriptillin and venlafaxine have no effect on SOD actvivity at therapeutic doses, while in higher doses they increase SOD activity.

3.3 Superoxide dismutase and depression

Depression is a complex and heterogeneous disorder that has a negative impact on quality of life, morbidity/mortality and cognitive function. Except several mechanisms involved in its pathogenesis oxidative stress has been proposed as a contributing factor in the pathogenesis of this disease [76]. Increased levels of ROS and altered levels of antioxidant defenses have been demonstrated [77]. Significantly lower plasma concentrations of several key antioxidants (vitamin E, Zn, Koenzyme Q) as well as lower antioxidant enzyme activity have been reported in major depression. Also, an association between depression and polymorphisms in genes including manganese superoxide dismutase and catalase has been demonstrated [78]. Lower SOD activity is accompanied by increased formation of hydrogen peroxides, which can generate other ROS including the very reactive hydroxyl or metal-associated radicals [79]. However, activity of antioxidant enzymes was found controversial in patients with depression. Oxidative stress and inflammation may enhance both SOD activity and its de novo synthesis especially in early stages of an injury [80]. The meta-analysis conducted by Liu et al. [81] reported a trend toward increased SOD levels and oxidative damage products in depression and no difference in catalase activity between patients with depression and controls. SOD, nitric oxide metabolites and lipid peroxides were found significantly higher in major depressive patients than in BD or healthy controls suggesting that biomarkers related to oxidative and nitrosative stress could aid in the differentiation of major depressive disorder and BD [82]. This study also showed that there were no significant sex-linked differences in SOD or catalase activities and that education was inversely associated with SOD activity and lipid peroxides formation suggesting that education has a protective effect on the generation of ROS. Only mild association was found between nicotine dependence and increased lipid hydroperoxide levels, but not between age and measured parameters. Another study also showed a significant increase in serum SOD, serum lipid peroxides and decrease in plasma ascorbic acid levels in patients of major depression as compared to control subjects. The trend reversed significantly after treatment with fluoxetine and citalopram [83]. All of these data support the concept that depression is accompanied with elevated oxidative stress and that antidepressant treatment may reduce oxidative stress, suggesting that augmentation of antioxidant defense is one of the mechanisms underlying the neuroprotective effects of antidepressants.

3.4 Superoxide dismutase and Alzheimer's disease

Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by the neuropathological deposition of extracellular amyloid-β peptide (Aβ) plaques and intracellular neurofibrillary tangles of hyperphosphorylated τ protein, and neurophil threads, loss of synapses and dendritic spines, cholinergic denervation,

hypoperfusion and hyperemia [84, 85]. Neurofibrillary tangles mainly contain self-aggregated hyperphosphorylated tau [86]. Neurotoxic peptides of varying lengths (Aβ42, Aβ40) formed by proteolitic cleavage of amyloid beta precursor protein (APP) can also form aggregates. Accumulation in brain tissue of Aβ42 aggregates is the major pathogenetic event in Alzheimer's disease and together with τ protein are mediators of the neurodegeneration that is among the main causative factors. Besides genetic abnormalities, oxidative stress is one of the causes of Aβ accumulation in AD. There is evidence of the leading contribution of oxidative damage to neurodegenerative disease in contrast to other diseases where oxidative stress plays a secondary role. Further, Aβ can be induced by oxidative stress [87], and in turn Aβ has the ability to induce oxidative stress. It is believed that oxidative stress occurs mainly as a result of overproduction of ROS by mitochondria.

Using the Alzheimer's disease mouse model in combination with a mouse that overexpresses the mitochondrial SOD, Massaad et al. [88] showed that severe deficits in the spacial and associative memory of AD mice could be prevented by scavenging of superoxide. MnSOD overexpression also resulted in a reduction in Aβ plaque deposition without affecting the levels of soluble and fibrillar Aβ. This finding show that quenching mitochondrial superoxide could be a preventive approach to the occurrence of AD. On the other hand, MnSOD reduction decreased amyloid plaques in the brain parenchyma but promoted the development of cerebrovascular amyloidosis, gliosis, and plaque-independent neuritic dystrophy suggesting that MnSOD protects the aging brain against Aβ-induced impairmets [89]. In addition, CuZnSOD activity was found significantly increased in fibroblast cell lines derived from AD patients [90]. De Leoa et al. [91] showed significantly increased CuZnSOD activity in red blood cells as well as the MnSOD mRNA levels in lymphocytes of AD patients. Total SOD activity was increased, whereas total GPX, catalase and peroxiredoxin activities were decreased in the superior temporal gyrus of AD patients, suggesting that hydrogen peroxide accumulates in this brain region [92]. It was also found that higher cerebrospinal fluid (CSF) CuZnSOD correlated with better, global cognition scores, yet less gray matter, and glucose metabolism in AD-sensitive parietal and frontal regions. Higher CSF CuZnSOD also associated with more CSF total tau and phosphorylated tau-181, but not beta-amyloid 1–42 [93]. These authors hypothesized that CuZnSOD antioxidation reflects tau but not amyloid accumulation, which may lead to pro-oxidant-based neurodegeneration and cognitive dysfunction. Morever, Rs2070424 polymorphism in CuZnSOD itself might be associated with AD in Chinese han population [94]. Further, it was demonstrated that S-adenosylmethionine and SOD supplementation prevents the exacerbation of AD-like features induced by B vitamin deficiency acting synergistically. They also contrasts the amyloid deposition typically observed in TgCRND8 mice [95]. On the basis of these resulta it was suggested that the combination of S-adenosylmethionine and SOD could be carefully considered as co-adjuvant of current AD therapies.

Similarly, extensive oxidative damage has been reported in mild cognitive impairment compared to those of normal aging subjects that is confirmed by decreased plasma levels of non-enzymatic antioxidants and activity of antioxidant enzymes [96]. These results show that ROS may act as important mediators of synaptic loss and inductors of neurofibrillary tangles and senile plaques formation [97]. The accumulation of oxidatively modified biomolecules is a hallmark of brain aging and could be an early event in the progression of AD. In 2019 Kelsey McLimans said: "In individuals with Alzheimer's or mild cognitive impairment, SOD1 was related to more gray matter, which is significant for memory. However, our results show 90 percent

of this positive association is negated by tau. This bolsters our hypothesis that CuZnSOD itself isn't detrimental; it's just trying to limit the oxidative damage caused by tau" [98].

4. Conclusion

The biochemical characteristics and physiology of the CNS strongly suggest that the brain is susceptible to oxidative damage and that oxidative stress is part of the pathophysiological mosaic of numerous neurological and psychiatric diseases. More importantly, oxidative stress is one of several biochemical mechanisms responsible for neurodegeneration in mental disorders, which can be limited as in schizophrenia, bipolar disorder, and depression, or reflected in massive apoptosis as in Alzheimer's disease. As the primary enzyme of antioxidant protection, SOD plays a significant role in preventing amplification of oxidative stress, production of more toxic free radicals and initiation of the internal apoptosis pathway. Although the results are inconsistent, in most studies the activity of SOD in major psychiatric illnesses has been altered. The inconsistency of the results may be a consequence of the heterogeneity of the disorders themselves, testing at different stages of the disease or the influence of psychopharmaceuticals on the enzyme activity and expression. In view of all the above, modulation of SOD activity can be considered in the light of a potential therapeutic target in major psychiatric illnesses.

Author details

Vladimir Djordjević[1,2]

1 Clinic for Mental Health Protection, Clinical Centre Niš, Serbia

2 Department of Psychiatry, Faculty of Medicine, University in Niš, Niš, Serbia

*Address all correspondence to: vladimir_dj@mts.rs

IntechOpen

References

[1] Riley PA. Free radicals in biology: oxidative stress and the effects of ionizing radiation. Int J Radiat Biol, 1994;65(1):27-33.

[2] Turrens JF. Mitochondrial formation of reactive oxygen species. J Physiol, 2003; 552 :335-344.

[3] Inoue M, Sato EF, Nishikawa M, et al. Mitochondrial generation of reactive oxygen species and its role in aerobic life. Curr Med Chem, 2003;10:2495-2505.

[4] Murphy MP. How mitochondria produce reactive oxygen species. Biochem J, 2009;417:1-13.

[5] Halliwell B. Phagocyte-derived reactive species: salvation or suicide? Trends Biochem Sci, 2006;31: 509-515.

[6] Valko M, Leibritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol, 2007;39:44-84.

[7] Gupta RK, Patel AK, Shah N, Chaudhary AK, Jha UK, Yadav UC, Gupta PK, Pakuwal U. Oxidative stress and antioxidants in disease and cancer: a review. Asian Pac J Cancer Prev, 2014;15(11):4405-4409.

[8] Kohen R, Nyska A. Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. Toxicol Pathol, 2002; 30(6):620-650.

[9] Berg D, Youdim MB, Riederer P. Redox imbalance. Cell Tissue Res, 2004;318: 201-213.

[10] Rabinovic AD, Hastings TG. Role of endogenous glutathione in the oxidation of dopamine. J Neurochem, 1998;71: 2071-2078.

[11] Choi YB, Lipton SA. Redox modulation of the NMDA receptor. Cell Mol Life Sci, 2000;57: 1535-1541.

[12] Ghafourifar P, Bringold U, Klein SD, Richter C. Mitochondrial nitric oxide synthase, oxidative stress and apoptosis. Biol Signals Recept, 2001; 10: 57-65.

[13] Pardini RS. Toxicity of oxygen from naturally occuring redox-active prooxidants. Arch Insect Biochem Physiol, 1995;29:101-118.

[14] Wolff SP, Dean RT. Glucose autoxidation and protein modification. The potential role of „autoxidative glycosylation“ in diabetes. Biochem J, 1987;245:243-250.

[15] Rosen H, Klebanoff S. Chemiluminescence and superoxide production by myeloperoxidase-deficient leukocytes. J Clin Invest, 1976;58:50-60.

[16] Stirpe F, Della Corte E. The regulation of rat liver xanthine oxidase. Conversion in vitro of the enzyme activity from dehydrogenase (type D) to oxidase (type O). J Biol Chem, 1969;244:3855-3863.

[17] Della Corte E, Stirpe F. The regulation of rat liver xanthine oxidase. Involvement of thiol groups in the conversion of the enzyme activity from dehydrogenase (type D) into oxidase (type O) and purification of the enzyme. Biochem J, 1972;126:739-745.

[18] Hille R, Massey V. Studies on the oxidative half-reaction of xanthine

oxidase. J Biol Chem, 1981;256: 9090-9095.

[19] Crapo JD, Oury TD, Rabouille C, Clot JW, Chang LY. Copper, zinc superoxide dismutase is primarily a cytosolic protein in human cells. Proc Natl Acad Sci, 1992;89:10405-10409.

[20] Hodgson EK, Fridovich I. The interaction of bovine erythrocyte superoxide dismutase with hydrogen peroxide: Inactivation of the enzyme. Biochemistry, 1975;14:5294-5299.

[21] Marklund SL. Regulation by cytokines of extracellular superoxide dismutase and other superoxide dismutase isoenzymes in fibroblasts. J Biol Chem, 1992;267:6696-6701.

[22] Tang L, Ou X, Henkle-Duhrsen K, Selkirk ME. Extracellular and cytoplasmic CuZn superoxide dismutase from Brugia lymphatic filaria nematode parasites. Infect Immun, 1994;62: 961-967.

[23] Yoshioka T, Homma T, Meyrick B, Takeda M, Moore-Jarrett KV, Ichikawa I. Oxidants induce transcriptional activation of manganese superoxide dismutase in glomerular cells. Kidney Int, 1994;46:405-413.

[24] Rezin GT, Amboni G, Zugno AI, Quevedo J, Streck EL. Mitochondrial dysfunction and psychiatric disorders. Neurochem Res, 2009;34:1021-1029.

[25] Mico JA, Rojas-Corrales MO, Gibert-Rahola J, Parellada M, Moreno D, Fraguas D et al. Reduced antioxidant defense in early onset first-episode psychosis: a case-control study. BMC Psychiatry, 2011;11:26. doi: 10.1186/ 1471-244X-11-26.

[26] Gunesa M, Altindagb A, Buluta M, Demira S, Ibiloglua AO, Kayaa MC, et al. Oxidative metabolism may be associated with negative symptoms in schizophrenia. Psychiatry Clin Psychopharmacol, 2017;27(1):54-61.

[27] Gonzalez-Liencres C, Tas C, Brown EC, Erdin S, Onur E, Cubukcoglu Z, et al. Oxidative stress in schizophrenia: a case-control study on the effects on social cognition and neurocognition. BMC Psychiatry, 2014; 14: 268 (doi:10.1186/s12888-014-0268-x)

[28] Abdala DS, Monteiro HP, Oliveira JA, Bechara EJ. Activities of superoxide dismutase and glutathione peroxidase in schizophrenic and manic-depressive patients. Clin Chem, 1986;32:805-807.

[29] Sarandol A, Kirli S, Akkaya C, Altin A, Demirci M, Sarandol E. Oxidative-antioxidative systems and their relation with serum S100 B levels in patients with schizophrenia: effects of short therm antipsychotic treatment. Prog Neuropsychopharmacol Biol Psychiatry, 2007;31:1164-1169.

[30] Padurariu M, Ciobica A, Dobrin I, Stefanescu C. Evaluation of antioxidant enzymes activities and lipid peroxidation in schizophrenic patients treated with typical and atypical antipsychotics. Neurosci Lett, 2010;479:317-320.

[31] Wu Z, Zhang XY, Wang H, Tang W, Xia Y, Zang F, et al. Elevated plasma superoxide dismutase in first-episode and drug naïve patients with schizophrenia: Inverse association with positive symptoms. Prog Neuropsychopharmacol Biol Psychiatry, 2012;36:34-38.

[32] Djordjevic VV, Lazarević D, Ćosić V, Knežević MZ, Djordjević BV. Age-related changes of superoxide dismutase activity in patients with schizophrenia.

Vojnosanit Pregl, 2016; UDC: 577.15: [616.895.8:615.214 DOI: 10.2298/ VSP141202142D.

[33] Cabungcal JH, Counotte DS, Lewis EM, Tejeda HA, Piantadosi P, Pollock C, et al. Juvenile antioxidant treatment prevents adult deficits in a developmental model of schizophrenia. Neuron, 2014; 83(5):1073-1084.

[34] Yao JK, Reddy R. Oxidative stress in schizophrenia: pathogenetic and therapeutic implications. Antioxid Redox Signal, 2011;15:1999-2002.

[35] Altuntas I, Aksoy H, Coskun I, Caykoylu A, Akcay F. Erythrocyte superoxide dismutase and glutathione peroxidase activities, and malondialdehyde and reduced glutathione levels in schizophrenic patients. Clin Chem Lab Med, 2000;38(12):1277-1281.

[36] Rukmini MS, D'Souza B, D'Souza V. Superoxide dismutase and catalase activities and their correlation with malondialdehyde in schizophrenic patients. Indian J Clin Biochem, 2004;19(2):114-118.

[37] Lang XE, Wang DM, Du XD, Jia QF, Chen DC, Xiu M, Wang L, Zhang XY. Elevated activity of plasma superoxide dismutase in never-treated first-episode schizophrenia patients: Associated with depressive symptoms. Schizophr Res, 2020;222:291-296. doi: 10.1016/j. schres.2020.05.032.

[38] Vidović B, Milovanović S, Đorđević B, Kotur-Stevuljević J, Stefanović A, Ivanišević J, et al. Effect of alpha-lipoic acid supplementation on oxidative stress markers and antioxidative defense in patients with schizophrenia. Psychiatria Danubina, 2014; 26(3): 205-213.

[39] Gonzalez-Liencres C, Tas C, Brown EC, Erdin S, Onur E, Cubukcoglu Z, et al. Oxidative stress in schizophrenia: a case-control study on the effects on social cognition and neurocognition. BMC Psychiatry, 2014; 14: 268 (doi:10.1186/s12888-014-0268-x)

[40] Dadheech G, Mishra S, Gautam S, Sharma P. Evaluation of antioxidant deficit in schizophrenia. Indian J Psychiatry, 2008; 50: 16-20.

[41] Inal ME, Kanbak G, Sunal E. Antioxidant enzyme activities and malodialdehyde levels related to aging. Clin Chem Acta, 2001;305(1-2):75-80.

[42] Ademoglu E, Ozcan K, Kucuk ST, Gurdol F. Age-related changes in the activity and expression of manganese superoxide dismutase, and mitochondrial oxidant generation in female and male rats. Turk J Biochem, 2013; 38(4):445-450.

[43] Wu JQ, Chen da C, Tan YL, Soares JC, Zhang XY. Mn-superoxide dismutase activity is associated with orofacial involuntary movements in schizophrenia patients with tardive dyskinesia. Hum Psychopharmacol, 2015;30(1):57-63.

[44] Michel TM, Thome J, Martin D, Nara K, Zwerina S, Tatschner T, Weijers HG, Koutsilieri E. Cu, Zn- and Mn-superoxide dismutase levels in brains of patients with schizophrenic psychosis. J Neural Transm, 2004;111:1191-1201.

[45] Raffa M, Mechri A, Othman LB, Fendri C, Gaha L, Kerekeni A. Decreased glutathione levels and antioxidant enzyme activities in untreated and treated schizophrenic patients. Prog Neuropsychopharmacol Biol Psychiatry, 2009;33(7):1178-1183.

[46] Padurariu M, Ciobica A, Dobrin I, Stefanescu C. Evaluation of antioxidant enzymes activities and lipid peroxidation in schizophrenic patients treated with typical and atypical antipsychotics. Neurosci Lett, 2010;479:317-320.

[47] Zhang XY, Tan YL, Cao LY, Wu GY, Xu Q, Shen Y, Zhou DF. Antioxidant enzymes and lipid peroxidation in different forms of schizophrenia treated with typical and atypical antipsychotics. Schizophr Res, 2006; 81: 291-300.

[48] Yang ZX, Feng ZD, Yuan CL, Yan ZP, Ying WG, Cun SY. The effect of risperidone treatment on superoxide dismutase in schizophrenia. J Clin Psychopharmacol, 2003; 23(2): 128-131.

[49] Ben Othmen L, Mechri A, Fendri C, Bost M, Chazot G, Gaha L, Kerkeni A. Altered antioxidant defense system in clinically stable patients with schizophrenia and their unaffected siblings. Prog Neuropsychopharmacol Biol Psychiatry, 2008;32:155-159.

[50] Dasgupta J, Dasgupta S, Rajni, Singh RK. Role of free radicals and antioxidants in schizophrenia. IJPCBS, 2014;4(4):825-828.

[51] Zhang M, Zhao Z, He L, Wan C. A meta-analysis of oxidative stress markers in schizophrenia. Sci China Life Sci, 2010;53:112-124.

[52] Hitzeroth A, Niehaus DJH, Koen L, Botes WC, Deleuze JF, Warnich L. Association between the MnSOD Ala-9Val polymorphism and development of schizophrenia and abnormal involuntary movements in the Xhosa population. Prog Neuro-Psychopharmacol Biol Psychiatry, 2007; 31(3): 664-672.

[53] Hori H, Ohmori O, Shinkai T, Kojima H, Okano C, Suzuki T, et al. Manganese superoxide dismutase gene polymorphism and schizophrenia: Relation to tardive dyskinesia. Neuropsychopharmacol, 2000; 23(2): 170-177.

[54] Emiliani FE, Sedlak TW, Sawa A. Oxidative stress and schizophrenia: recent breakthroughs from an old story. Curr Opin Psychiatry, 2014;27(3): 185-190.

[55] Wu JQ, Kosten TR, Zhang XY. Free radicals, antioxidant defense systems, and schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry, 2013;46:200-206. doi: 10.1016/j.pnpbp.2013.02.015.

[56] Manji H, Kato T, Di Prospero NA, Ness S, Beal MF, Krams M, Chen G. Impaired mitochondrial function in psychiatric disorders. Nat Rev Neurosci, 2012;13(5):293-307.

[57] Sun X, Wang J-F, Tseng M, Young LT. Downregulation in components of the mitochondrial electron transport chain in the postmortem frontal cortex of subjects with bipolar disorder. J Psychiatry Neurosci, 2006; 31(3): 189-196.

[58] Andreazza AC, Shao L, Wang J-F, Young LT. Mitochondrial complex I activity and oxidative damage to mitochondrial proteins in the prefrontal cortex of patients with bipolar disorder. Arch Gen Psychiatry, 2010;67(4): 360-368.

[59] Shao L, Young LT, Wang JF. Chronic treatment with mood stabilizers lithium and valproate prevents excitotoxicity by inhibiting oxidative stress in rat cerebral cortical cells. Biol Psychiatry, 2005;58: 879-884.

[60] Lovell MA, Xie C, Markesbery WR. Acrolein, a product of lipid peroxidation,

inhibits glucose and glutamate uptake in primary neuronal cultures. Free Radic Biol Med, 2000;29:714-720.

[61] Miyazaki I, Asanuma M. Dopaminergic neuron-specific oxidative stress caused by dopamine itself. Acta Med Okayama, 2008;62:141-150.

[62] Nocito A, Dahm F, Jochum W, Jang JH, Georgiev P, Bader M, et al. Serotonin mediates oxidative stress and mitochondrial toxicity in a murine model of nonalcoholic steatohepatitis. Gastroenterol, 2007;133:608-618.

[63] Andreazza AC, Kauer-Sant'anna M, Frey BN, Bond DJ, Kapczinski F, Young LT, Yatham LN. Oxidative stress markers in bipolar disorder: a meta-analysis. J Affect Disord, 2008;**111**(2-3): 135-144.

[64] Selek S , Savas HA, Gergerlioglu HS, Bulbul F, Uz E, Yumru M. The course of nitric oxide and superoxide dismutase during treatment of bipolar depressive episode. J Affect Disord, 2008; 107(1-3):89-94. doi: 10.1016/j.jad.2007.08.006

[65] Abdala DS, Monteiro HP, Oliveira JA, Bechara EJ. Activities of superoxide dismutase and glutathione peroxidase in schizophrenic and manic-depressive patients. Clin Chem, 1986;32:805-807.

[66] Kuloglu M, Ustundag B, Atmaca M, Canatan H, Tezcan AE, Cinkilinc N. Lipid peroxidation and antioxidant enzyme levels in patients with schizophrenia and bipolar disorder. Cell Biochem Func, 2002;20(2):171-175.

[67] Savas HA, Gergerlioglu HS, Armutcu F, Herken H, Yilmaz HR, Kocoglu E. Elevated serum nitric oxide and superoxide dismutase in euthymic bipolar patients: impact of past episodes. World J Biol Psychiatry, 2006;7(1):51-55.

[68] Gergerlioglu HS, Savas HA, Bulbul F, Selek S, Uz E, Yumru M. Changes in nitric oxide level and superoxide dismutase activity during antimanic treatment. Prog Neuropsychopharmacol Biol Psychiatry, 2007;31(3):697-702. doi: 10.1016/j.pnpbp.2006.12.020.

[69] Kunz M , Gama CS, Andreazza AC, Salvador M , Ceresér KM, Gomes FA, et al. Elevated serum superoxide dismutase and thiobarbituric acid reactive substances in different phases of bipolar disorder and in schizophrenia. Prog Neuro-Psychopharmacol Biol Psychiatry, 2008; 32:1677-1681.

[70] Jiménez-Fernández S, Gurpegui M, Garrote-Rojas D, Gutiérrez-Rojas L, Carretero MD, Correll CU. Oxidative stress parameters and antioxidants in patients with bipolar disorder: Results from a meta-analysis comparing patients, including stratification by polarity and euthymic status, with healthy controls. Bipolar Dis, 2021;23:117-129.

[71] Zhang XY, Tan YL, Cao LY, Wu GY, Xu Q, Shen Y, Zhou DF. Antioxidant enzymes and lipid peroxidation in different forms of schizophrenia treated with typical and atypical antipsychotics. Schizophr Res, 2005; 81: 291-300.

[72] Zerin Khan F, Sultana SP, Akhter N, Mosaddek AS. Effect of olanzapine and risperidone on oxidative stress in schizophrenia patients. Int Biol Biomed J Spring, 2018;4(2):89-97.

[73] Parikh V, Khan MM, Mahadik SP. Differential effects of antipsychotics on expression of antioxidant enzymes and membrane lipid peroxidation in rat brain. J Psychiatric Res, 2003; 37(1): 43-51.

[74] Li ML, Chlan-Fourney J, Juorio AV, Bennett VL, Shrikhande S, Keegan DL et al. Differential effects of olanzapine on the gene expression of superoxide

dismutase and the low affinity nerve growth factor receptor. J Neurosci Res, 1999;56(1):72-75.

[75] De Sousa RT, Zarate CA, Zanettia MV, Costa AC, Taliba LL, Gattaza WF, Vieira RM. Oxidative stress in early stage bipolar disorder and the association with response to litium. J Psychiatr Res, 2014;50:36-41.

[76] Ng F, Berk M, Dean O, Bush AI. Oxidative stress in psychiatric disorders: evidence base and therapeutic implications. Int J Neuropsychopharmacol, 2008;11(6):851-876.

[77] Gawryluk JW, Wang JF, Andreazza AC, Shao L, Young LT. Decreased levels of glutathione, the major brain antioxidant, in post-mortem prefrontal cortex from patients with psychiatric disorders. Int J Neuropsychopharmacol, 2011;14(1):123-130.

[78] Maes M, Galecki P, Chang YS, Berk M. A review on the oxidative and nitrosative stress (O&NS) pathways in major depression and their possible contribution to the (neuro) degenerative processes in that illness. Prog. Neuro-psychopharmacol. Biol Psychiatry, 2011;35(3):676-692.

[79] Fukai T, Ushio-Fukai M. Superoxide dismutases: role in redox signaling, vascular function, and diseases. Antioxid Redox Signal, 2011;15(6):1583-1606.

[80] Terrazzano G, Rubino V, Damiano S, Sasso A, Petrozziello T, Ucci V, et al. T cell activation induces CuZn superoxide dismutase (SOD)-1 intracellular re-localization, production and secretion. Biochim Biophys Acta, 2014;1843(2):265-274.

[81] Liu T, Zhong S, Liao X, Chen J, He T, Lai S, Jia Y. A metaanalysis of oxidative stress markers in depression. PLoS One, 2015; 10(10):e0138904

[82] Maes M, Bonifacio KL, Morelli NR, Vargas HO, Barbosa DS, Carvalho AF, Nunes SOV. Major differences in neurooxidative and neuronitrosative stress pathways between major depressive disorder and types I and II bipolar disorder. Mol Neurobiol, 2018; 56(1):141-156 DOI: 10.1007/ s12035-018-1051-7

[83] Khanzode SD, Dakhale GN, Khanzode SS, Saoji A, Palasodkar R. Oxidative damage and major depression: the potential antioxidant action of selective serotonin re-uptake inhibitors. Redox Rep, 2003;8(6):365-370.

[84] Harkany T, Penke B, Luiten PG. β-Amyloid excitotoxicity in rat magnocellular nucleus basalis. Effect of cortical deafferentation on cerebral blood flow regulation and implications for Alzheimer's disease. Ann N Y Acad Sci, 2000;903:374-386.

[85] Tanzi R, Betram L. Twenty years of the Alzheimer's disease amyloid hypothesis: a genetic perspective. Cell, 2005;120:545-555.

[86] Goedert M, Spillantini MG. A century of Alzheimer's disease. Science, 2006;314(5800): 777-781.

[87] Tong Y, Zhou W, Fung V, Christensen MA, Qing H, Sun X, Song W. Oxidative stress potentiates BACE1 gene expression and Abeta generation. J Neural Transm, 2005;112:455-469.

[88] Massaad CA, Pautler RG, Klann E. Mitochondrial superoxide: a key player in Alzheimer's disease. Aging, 2009;1(9):758-761.

[89] Esposito L, Raber J, Kekonius L, Yan F, Yu G-Q, Ly NB, et al. Reduction in

mitochondrial superoxide dismutase modulates Alzheimer's disease-like pathology and accelerates the onset of behavioral changes in human amyloid precursor protein transgenic mice. J Neurosci, 2006;26(19):5167-5179.

[90] Zemlan FP, Thienhaus OJ, Bosmann HB. Superoxide dismutase activity in Alzheimer's disease: possible mechanism for paired helical filament formation. Brain Res, 1989;476(1): 160-162.

[91] De Leoa ME, Borrelloa S, Passantinoa M, Palazzottia B, Mordenteb A, Danielec A, et al. Oxidative stress and overexpression of manganese superoxide dismutase in patients with Alzheimer's disease. Neurosci Lett, 1998;250:173-176.

[92] Youssef P, Chami B, Lim J, Middleton T, Sutherland GT, Witting PK. Evidence supporting oxidative stress in a moderately affected area of the brain in Alzheimer's disease. Sci Rep, 2018;8:11553 DOI:10.1038/s41598-018-29770-3

[93] McLimans KE, Clark BE, Plagman A, Pappas C, Klinedinst B, Anatharam V, et al. Is cerebrospinal fluid superoxide dismutase 1 a biomarker of tau but not amyloid-induced neurodegeneration in Alzheimer's disease? Antioxid Redox Signal, 2019;31:572-578.

[94] Li Y, Ma C, Wei Y. Relationship between superoxide dismutase 1 and patients with Alzheimer's disease. Int J Clin Exp Pathol, 2017;10(3):3517-3522.

[95] Cavallaro RA, Nicolia V, Fiorenza MT, Scarpa S, Fuso A. S-adenosylmethionine and superoxide dismutase 1 synergistically counteract Alzheimer's disease features progression in TgCRND8 mice. Antioxidants, 2017;6(4):76; https://doi.org/10.3390/antiox6040076

[96] Sultana R, Perluigi M, Butterfield DA. Oxidatively modified proteins in Alzheimer's disease (AD), mild cognitive impairment and animal models of AD: role of Abeta in pathogenesis. Acta Neuropathol, 2009;118:131-150.

[97] Kern A, Behl C. The unsolved relationship of brain aging and late-onset Alzheimer disease. Biochim Biophys Acta, 2009;1790(10):1124-1132 doi: 10.1016/j.bbagen.2009.07.016..

[98] Iowa State University. Antioxidant puts up fight, but loses battle against protein linked to Alzheimer's disease. Science Daily. ScienceDaily, 2019. Available from: http//www.sciencedaily.com/releases/2019/06/190617164701.htm.

Chapter 5

Reactive Oxygen Species in the Development and Resolution of Autoimmune and Inflammatory Disease

Joshua Banda and Allan K. Chang

Abstract

Reactive oxygen species (ROS) have been associated with a wide variety of human diseases and disorders. The ability of these molecules can incapacitate antioxidant activity leading to an imbalance between oxidants and anti-oxidants, with the latter being more pronounced. ROS are no strangers to immune cell relationships and function and consequently the development of autoimmune and inflammatory diseases. The collateral damage of excessive ROS (collectively called Oxidative stress) to the cells or tissue due to nucleic acid damage and oxidation of macromolecules such as proteins and lipids is linked to the manifestation, malfunction and translation to the disease state of cells. Contrary to this view, recent studies have shown that ROS have protective roles in certain autoimmune and inflammatory diseases. Despite significant advances in our understanding of inflammatory and autoimmune diseases, therapeutics for these diseases still need further development and identification of new targets for improved therapeutic effect. ROS molecules and inflammation modulators appear before disease development making them great therapeutic targets with the potential to inhibit disease manifestation.

Keywords: reactive oxygen species, antioxidants, immune cell, autoimmune, inflammatory disease, oxidative stress

1. Introduction

1.1 Reactive oxygen species

Reactive Oxygen Species (ROS) are by-products of chemical reactions that involve a one-electron reduction of oxygen, leading to the production of a diatomic oxygen radical known as superoxide. Superoxide serves as a precursor for multiple ROS generation. The oxygen anion can be produced mainly by enzymatical or non-enzymatical means. Enzymes such as Phagocytic Nicotinamide Adenine Dinucleotide Phosphate Oxidase (NADPH Oxidase), cytochrome P450–dependent oxygenase,

and the proteolytic conversion of cytosolic Xanthine dehydrogenase can produce singlet-oxygen molecules [1, 2]. The enzymatic generation of superoxide is the main generator of superoxide, especially NADPH oxidase. However, non-enzymatic generation can also cause a significant increase in reactive species. This occurs mostly in the mitochondria during ATP synthesis. The electron transport chain (ETC) has been reported to consist of centers that can leak electrons to oxygen, thereby causing a reduction of the oxygen [1]. Additionally, reduced coenzymes and prosthetic groups such as flavins and metal ions can directly transfer an electron to oxygen. The produced singlet-oxygen undergoes dismutation, catalyzed by the enzyme superoxide dismutase, to produce hydrogen peroxide, a more stable and less reactive molecule. The increased availability of ROS can lead to an imbalance between oxidants and antioxidants, resulting in a state that is known as oxidative stress [2]. During this state, the various deleterious effects of ROS such as disruption of cellular homeostasis, structures and function are manifested in the cell. Therefore, ROS overproduction has a pathological role in the development of various conditions and diseases, including inflammation and autoimmune disease.

1.2 Inflammation

Inflammation is a none-specific immune response of cells or tissues to a stimulus such as a pathogen, cell damage, or toxicity that plays an important role in host defense. This response involves the signaling molecules, immune cells and blood vessels. The process is marked by the proliferation of inflammatory cells like monocytes, neutrophils, and lymphocytes. These cells release various molecules such as ROS, pro-inflammatory cytokines and various enzymes that have the ability to induce oxidative stress in the target tissues [3]. Therefore, inflammation and oxidative stress can co-occur, and one process can induce the other and vice versa.

Inflammatory responses can be complex as they involve multiple interactions of many cells and mediators. There are four major patterns of inflammatory response that are also common mechanisms of diseases. These patterns depend on the type of hypersensitivity. Two or more types of hypersensitivity can occur at once in a patient.

1. Type 1: This type of hypersensitivity is mediated by immunoglobulin (Ig) E that is found on the surface of mast cells. The interaction between this antibody and a respective antigen causes the release of inflammation mediators such as serotonin and histamine, among others. The release of these molecules causes diminished blood pressure due to vasodilation, increased vascular endothelium permeability, and bronchoconstriction [4]. Interestingly, increased activation of mast cells can cause a systemic response that causes anaphylactic shock. Mediators that are released by mast cells can also recruit inflammatory cells such as eosinophils and increase the production of PAF, leukotrienes, prostaglandins and cytokines like TNF-α, IL-1, IL-3 and IL-5 [5]. This results in a sustained inflammatory response and reduces transient respiratory efficiency.

2. Type 2: This type of hypersensitivity is triggered by the binding of antibodies, IgG and IgM to the cellular antigens, causing tissue destruction. The binding of antibodies results in lysis of the cell via the in-situ fixation complement. Ig M is an effective inducer of this process [5]. Fixation of complement is an important cause of cell injury, and it can cause opsonization as well as being an active recruiter of inflammatory cells.

3. Type 3: This is heavily marked by serum sickness due to increased circulating immunocomplexes. The deposition of these immunocomplexes can lead to injury at the site of deposition (usually vascular beds). This is because the complexes are efficient activators of the classical pathway of the complement cascade [6]. The properties of the complexes determine which vascular bed they are deposited in. This deposition is precise and distinct in pathophysiology of different diseases such chronic autoimmune diseases such as systemic lupus erythematosus [5, 6]. Certain inflammatory responses have a confection of features of type 2 and type 3 hypersensitivity.

4. Type 4: This type of hypersensitivity usually takes longer to occur after antigen exposure and is also referred to as delayed-type hypersensitivity. It is typified by antigen-specific T cells activation after 24–48 hours of exposure to antigen. The antigens modify cellular proteins and these proteins are targeted by T cells. CD8+ T cells interact with the antigen presented by MHC-I and become activated [7]. This causes the T cell to kill all the cells that are presenting that antigen. Antigen-presenting cells also display antigens via the MHC-II that activates CD4+ T cells and causes the release of cytokines [5]. Activation of the two T cell activation leads to an increase in antigen-specific response, leading to hypersensitivity reaction over time. In some events, an immediate inflammatory response is initiated due to a positive response. The positive response occurs when the T cells are pre-primed as a result of prior ongoing exposure to the pathogenic antigen [6]. During this event, the interaction between the T cells and macrophages causes the release of TNF- and IFN, which are responsible for the inflammatory response. Other types of hypersensitivity responses that are triggered by cellular components of the immune system can also be referred to as type 4 even without being antigen specific (**Table 1**).

Inflammation is triggered to eliminate harmful agents so as to minimize the effects of the injuries. However, minimal inflammation (acute inflammation) usually does not inhibit these effects and prolonged inflammation (chronic inflammation) is associated with multiple diseases and conditions [3]. Acute inflammation is known to be part of the innate immune response as it is the initial response to a stimulus. In this phase of inflammation, platelets and granulocytic cells like basophils, eosinophils and mast cells are activated. These cells migrate from the blood vessels to the site of injury and release molecules that initiate, stimulate and attenuate inflammation for a short period [8]. For this to occur, the blood vessel becomes more permeable, consequently leading to the escape of proteins, outflow of fluids known as exudate and migration of other blood cells from the vessels to the site of inflammation, causing a swelling known as edema on the site. Neutrophils are the primary cells during this phase and they tend to engulf the foreign materials and organisms together with other debris. As neutrophils are short-lived cells, they are replaced by monocytes that differentiate into macrophages [8]. The acute phase usually resolves after hours or days, or even within a week. The persistent presence of monocytic cells due to ongoing and long-term response to stimuli leads to the development of chronic inflammation.

During chronic inflammation, there is a continuous accumulation of macrophages and lymphocytes at the site of inflammation due a persistent stimulus from the immune system [9]. The prolonged inflammatory response that might last for a week, months and in some cases a lifetime, will eventually lead to tissue injury. This can be induced by viral or certain bacterial infection and in some individuals,

Type	Mediator	Components	Antigen & Antibody	Principle	Phenotype	Time/Period	Example
1	IgE	Mast cells, Basophils, Histamine	Antigen: exogenous, free in circulation Antibody: fixed on mast and basophil cells	Destruction of cells via antibody mediated degranulation of granulocytes.	Weal and Flare	Early ≤3 hours	Asthma, Bee sting, Rhinitis
2	IgG/IgM	Neutrophils, Complement system components	Antigen: endogenous or exogenous, fixed on cells Antibody: Free in circulation	Annihilation of normal tissue cells via antibody mediated pathway.	Lysis and Necrosis	Intermediated 5–8 hour	Rhesus hemolytic disease, autoantigen-induced cell damage, Drug-induced anemia
3	IgG/IgM	Compliment system components, Phagocytes, Killer cells	Antigen: free in circulation, with exogenous or endogenous origin Antibody: free in circulation	Antigen–antibody complex mediated cell death	Erythema and Edema	Early - Intermediate depending on stimulant 2–8 hours	SLE, Lung arthritis, glomerulonephritis, vasculitis
4	$CD4^+$ Th cells, $CD8^+$ Tc cells	Antigen presenting cells (APC), Macrophages, Cytokines	Antigen: from exogenous or endogenous sources, soluble or fixed on cell. Antibody: none	T lymphocytes mediated cell damage	Erythema and Induration	Delayed ≥ 24 hrs from stimulation	Granuloma formation, Diabetes Mellitus,

Table 1.
Summary of types of hypersensitivity.

genetic polymorphism of inflammation mediators and cell receptors can also induce and favor extensive chronic inflammation [10]. In addition to the accumulation of macrophages, chronic inflammation is also marked by the proliferation of fibroblasts and small blood vessels. In rare cases when the inflammatory response encounters an agent that is difficult to eliminate, the response proceeds to granulomatous inflammation.

Granulomatous inflammation is a specific type of chronic inflammation that is marked by the presence of crystalline materials embedded into the tissues. Macrophages are the predominant effectors and they are recruited by T cells. Th1 cells have been reported to be the major mediators of granulomatous inflammation reactions [11]. Following the activation of Th1 cells by the antigens presented on MHC-II on the macrophages, Th1 secretes IFN-γ and other cytokines. These molecules then transform macrophages into activated tissue macrophages. Although the activated macrophages have increased capacity to eliminate foreign pathogens, they have a tendency to fuse and form multinucleated giant cells which forms concentric nodules that are known as granulomas [12]. Central necrosis can develop in these granulomas. This phenomenon has have been observed in infections caused by *M. tuberculosis* and other pathogens [13]. Such a phenomenon has also been reported in some autoimmune diseases.

Although inflammation is an essential process to the host defense, it can easily induce excessive tissue damage, resulting in acute or chronic tissue damage, organ or system dysfunction with fatal outcomes. Studies have shown increasing evidence of the involvement of chronic inflammation in autoimmune diseases, including but not limited to rheumatoid arthritis (RA), inflammatory bowel disease (IBD) systemic lupus erythematosus (SLE), gout, and diabetes [14]. This happens when inflammatory activity causes the production of autoimmune molecules and reactive species that sensitizes the immune system to a non-pathogenic component of the body.

1.3 Autoimmune diseases

Autoimmune diseases are chronic conditions that result from the loss of immune tolerance to self-antigens, causing the immune system to attack the organisms' healthy cells, tissues, and/or organs. Autoreactive T cells and autoantibodies are identified as the major attackers of self-antigen [15]. The differentiation and activation of these key attackers are still not fully elaborated. These disorders can be classified into two major groups, organ-specific autoimmune diseases and multiple organs or systemic autoimmune diseases. Different types of autoimmune diseases have been found to share common phenotypic features, from clinical signs and symptoms to genetic factors and pathophysiology mechanisms [16–18]. However, it is likely that some inducing factors may differ as different autoimmune diseases target different cells, organs and systems. Additionally, environmental factors also play a role in the onset of these diseases.

Factors that are common in different autoimmune diseases include but is not limited to the following features.

1. Pathology: The phenotypic manifestation of autoimmune varies, depending on the affected or target cell or system. However, the major pathogenic role is contributed by the phagocytic T cells and B cells. Other predominant cells include macrophages, neutrophils, and CD8+ T cells. Other cells that also contribute to the pathogenesis of autoimmune diseases are T helper cells, especially Th1, Th9

and Th17 [19]. The array of complex biological functions displayed by these cells such as cytokine production, antigen presentation, exosome release, the release of neutrophil extracellular traps (NETs), ROS, Arginase 1 and programmed death-ligand 1 have been implicated in the induction of autoreactive T cells and B cells as well as tissue damage and inflammation [20]. Abnormalities in the function of cells that participate in the classic immune response such as higher expression of IL-6, interferon-α, APRIL and BAFF can cause the dysregulation of adaptive immune cells [21]. These cells then go on to cause cell death either directly or indirectly by releasing cytokines, ROS, RNS and prostaglandins. Interestingly, specialized pro-inflammatory neutrophils with enhanced NETs and inflammatory cytokine production capacity have been found in the peripheral blood of patients suffering from different autoimmune diseases [15]. These cells have a low density due to altered buoyancy properties.

Peripheral tissues contain activated regulatory T cells that control inflammation and autoimmunity responses by eliminating malfunctioning neutrophils, lymphocytes and macrophages. Cells such as CD25+ and CD4+ T cells can secrete anti-inflammatory cytokines that can reduce Th1, Th9 and Th17 activity, thereby preventing autoimmune disease development [22]. However, their function can be inhibited by environmental agents such as pathogenic toxins or smoking.

2. Risk factors

   a. Gender: Statistically women are more susceptible to autoimmune diseases than men. With nearly 5% of the total global population suffering from these diseases, 80% of the reported cases are women [23]. Pathophysiology in the progression of autoimmune diseases in women tends to be different from men and can cause polyautoimmunity. Factors that contribute to this are differences in hormonal orientation and genetic factors [24]. High levels of estrogen, progesterone and prolactin have been implicated in the development of autoimmune diseases.

   b. Age: Diseases like systemic lupus erythematosus (SLE) and type1 diabetes mellitus tend to have a high severity when the onset is early [25]. However, other autoimmune diseases are not influenced by the time of onset, e.g. rheumatoid arthritis and Sjogren's syndrome.

   c. Environmental agents: One of the most crucial environmental autoimmunity triggers is infectious agents. Viruses like the Epstein–Barr virus and cytomegalovirus have been implicated in multiple autoimmune inductions [26]. However certain viruses like the hepatitis B virus have the putative ability to protect against autoimmune disease development.

   d. Genetics: The genetic risk factors of autoimmune diseases can be divided into two groups, the common factors and the specific factors. Autoimmune phenotype is determined by a combination of common and disease-specific alleles interacting with environmental and epigenetic factors [27]. Autoimmune phenotypes have also been confirmed to be the outcomes of nonspecific diseases genes [28]. Additionally, genetic ancestry can also influence the heterogeneity and variation of the clinical manifestation disease [29]. Certain population

subgroups and races have been associated with having a relatively high frequency of autoimmune diseases risk alleles. However, genetic risk factors only confer a small risk and can only explain a limited proportion of heritability in relation to autoimmune diseases [30]. Investigated population and additive and non-additive factors should be considered when assessing heritability in autoimmunity.

e. Other autoimmune conditions: People with a history of autoimmunity or who have an existing autoimmune infection are at higher risks of developing another autoimmune disease [31]. Diverse manifestation of disease phenotype originating from the same gene causes a condition known as polyautoimmunity [17]. The coexistence of more than two autoimmune diseases in a patient leads to a syndrome known as multiple autoimmune syndromes (MAS). MAS is known to favor the pathogenesis of other autoimmune diseases. Dermatological autoimmune disease is present in most MAS. Aside the existence of other autoimmune conditions, the development of MAS is associated with genetic, immunologic, infectious, and psychological factors.

3. Subphenotype: Signs and symptoms of autoimmunity are shared across a wide range of autoimmune diseases. Symptoms such as fatigue, dizziness, arthritis, alopecia, and Raynaud's phenomenon and high levels of cytokines are common in most autoimmune diseases [32]. However, these diseases can have a heterogeneous spectrum if the disease course is dependent on the patient. Additionally, the disease phases differ from one patient to another and even within the same

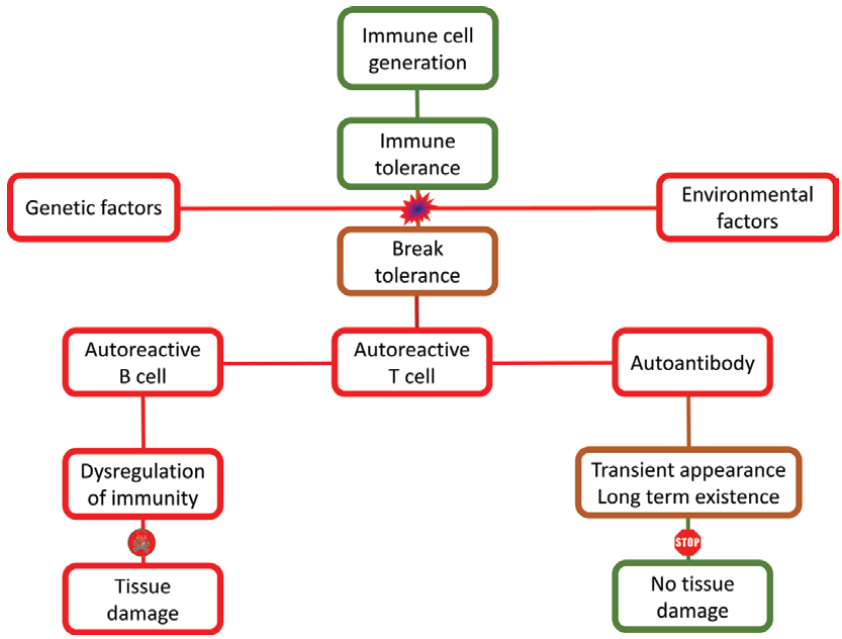


Figure 1.
Development of autoimmune disease. Following the generation and maturation of immune cells in the bone marrow and thymus, the cells undergo a series of processes to produce immuno tolerant cells. A small number of T and B cells evade this process and form autoreactive T and B cells. However these cells are harmless until acted upon by genetic or environmental factors autoantibody can trigger autoimmunity for long term.

patient [33]. This can increase the challenge in acquiring a better understanding of autoimmune diseases because although subphenotypes are similar, they can change depending on the diseases activity and duration [34].

4. Recurrence risk: Complex diseases such as autoimmune diseases tend to cause phenotype clusters in the family of the infected individual. This aggregation usually occurs in a higher frequency than what is observed in the general population [29]. The presence of different autoimmune diseases in the members of the nuclear family is known as familial autoimmunity. When a specific autoimmune disease occurs in the family, it is known as familial autoimmune diseases [18]. Familial autoimmunity is common than a familial autoimmune disease (**Figure 1**).

2. Pathogenic foreign bodies, ROS, inflammation and autoimmunity

Production of ROS in phagocytic cells during the oxidative burst is essential to the elimination of pathogens during an immune response. However, it is also connected with the promotion of inflammation and tissue damage. Interestingly, recent studies have demonstrated that phagocyte derived ROS plays a role in the regulation of inflammation as well as providing protection against autoimmunity. This is mainly because chronic inflammation in multiple pathologic conditions has been associated with ROS deficiency.

The activation of the oxidative burst is highly marked by increased uptake of oxygen in the neutrophils. The consumed oxygen is primarily converted to singlet-oxygen molecules. This leads to an increase of the membrane-permeable hydrogen peroxide due to elevated dismutation activity within the phagocytes. This initiates the generation of various radical and non-radical ROS molecules via the activity of myeloperoxidase. In the events of oxidative burst, the NADPH oxidase complexes serve as one of the major generators of ROS but the localization and timing of the products depend on the stimulus [35]. Despite the localization and efficiency of ROS production, a high concentration of the molecules has been reported to cause the inactivation of proteins and enzymes via the adduct formation due to lipid peroxidation products [36]. Among the enzymes that are inactivated by this mechanism is NOX2, a potent producer of superoxide molecules. To counter this inhibition, phagocytic cells recruit cytochrome-b558 from the lysosomal pool via the soluble NSF attachment receptor 23 (SNARE) [37]. This enables the continuous production of ROS even after the inactivation of NOX2. This mechanism ensures the efficient eradication of pathogens and has been reported to potentially play a vital role in the regulation of autoimmunity [38]. The failure to move the NOX2 to the endosomes due to decreased Ncf4 expression in phagocytic cells can cause autoimmune responses but without the elimination of pathogenic molecules. Notably, increased activity of NOX2 is known to cause the destruction of membranes because of lipid peroxidation and this results in the leakage of the endosome contents such as antigens [39]. This process allows the presentation as well as cross-presentation of antigens to MHC-I. ROS production is therefore essential for the eradication of intracellular pathogens [39, 40].

Some evidence of the role of ROS in the maturation of dendritic cells and the increased expression of MHC-II molecules has been proposed, however, this is met with conflicting research results that NOX2-ROS production does not play a role in the maturation, differentiation and production of cytokines. This is despite its

important role in the elimination of intracellular foreign stimuli. Nevertheless, ROS enables regulated presentation of antigen by MHC-II via the oxidation of cathepsin cysteinyl thiols which prevents excessive protein degradation in early phagosomes. Intracellular foreign pathogen infections such as bacterial and fungal infections have been reported to be persistent in conditions where NOX2 activity is lacking [41]. NOX2 is also involved in backup mechanisms of pathogen capturing such as the formation of neutrophil extracellular traps (NETs) [42]. NETs are mainly composed of chromatin coated in antimicrobial peptides and proteases. During NETs formation, ROS are required for the release of elastase while myeloperoxidase participates in the formation of azurophilic neutrophils granules that facilitate histone degradation in the nucleus [42].

2.1 ROS in inflammatory and autoimmune development

Oxidative damage caused by ROS can generate deleterious byproducts consisting mainly of proteins and lipids that are modified into peroxides. These molecules play important roles in the pathogenesis of several diseases. These molecules have also been implicated in the pathophysiology of cell death and tissue damage. Some of these have the ability to cause immunogenic reactions by inducing pathogenic antibody release in diseases such as systemic lupus erythematous, alcoholic liver disease, diabetes mellitus, inflammation, degenerative diseases, and rheumatoid arthritis. Aldehydic by-products that form adducts with proteins make up two-thirds of molecules that have been implicated in these conditions.

Stress-induced by ROS or any other factor compromises the antioxidant activity within a cell can lead leading to an imbalance in the pro-oxidant/antioxidant balance. The prevalence of this phenomenon has been shown to increase lipid peroxidation. Lipid peroxidation is the degeneration of polyunsaturated fatty acids by free radical activity. The process involves three steps: initiation, propagation and termination. In the first step, a reactive radical extracts a hydrogen molecule from the methylene group. This leaves an unpaired electron on the carbon that combines with molecular oxygen in the propagation phase which then forms reactive peroxyl radicals that react with other lipids thereby forming hydroperoxides. Notably, peroxyl can produce fatty acid radicals and this can cause a chain reaction that causes lipid peroxide toxicity. Lipid peroxidation can also be induced by incidences of exacerbated conjugated dienes, 4-hydroxyl-2-nonenal modified proteins, malondialdehyde modified proteins and 4-hydroxynonenal among other molecules. Products of lipid peroxidation such as 4-hydroxy-2-alkenals can form the an adduct with the amino groups of proteins, leading to ROS induced protein modification. Modified proteins that gain the function of an aldehyde are highly immunogenic. ROS-induced lipid peroxidation and protein modification are likely to co-occur, and the two processes can mutually induce each other. Some products of these processes can avidly react with antioxidants including glutathione and cofactors of ketoglutarate dehydrogenase causing further damage to the antioxidant response.

Lipid peroxides are not bystanders when it comes to the destruction of cellular membranes, cell-matrix and the accelerators in the development of conditions such as atherosclerosis in arthritis especially rheumatoid arthritis. The destruction of the cell membrane can cause the leakage of cellular content, thereby inducing an inflammatory response as the phagocytic cells attempt to clean the debris. The process of debris clearance can percussively cause tissue damage. During this process two sets of macrophages are activated, the first set is M1 which is classically activated, and the

second set is M2, which is alternatively activated. M1 is known for excessive production of toxic production which M2 tries to resolve by producing molecules like EVG and VEGF. The difference in function is made vivid by the distinction in the cytokine profile of the two sets. M1 releases excessive proinflammatory cytokines IL1, IL6 and ROS which ultimately causes cell death by activating death receptors and/or caspases. M2 on the other hand releases anti-inflammatory cytokines like IL-4 and IL-10. In situations that lead to excessive tissue injury, there is little to no anti-inflammatory response as compared to proinflammatory. Aside from this, oxidation of low-density lipoproteins can cause the upregulation of chemokines, adhesion molecules and glycan end-products, thereby inducing an increased inflammatory response. Inflammation in the presence of oxidative stress is known to result in the nonenzymatic degradation of proteins through glycoxidation. Glycoxidation of immunoglobins produces modified immunoglobins which can induce a systemic inflammatory response. Neo-epitopes created by protein modifications can be recognized by toll-like receptor-4, advanced glycan end-product receptors, and scavenger receptors as invasive and can induce pathogen-associated molecular patterns in the immune system that will ultimately lead to autoimmunity. Additionally, there is a correlation between ROS-altered biomolecules and the severity score of autoimmune and inflammatory diseases.

2.2 Examples of inflammatory and autoimmune diseases and the ROS

2.2.1 Systemic lupus erythematosus

SLE is a complex autoimmune disease that affects at least 0.04% - 0.2% of every 100,000 people. The disease has a high prevalence in childbearing age women. This disease is marked by the increased presence of autoantibodies that target nuclear components and inflammation. This usually occurs in organs like the lungs, kidney, and joints. Although the exact cause of SLE remains not fully understood, a genetic predisposition that promotes the formation of lupus has been purported. Additionally, single nucleotide polymorphism in the Ncf2 gene that causes reduced ROS production is known to increase the likelihood of SLE occurrence. The promoting role of ROS deficiency in lupus-like phenotype has been demonstrated in mice where mutation of the Ncf1 shows high levels of anti-DNA, anti-histone, and anti-RNA antibodies with elevated deposits of Ig G and complement C3 in the glomeruli [43]. This contributes to the development of clinical signs of Arthritis, lung hemorrhage and enhanced glomerulonephritis. Increased risk of atherosclerosis is another feature of SLE. Atherosclerosis is developed due to endothelial dysfunction which is linked to a diminished bioavailability of nitric oxide and an increased generation of ROS. Studies in lupus-prone mice have shown increased activity of NADPH oxidase coupled with elevated systolic blood pressure and renal disease, a typical symptom of lupus [44]. ROS in SLE can play a double role depending on the stage of the disease. On one hand, it can be essential in the prevention of autoimmune diseases during the early stage, but it may exacerbate damage during the late stage of the disease.

2.2.2 Type 1 diabetes (diabetes mellitus)

Type 1 diabetes is a metabolic disease that results from the dysregulation of insulin due to the autoimmune destruction of β-cells in the pancreas. The disease emerges at an early age and is manifested as high levels of blood sugar.

The hyperglycemia-induced onset of diabetes has been linked to excessive oxidative stress damage. Mouse model-based experiments have demonstrated that ROS plays a key role in disease development of the disease [45]. For example, non-obese diabetic mice that produce is prone to type1 diabetes, contrary to their ROS deficient counterparts [46]. Reducing the ROS levels is known to help in transforming macrophages to M2 phenotype. Unlike M1, M2 is not a proinflammatory phenotype and does not cause diabetes mellitus. Cells taken from diabetic patients have been found to exhibit increased production of singlet oxygen molecules and a depletion of antioxidants or loss of antioxidant enzymes activity. Hyperglycemia coupled with oxidative stress can lead to macromolecule damage such as protein damage, DNA, and lipids. Islet β-cells are highly susceptible to this damage, which displaces the activation of signaling pathways [47].

2.2.3 Rheumatoid arthritis

Rheumatoid arthritis is an autoimmune and inflammatory disorder characterized by persistent joint inflammation, which can result in the production of autoantibodies, destruction of the bone and cartilage at the site of inflammation. Mutations in the genes that encode components involved in oxidative stress have been found to play a role in the progression of rheumatoid arthritis. By using murine-induced arthritis, pristine-induced arthritis (PIA), gene regions that are involved in the regulation of different phases of the disease and its severity have been identified using mouse models [48]. The loss of NOX2 function due to polymorphism of the *Ncf1* gene is associated with the manifestation of the arthritis phenotype. Recovery of NOX2 function shows protection against the development of the disease. Sever collagen-induced arthritis with excessive cartilage destruction has also been found in mice with a mutated *Ncf1* gene [43]. Additionally, female mice with a mutated *Ncf1* gene develop spontaneous arthritis postpartum. Autoimmune responses that target cartilage-derived molecules play a vital role in the development of arthritis. The recognition of type II collagen bound to MHC-II by T cells is important for disease initiation. However, this alone is not enough to break the immune tolerance. When the recognition of type II collagen by T cells is coupled with the dysregulation of NOX2, chronic collagen-induced arthritis is initiated [49]. Notably, tolerance break can still occur by modulation of T cell activation. Single nucleotide polymorphism in genes that encode for components of NADPH oxidase complex such as *Ncf4*, *Ncf2*, and *Rac2* and genes of antioxidants enzymes have been linked to arthritis incidence. Low copy numbers of the *Ncf1* gene are a common occurrence in patients with rheumatoid arthritis. Therefore, an increased copy number of the gene can lead to protection against the development of rheumatoid arthritis.

The protective role of ROS in rheumatoid arthritis seems to oppose its destructive role in joint inflammation. During arthritic inflammation, reactive species can exacerbate inflammation while contributing to tissue damage via collagen degradation. Overproduction of ROS is also known to cause cartilage degradation in osteoarthritis. This is achieved by inducing apoptosis in the chondrocytes, cells that are essential for the formation and function of the cartilage [50]. The prevalence of ROS coupled with inflammation causes the disruption of tissue homeostasis and depletion of antioxidants. Hydrogen peroxide and hydroxyl species react with membrane lipids, causing lipid peroxidation and promoting cartilage degeneration while inhibiting self-repair [51]. Cartilage degeneration is achieved by affecting the structures of the structural proteins found on the joint, such as collagen and proteoglycan, causing

the chondrocytes to enter the stage of senescence or cell death, which can eventually lead to subchondral sclerosis and meniscal and ligament damage. ROS and inflammatory cells are contained in the synovial fluid in the joint. Their intense activity not only causes the thinning of the proteoglycan layer and collagen fiber but can also cause functional impotence to the DNA mismatch repair system. This activates the NF-kB and the overproduction of metalloproteinase and DNA adducts such as 8-oxo-hydroxy-7,8-dihydro-2′-deoxyguanosine there by contributing to DNA damage and cell arrest. With reduced antioxidant activity, arthritis progression is favored.

2.2.4 Chronic granulomatous disease

Chronic granulomatous disease (CGD) also known as Bridges-Good or Quie syndrome is a rare inheritable immunodeficiency disorder that affects phagocytes such as neutrophils, monocytes and macrophages. These phagocytes lose their capability to form reactive oxygen compounds such as superoxides, and their capability to eliminate invasive pathogens will therefore, become greatly reduced. CGD is characterized by recurrent bacterial or fungal infection and other dysregulated inflammatory responses, leading to the formation of granulomatous and development of other inflammatory disorders such as colitis. Clinical manifestation of CGD includes pneumonia, adenitis, subcutaneous and hepatic cellulitis, lymphadenitis, osteomyelitis, and sepsis. Clinical studies have also shown the involvement of the genitourinary system and gastrointestinal tract in granulomas [52]. CGD is caused by a reduction in ROS production due to defective activity of the NADPH complex's NOX2. The defect is a result of mutations in the NOX2 catalytic subunit gene gp91phox. About 70% of all reported CGD cases are found in males and they are X-linked. Mutations in the Ncf1 and Ncf2 which are recessive autosomal inherited account for a large number of cases [53]. These mutations can cause reduced ROS production and disable the ability to form NETs, leading to reduced efficiency in pathogen elimination. Interestingly, CGD patients have reduced long term memory immunity. A reduced long term memory in CGD patients and mice may can be due to the relation between the number of neutrophils, NOX2 normal activity and percentage of memory B cells. ROS is known to directly influence the process of memory B cells, and hence a loss of function of NOX2 will reduce this process. Contrary to a reduced number of memory B cells, Cd19+ B cells and immature Ig, the D + CD27- B cells availability increases in patients [54]. ROS also plays a role in the activation and proliferation of B cells. According to *in vitro* studies, the neutralization of ROS in B cells is associated with the attenuation of B cell receptor signaling. CGD patients with Ncf1 mutation have increased expression of type 1 IFNs, which is comparable to 1 IFNs in SLE patients. Type 1 IFNs are known to cause autoimmune disease by inducing the differentiation of dendritic cells capable of presenting organisms own materials to the phagocytic immune cells [55]. This puts CGD patients at a high risk of developing SLE and other autoimmune diseases, including juvenile idiopathic arthritis, antiphospholipid syndrome, and IgG nephropathy.

2.2.5 Sepsis

In septic patients, the loss of redox balance is usually the common cause of severe inflammatory response syndrome. The inflammation is caused by the reactive species from neutrophils and macrophages. The inflammation phase of the disease is marked by reduced mitochondrial ATP synthesis and continuous uptake of antioxidants by

affected cells. The antioxidants aim to fight the deleterious activity of ROS and RNS such as reversible and irreversible oxidative modifications of nucleic acids, lipids, and proteins. The oxidative modification of complex lipids of the mitochondrial inner membrane such as cardiolipin worsens the mitochondrial dysfunction by causing the release of cytochrome c [56, 57]. This causes further reduction of ATP synthesis while elevating the production of reactive species. Excessive and persistent ROS in turn inhibits the translocation of Nrf2, which jeopardizes the antioxidant response mechanism.

2.2.6 Psoriasis

Psoriasis is an immune-mediated chronic inflammatory skin disease that speeds up the growth cycle of skin cells. The disease comprises numerous comorbidities and the multifactorial etiology of cardiovascular diseases, metabolic syndrome, and type 2 diabetes [58]. The pathogenesis of psoriasis is heavily marked by oxidative stress. However, ROS is reported to have protective effects in psoriasis. In mice models with induced psoriasis, elevated levels of ROS will increase the functionality of T regulator cells as well as the expression of indoleamine 2,3-dioxygenase. Increased functionality of T regulator cells results in the reduction of circulating Th17 cells [20]. The protective effects of ROS are further supported by evidence showing the exacerbation of Psoriasis in Ncf1 mutated mice [59]. This suggests that the normal functionality of NOX2 plays a role in the attenuation of psoriasis.

2.2.7 Gout

Gout is one of the most understood and manageable systemic rheumatoid diseases. It is a disorder of purine metabolism that results in the formation of monosodium urate crystals that are deposited in and around the joints. This is mostly due to longstanding hyperuricemia. The urate crystals can induce the release of inflammatory cytokines from monocytes and neutrophils, which cause immense inflammation [60]. The onset of gout attack can last for at most 10 days and then disappear, but the crystals remain present in the joint area. These crystals can induce the formation of large NETs aggregates that end up trapping and degrading the pro-inflammatory mediators. This in turn will limit and resolve the inflammation [61]. The formation of NETs in gout is dependent on the availability of ROS, and a deficiency in ROS can result in a chronic state for the disease. Therefore, a functional oxidative burst is critical for the maintenance of immune tolerance and the resolution of inflammation in gout.

3. ROS in inflammation resolution and autoimmune regulation

The effects of ROSs autoimmunity appears to be more complicated than previously anticipated. ROS is thought to be solely a by-product of the process involved in the cellular response to inflammation or infectious stimuli. However, recent findings have attributed ROS to have vital roles in cellular regulatory and inflammation restraining processes. As their role in numerous cell functions is elaborated, it is now understood that these functions cannot occur without the presence of ROSs. Despite this, the tissue, cell type and time point on which ROS act as anti-inflammatory and immunoregulators are not yet elucidated.

The deficiency of NOX2 has been found to play a key role in the induction of multiple autoimmunity conditions. This is true even in severe bacterial and fungal infections. Inflammatory and autoimmune diseases such as Corhn-like inflammatory disease, idiopathic arthritis and CGD can co-occur. Animal model experiments as well as genome-wide studies have shown a relationship between the polymorphism of Ncf1 and the occurrence of many arthritic diseases [62]. Ncf1 is a cytosolic subunit of NOX2 whose polymorphism signifies diminished production of superoxide. Mutation in the Ncf1 gene which results in loss of NOX2 function has been reported to increase susceptibility to T cell autoreactive activation, cartilage oligomeric matric protein-induced arthritis, and collagen-induced arthritis among others [28]. Additionally, NOX2 derived ROS have been found to have regulatory roles in T-cell dependent nervous system diseases such as multiple sclerosis and Guillan-barre syndrome. Interestingly, NOX2 is also a regulator of autoantibody production and autoimmune inflammation. Therefore, NOX2 activity, especially ROS generation has a crucial preventative effect on the development of autoimmunity and can regulate chronic inflammation [63]. However, it is important that NOX2 is viewed not as a regulator of disease susceptibility but as a regulator of disease severity.

The protective role of ROS in inflammatory and autoimmunity is not universal. In type 1 diabetes, ROS deficiency is associated with safeguarding from diseases, especially in non-obese diabetic animal model. Thayer et al. reported the essential role of macrophage ROS in mediating effector function for CD4+ T cells autoreactivity and autoimmune diabetes pathogenesis [64]. NOX2 generated ROS is also vital in the execution of islet reactivity. However, mutations in the Ncf1 gene which eventually leads to NOX2 malfunction and reduced ROS availability has been found to significantly alter the effector function of macrophages and T-cell subsets [43]. Additionally, collagen antibody transfer which develops independently of phagocytic immune cells can induce arthritis. This phenomenon can be exacerbated by an increase of Ncf1 gene expression. Furthermore, non-classical autoimmune diseases such as monosodium urate crystal-induced inflammation and zymosan show signs of increased inflammation even in the absence of NOX2 and ROS [41, 63]. This shows that certain cases ROS deficiency can be linked to the protection against disease.

4. Conclusion

The development of autoimmunity is defied by a wide range of mechanisms, regulatory cells and tolerance mechanism, which when not properly functioning or are inefficient, fail to decelerate the effects of the interplay of environmental, genetic, and immunological factors. Faults in the immune tolerance mechanism consequentially leads to inappropriate cell death or survival or failure to clear debris which are involved in the pathogenesis of autoimmune diseases. The failure of these systems and the eventual development of autoimmune diseases is aided by inflammatory modulators. Interestingly, this process occurs before the full development of diseases, making the modulators a potential therapeutic target. As such, they can also be used as control points to prevent the exacerbation of the autoimmune diseases. Targeting inflammatory modulators can, therefore, offer opportunities for the development of novel diagnostic methods and effective therapy in the future.

DOI: http://dx.doi.org/10.5772/intechopen.99988

Author details

Joshua Banda and Allan K. Chang*
College of Life and Environmental Science, Wenzhou University, Wenzhou, China

*Address all correspondence to: akcchang@163.com

IntechOpen

References

[1] Oyewole, A.O. and M.A. Birch-Machin, Mitochondria-targeted antioxidants. FASEB J, 2015. 29(12): p. 4766-4771.

[2] Bedard, K. and K.H. Krause, The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. Physiol Rev, 2007. 87(1): p. 245-313.

[3] Medzhitov, R., Origin and physiological roles of inflammation. Nature, 2008. 454(7203): p. 428-435.

[4] Abbas, M., M. Moussa, and H. Akel, Type I Hypersensitivity Reaction, in StatPearls. 2021: Treasure Island (FL).

[5] Uzzaman, A. and S.H. Cho, Chapter 28: Classification of hypersensitivity reactions. Allergy Asthma Proc, 2012. 33 Suppl 1: p. 96-99.

[6] Actor, J.K., Chapter 8 - Immune Hypersensitivities, in Introductory Immunology (Second Edition), J.K. Actor, Editor. 2019, Academic Press. p. 103-110.

[7] Gaspari, A.A., S.I. Katz, and S.F. Martin, Contact Hypersensitivity. Curr Protoc Immunol, 2016. 113: p. 4 2 1-4 2 7.

[8] Oishi, Y. and I. Manabe, Macrophages in inflammation, repair and regeneration. Int Immunol, 2018. 30(11): p. 511-528.

[9] Suzuki, K., Chronic Inflammation as an Immunological Abnormality and Effectiveness of Exercise. Biomolecules, 2019. 9(6).

[10] Germolec, D.R., et al., Markers of Inflammation. Methods Mol Biol, 2018. 1803: p. 57-79.

[11] Roos, D., Chronic Granulomatous Disease. Methods Mol Biol, 2019. 1982: p. 531-542.

[12] Zumla, A. and D.G. James, Granulomatous infections: etiology and classification. Clin Infect Dis, 1996. 23(1): p. 146-158.

[13] Giorgio, S., et al., Granulomas in parasitic diseases: the good and the bad. Parasitol Res, 2020. 119(10): p. 3165-3180.

[14] Abou-Raya, A. and S. Abou-Raya, Inflammation: a pivotal link between autoimmune diseases and atherosclerosis. Autoimmun Rev, 2006. 5(5): p. 331-337.

[15] Selmi, C., Autoimmunity in 2009. Autoimmun Rev, 2010. 9(12): p. 795-800.

[16] Triolo, T.M., et al., Identical and Nonidentical Twins: Risk and Factors Involved in Development of Islet Autoimmunity and Type 1 Diabetes. Diabetes Care, 2019. 42(2): p. 192-199.

[17] Perga, S., et al., The Footprints of Poly-Autoimmunity: Evidence for Common Biological Factors Involved in Multiple Sclerosis and Hashimoto's Thyroiditis. Front Immunol, 2018. 9: p. 311.

[18] Anaya, J.M., et al., The kaleidoscope of autoimmunity: multiple autoimmune syndromes and familial autoimmunity. Expert Rev Clin Immunol, 2007. 3(4): p. 623-635.

[19] Jager, A. and V.K. Kuchroo, Effector and regulatory T-cell subsets in autoimmunity and tissue inflammation. Scand J Immunol, 2010. 72(3): p. 173-184.

[20] Singh, R.P., et al., Th17 cells in inflammation and autoimmunity. Autoimmun Rev, 2014. 13(12): p. 1174-1181.

[21] MacLennan, I.C.M. and C.G. Vinuesa, Dendritic Cells, BAFF, and APRIL: Innate Players in Adaptive Antibody Responses. Immunity, 2002. 17(3): p. 235-238.

[22] Tobon, G.J., et al., Are autoimmune diseases predictable? Autoimmun Rev, 2012. 11(4): p. 259-266.

[23] Quintero, O.L., et al., Autoimmune disease and gender: plausible mechanisms for the female predominance of autoimmunity. J Autoimmun, 2012. 38(2-3): p. J109-J119.

[24] Billi, A.C., J.M. Kahlenberg, and J.E. Gudjonsson, Sex bias in autoimmunity. Curr Opin Rheumatol, 2019. 31(1): p. 53-61.

[25] Amador-Patarroyo, M.J., A. Rodriguez-Rodriguez, and G. Montoya-Ortiz, How does age at onset influence the outcome of autoimmune diseases? Autoimmune Dis, 2012. 2012: p. 251730.

[26] Pordeus, V., et al., A latitudinal gradient study of common anti-infectious agent antibody prevalence in Italy and Colombia. Isr Med Assoc J, 2008. 10(1): p. 65-68.

[27] Ramos, P.S., A.M. Shedlock, and C.D. Langefeld, Genetics of autoimmune diseases: insights from population genetics. J Hum Genet, 2015. 60(11): p. 657-664.

[28] Sorrentino, R., Genetics of autoimmunity: an update. Immunol Lett, 2014. 158(1-2): p. 116-119.

[29] Anaya, J.M., L. Gomez, and J. Castiblanco, Is there a common genetic basis for autoimmune diseases? Clin Dev Immunol, 2006. 13(2-4): p. 185-195.

[30] Marson, A., W.J. Housley, and D.A. Hafler, Genetic basis of autoimmunity. J Clin Invest, 2015. 125(6): p. 2234-2241.

[31] Samanta, D., et al., Multiple Autoimmune Disorders in Aicardi-Goutieres Syndrome. Pediatr Neurol, 2019. 96: p. 37-39.

[32] Stojanovich, L. and D. Marisavljevich, Stress as a trigger of autoimmune disease. Autoimmun Rev, 2008. 7(3): p. 209-213.

[33] Anaya, J.M., et al., Lupus nephritis in Colombians: contrasts and comparisons with other populations. Clin Rev Allergy Immunol, 2011. 40(3): p. 199-207.

[34] Kapsogeorgou, E.K. and A.G. Tzioufas, Autoantibodies in Autoimmune Diseases: Clinical and Critical Evaluation. Isr Med Assoc J, 2016. 18(9): p. 519-524.

[35] Nguyen, G.T., E.R. Green, and J. Mecsas, Neutrophils to the ROScue: Mechanisms of NADPH Oxidase Activation and Bacterial Resistance. Front Cell Infect Microbiol, 2017. 7: p. 373.

[36] Brieger, K., et al., Reactive oxygen species: from health to disease. Swiss Med Wkly, 2012. 142: p. w13659.

[37] Aviello, G., et al., Colitis susceptibility in mice with reactive oxygen species deficiency is mediated by mucus barrier and immune defense defects. Mucosal Immunol, 2019. 12(6): p. 1316-1326.

[38] Mohsenzadegan, M., et al., Altered pattern of Naive and memory B cells and B1 cells in patients with chronic granulomatous disease. Iran J Allergy Asthma Immunol, 2014. 13(3): p. 157-165.

[39] Cachat, J., et al., Altered Humoral Immune Responses and IgG Subtypes in NOX2-Deficient Mice and Patients: A Key Role for NOX2 in Antigen-Presenting Cells. Front Immunol, 2018. 9: p. 1555.

[40] Hari, A., et al., Redirecting soluble antigen for MHC class I cross-presentation during phagocytosis. Eur J Immunol, 2015. 45(2): p. 383-395.

[41] Singel, K.L. and B.H. Segal, NOX2-dependent regulation of inflammation. Clin Sci (Lond), 2016. 130(7): p. 479-490.

[42] Ravindran, M., M.A. Khan, and N. Palaniyar, Neutrophil Extracellular Trap Formation: Physiology, Pathology, and Pharmacology. Biomolecules, 2019. 9(8).

[43] Zhao, J., et al., A missense variant in NCF1 is associated with susceptibility to multiple autoimmune diseases. Nat Genet, 2017. 49(3): p. 433-437.

[44] Morgan, P.E., A.D. Sturgess, and M.J. Davies, Increased levels of serum protein oxidation and correlation with disease activity in systemic lupus erythematosus. Arthritis Rheum, 2005. 52(7): p. 2069-2079.

[45] Thayer, T.C., et al., Superoxide production by macrophages and T cells is critical for the induction of autoreactivity and type 1 diabetes. Diabetes, 2011. 60(8): p. 2144-2151.

[46] Kurien, B.T., et al., Oxidatively modified autoantigens in autoimmune diseases. Free Radic Biol Med, 2006. 41(4): p. 549-556.

[47] Ahmed, N., et al., Degradation products of proteins damaged by glycation, oxidation and nitration in clinical type 1 diabetes. Diabetologia, 2005. 48(8): p. 1590-1603.

[48] Olofsson, P., et al., Identification and isolation of dominant susceptibility loci for pristane-induced arthritis. J Immunol, 2003. 171(1): p. 407-416.

[49] Backlund, J., et al., Genetic control of tolerance to type II collagen and development of arthritis in an autologous collagen-induced arthritis model. J Immunol, 2003. 171(7): p. 3493-3499.

[50] Blanco, F.J., et al., Osteoarthritis chondrocytes die by apoptosis. A possible pathway for osteoarthritis pathology. Arthritis Rheum, 1998. 41(2): p. 284-289.

[51] Tiku, M.L., R. Shah, and G.T. Allison, Evidence linking chondrocyte lipid peroxidation to cartilage matrix protein degradation. Possible role in cartilage aging and the pathogenesis of osteoarthritis. J Biol Chem, 2000. 275(26): p. 20069-20076.

[52] Leiding, J.W. and S.M. Holland, Chronic Granulomatous Disease, in GeneReviews((R)), M.P. Adam, et al., Editors. 1993: Seattle (WA).

[53] Lent-Schochet, D. and I. Jialal, Chronic Granulomatous Disease, in StatPearls. 2021: Treasure Island (FL).

[54] Pozo-Beltran, C.F., et al., B subset cells in patients with chronic granulomatous disease in a Mexican population. Allergol Immunopathol (Madr), 2019. 47(4): p. 372-377.

[55] Crow, M.K., Type I interferon in organ-targeted autoimmune and inflammatory diseases. Arthritis Res Ther, 2010. 12 Suppl 1: p. S5.

[56] Petit, P.X., et al., Tafazzin Mutation Affecting Cardiolipin Leads to Increased Mitochondrial Superoxide Anions and Mitophagy Inhibition in Barth Syndrome. Cells, 2020. 9(10).

[57] Petrosillo, G., F.M. Ruggiero, and G. Paradies, Role of reactive oxygen species and cardiolipin in the release of cytochrome c from mitochondria. FASEB J, 2003. 17(15): p. 2202-2208.

[58] Griffiths, C.E.M. and J.N.W.N. Barker, Pathogenesis and clinical features of psoriasis. The Lancet, 2007. 370(9583): p. 263-271.

[59] Hultqvist, M., et al., Lack of reactive oxygen species breaks T cell tolerance to collagen type II and allows development of arthritis in mice. J Immunol, 2007. 179(3): p. 1431-1437.

[60] Maueroder, C., et al., How neutrophil extracellular traps orchestrate the local immune response in gout. J Mol Med (Berl), 2015. 93(7): p. 727-734.

[61] Schauer, C., et al., Aggregated neutrophil extracellular traps limit inflammation by degrading cytokines and chemokines. Nat Med, 2014. 20(5): p. 511-517.

[62] van Dalen, S.C.M., et al., The role of NOX2-derived reactive oxygen species in collagenase-induced osteoarthritis. Osteoarthritis Cartilage, 2018. 26(12): p. 1722-1732.

[63] Hoffmann, M.H. and H.R. Griffiths, The dual role of Reactive Oxygen Species in autoimmune and inflammatory diseases: evidence from preclinical models. Free Radic Biol Med, 2018. 125: p. 62-71.

[64] Thayer, T.C., et al., Assessing Immune Responses in the Nonobese Diabetic Mouse Model of Type 1 Diabetes. Methods Mol Biol, 2020. 2128: p. 269-289.

Chapter 6

Reactive Oxygen Species (ROS) in the Pathophysiology of Rheumatoid Arthritis (RA)

Haseeb Ahsan, Mohammad Yusuf Hasan and Rizwan Ahmad

Abstract

Free radicals are highly reactive molecules that are unstable and have extremely short-short half-life. They are derived from either oxygen (reactive oxygen species, ROS) or nitrogen (reactive nitrogen species, RNS) in mitochondria, plasma membrane and endoplasmic reticulum due to oxidative stress and damage. ROS/RNS are physiologically useful at low concentrations and are responsible for the activation of redox-sensitive signaling pathways, phagocytosis of infected cells and removal of abnormal and aging cells. The endogenous sources of ROS are the electron transport chain, the respiratory burst of phagocytes and oxidation of lipids. These radicals react with biomolecules such as DNA, proteins and lipids and may cause pathophysiological conditions such as autoimmunity, carcinogenesis, and neurodegenerative diseases. The role of ROS in autoimmune response remains complex and they have been implicated in the initiation, generation and amplification of novel epitopes. ROS also appears to play a critical role in rheumatoid arthritis (RA), a systemic autoimmune disease of the joints also known as inflammatory arthritis (IA). ROS are involved in the initiation of various signaling pathways and have a significant role in the pathophysiology of RA.

Keywords: ROS, RNS, RA, autoimmunity, free radicals, rheumatoid arthritis

1. Introduction

A free radical is a "molecule containing one or more unpaired electron(s) and which is capable of independent existence". Free radicals are highly reactive species and are involved in several metabolic processes including oxidative reactions in mitochondria, and 'oxidative burst' of phagocytes, etc. In excess, free radicals lead to diseases including autoimmune, cardiovascular, neurodegenerative, cancers and must be reduced to minimize these pathological conditions. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are two types of free radicals formed in the body and consist of both radical and non-radical moieties [1]. Under normal conditions, living organisms utilize respiration to survive, they undergo a process of reduction of oxygen molecules through the addition of four electrons resulting in the formation of water. This process produces molecules such as superoxide anion (O_2—·), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH·) as a byproduct. During energy

transduction via electron transport of molecular oxygen, O_2—· is formed intracellularly within the mitochondria, which may potentially lead to the development of a variety of pathophysiological conditions. [2, 3] (**Figure 1**).

The O_2·– is converted into stable non-radical H_2O_2 by the enzyme superoxide dismutase (SOD). Catalase (CAT) and glutathione peroxidase (GPx) convert H_2O_2 to H_2O and O_2. Further, H_2O_2 is converted by myeloperoxidase (MPO) in the neutrophils to hypochlorous acid (HOCl), which is a strong oxidant acting as a bactericidal agent in phagocytic cells. The reaction of HOCl with H_2O_2 yields singlet oxygen ($^1O_2$) and water. Finally, H_2O_2 is converted in a spontaneous reaction catalyzed by Fe^{2+} (Fenton reaction) to the highly reactive ·OH (**Table 1**). The ·OH causes oxidative damage to biological molecules such as lipid, protein and DNA leading to the etiopathogenesis of autoimmune disorders [5]. The excess generation of OH· and peroxynitrite (ONOO—) causes oxidative damage to cell membranes and lipoproteins leading to lipid peroxidation resulting in the formation of harmful compounds such as malondialdehyde (MDA) and thiobarbituric acid reactive substances (TBARS) [6]. ROS/RNS damage nucleic acids leading to change in the function and conformation of DNA resulting in the formation of strand breaks, nitrogenous base damage-causing mutations [1]. The ROS are also produced from other sources such as NADPH oxidase (NOX) inactivated phagocytes and endothelial cells, macrophage and polymorphonuclear cells (PMN), lysosome and microsomes. ROS is also involved in inflammatory reactions through the activation of nuclear transcription factor kappa B (NF-κB), leading to upregulation of pro-inflammatory cytokines and leukocyte adhesion molecules (LAM) [5].

Oxidative stress results from reactive species from the biochemical reactions within the body including NADPH oxidases (NOXs), nitric oxide synthase (NOS), nitrate reductase (NR), mitochondrial electron transport chain (ETC) and the hydrogen sulphide (H_2S) producing enzymes cystathionine-β synthase (CBS) and cystathionine-γlyase (CSE). Superoxide undergoes a dismutation reaction to generate H_2O_2 which, in the presence of transition metal ions (e.g., ferrous ions), forms the ·OH. MPO, produces HOCl MPO is a heme-containing peroxidase expressed mainly in neutrophils and to a lesser degree in monocytes. MPO, in the presence of H_2O_2 and halides, catalyzes the

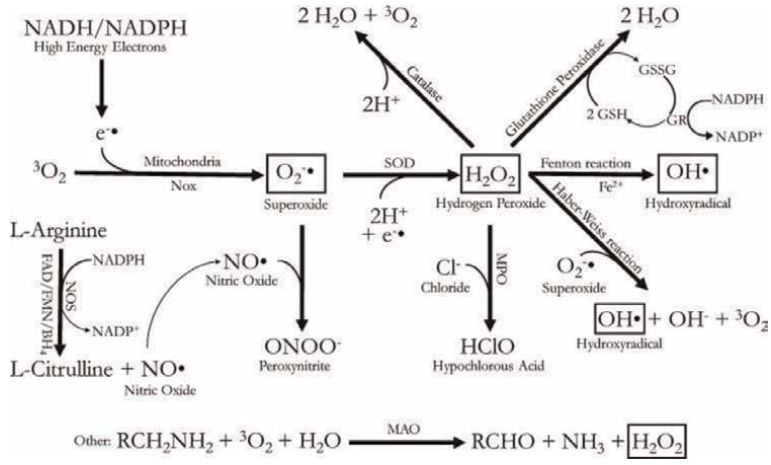


Figure 1.
*Pathways of ROS/RNS in the body. NADH, nicotinamide adenine dinucleotide; NADPH, nicotinamide adenine dinucleotide phosphate; GSH, glutathione; GSSG; glutathione disulfide; SOD, superoxide dismutase; NOS, nitric oxide synthases; MAO, monoamine oxidase; MPO, myeloperoxidase (*reprinted from *[3]).*

Equations	
Oxygen radical generation	
NADPH oxidase:	$2O_2 + NADPH \rightarrow 2O_2^{\bullet -}(\text{superoxide}) + NADPH^+ + H^+$
Spontaneous conversion:	$2O_2^{\bullet -} + 2H^+ \rightarrow \left[2HO_2^{\bullet}(\text{hydroperoxyl radical})\right] \rightarrow O_2 + H_2O_2$
Superoxide dismutase:	$2O_2^{\bullet -} + 2H^+ \rightarrow O_2 + H_2O_2$
Myeloperoxidase:	$Cl^- + H_2O_2 \rightarrow OCl^-(\text{oxidised halide}) + H_2O$
Reactive oxygen species secondary products	
	$H_2O_2 + Fe^{2+} \rightarrow OH^- + OH^{\bullet}(\text{hydroxyl radical}) + Fe^{3+}$
	$Fe^{3+} + O_2^{\bullet -} \rightarrow O_2 + Fe^{2+}$
	$O_2^{\bullet -} + HOCl \rightarrow O_2^{\bullet -} + OH^{\bullet} + Cl^-$
	$H_2O_2 + OCl^- \rightarrow {}^1O_2(\text{singlet oxygen}) + H_2O + Cl^-$
	$NH_3 + HOCl \rightarrow NH_2Cl\ (\text{chloramine}) + H_2O$
	$\underset{(\text{amino acids})}{R - CHNH_2 - COOH} + HOCl \rightarrow \underset{(\text{chloramines})}{R - CHNHCl - COOH} + H_2O$ $\rightarrow \underset{(\text{aldehydes})}{R - CHO} + CO_2 + NH_4^+ + Cl^-$
Nitrogen radical generation and secondary reactions	
Nitric oxide synthetase:	$\text{arginine} + O_2 + NADPH \rightarrow NO^{\bullet} + \text{citrulline} + NADP^+$
	$NO^{\bullet} + O_2^{\bullet -} \rightarrow ONOO^-\ (\text{peroxynitrite})$
	$ONOO^- + H^+ \rightarrow ONOOH\ (\text{peroxynitrous acid})$
	$ONOOH\ (\text{peroxynitrous acid}) \rightarrow NO_3^-\ (\text{nitrate ion})$
	$ONOOH \rightarrow NO_2^+\ (\text{nitronium ion})$
	$ONOOH \rightarrow NO_2^{\bullet}\ (\text{nitrogen dioxide radical})$
	$ONOOH \rightarrow OH^-$
	$ONOOH \rightarrow OH^{\bullet}\ (\text{hydroxyl radical})$
Lipid peroxidation:	$LH + \text{Radical}^{\bullet} \rightarrow L^{\bullet} + RH$
	$L^{\bullet} + O_2 \rightarrow LOO^{\bullet}\ (\text{lipid peroxyl radical})$
	$LH + LOO^{\bullet} \rightarrow L^{\bullet} + LOOH\ (\text{leading to lipid propagation})LOO^{\bullet}$ $+\text{TocOH}\ (\alpha - \text{tocopherol})$ $\rightarrow LOOH + \text{TocO}^{\bullet}\ (\text{chain termination})$

Table 1.
Equations showing products generated by oxygen/nitrogen radicals (reprinted from [4]).

formation of ROS such as HOCl. The MPO/HOCl system plays an important role in microbial killing by neutrophils. Moreover, it has also also been shown to be a local mediator of tissue damage and the inflammation in various inflammatory diseases. When ROS production exceeds the physiological antioxidant defense, oxidative stress occurs consequently leading to oxidative modification of proteins. This protein alteration may lead to the formation of neoepitopes resulting in the formation of autoantibodies [7, 8]. The pathogenesis of autoimmune diseases (ADs) such as RA is

characterized by the loss of peripheral tolerance to autoantigens, excessive activation of T and B cells, which leads to increased levels of cytokines and autoantibodies (rheumatoid factor, RF; anti-cyclic citrullinated peptide antibodies, ACPA; etc.) [9].

Studies have shown that anti-carbamylated proteins anti-CarP and anti-type II collagen antibodies can serve as a promising diagnostic such diagnostic tool in such ADs. The activation of endogenous cellular antioxidant defense systems (e.g., nuclear erythroid 2-related factor 2; Nrf2-dependent pathway), inhibition of ROS/RNS source (e.g., isoform-specific NOX inhibitors), etc., may become the potential future strategies for redox-based therapeutic compounds [7].

2. Pathophysiology of free radicals

When there is an imbalance between free radical production and removal, causing oxidative and resulting in aberrant metabolic processes in the body. To neutralize and eradicate the free radicals, enzymatic and non-enzymatic antioxidants such as vitamin E (tocopherols and tocotrienols), SOD, CAT, GPx, vitamin C (ascorbic acid), beta-carotene (β-carotene), coenzyme Q10 (CoQ10), play an important role in the body's defense system against these toxic species. Thus, these biological molecules demonstrate an essential role in the quenching or removal of harmful free radicals. Antioxidants such as polyphenolic compounds can reduce the concentration of ROS by scavenging them, and hence potentially averting any deleterious oxidative damage to macromolecules [2]. Several degenerative conditions such as cardiovascular, neurological, diabetes, ischemia-reperfusion injury and aging have been shown to be caused by pathophysiology of free radicals. Autoimmune diseases (ADs) such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) are prominent examples where free radical-induced damage occurs in cells and tissues [10]. The diseases, which are caused by free radical, are usually characterized into two categories: one relating to cancer and diabetes involving “mitochondrial oxidative stress” and occur due to impaired glucose tolerance while the second category involves inflammatory oxidative conditions resulting in atherosclerosis and chronic inflammation. Mutation in DNA, which more often occurs due to oxidative damage is strongly correlated to the etiology of cancer, and is one of the initiators of carcinogenesis in which elevated levels of DNA lesions become apparent in many tumors. ROS-induced changes are also found in various diseases of the heart such as cardiomyopathy, ischemic heart disease, hypertension, atherosclerosis, and heart failure [1]. Several studies have established the key role of oxidative stress in the pathology of the joints which includes inflammatory infiltration, synovial proliferation, and angiogenesis [11, 12].

3. Rheumatoid arthritis

RA is a systemic AD of the joints also known as inflammatory arthritis (IA), in which several additional organ systems are known to be involved, including the pulmonary, cardiovascular, ocular, and cutaneous systems. However, the presence of IA, defined as tenderness and swelling consistent with underlying synovitis is the hallmark for the clinical diagnosis of RA. It is characterized by persistent joint inflammation with accompanying bone and cartilage damage and the formation of autoantibodies [13] (**Figure 2**). Most of the RA patients have rheumatoid factor (RF,autoantibody against the Fc portion of IgG) and anti-citrullinated peptide antibodies (ACPA) [14–16]. The exact cause of RA

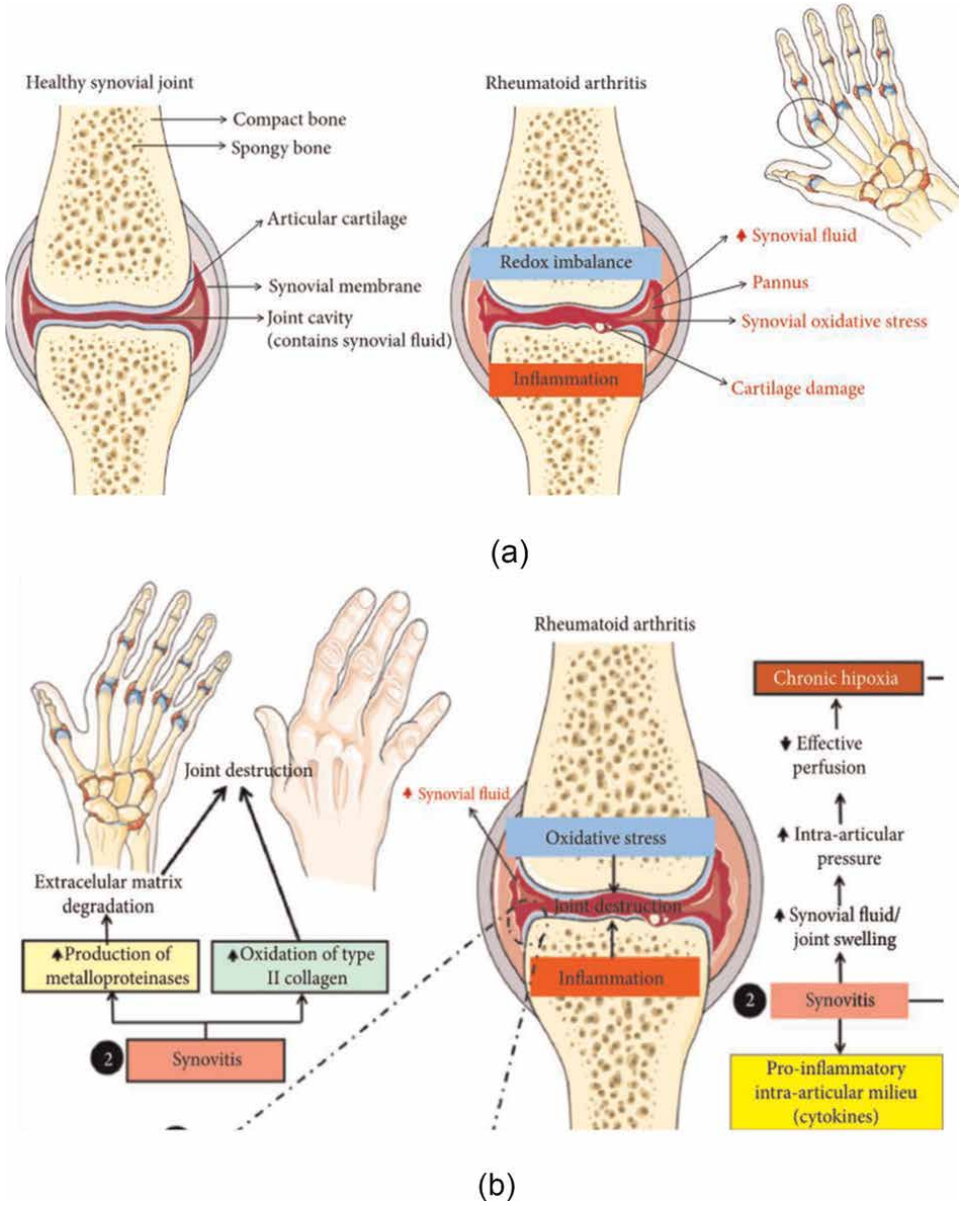


Figure 2.
a. Rheumatoid arthritis. The oxidative stress and local inflammatory mediators manifest mainly in the joint resulting in pain and pain and inflammation which may lead to and joint damage and disability at a later stage (reprinted and modified from *[13]). b. Oxidative stress and inflammation in rheumatoid arthritis* (reprinted and modified from *[13]).*

is still not completely understood, although there is a strong evidence of the genetic involvement in disease process. The class II major histocompatibility complex (MHC) molecules, HLA-DR1 and HLA-DR4 are regarded as the major genetic risk factors in the etiopathogensis of RA [17–19].

Several studies suggest that oxidative stress leads to defective redox signaling and damage to biomolecules in the pathogenesis of RA [12]. The mutations in genes that

encode inflammatory and enzymatic molecules involved in the oxidative burst are responsible for the development of RA. In arthritic inflammation, ROS acts as an inflammatory mediator and contributes to destruction of collagen tissues and the overproduction of ROS is associated with damage leading to cartilage degradation. The damaged chondrocytes, which are essential for the structure and function of cartilage, are stimulated to undergo apoptosis. Fewer chondrocytes are unable to self-repair the cartilage and therefore promote the breakdown of the extracellular matrix in joints. Furthermore, H_2O_2 leads to chondrocyte lipid peroxidation which is also linked to protein oxidation and degradation of cartilage matrix [20].

Additionally, the functional genetic analysis showed that the single nucleotide polymorphisms (SNPs) in the neutrophil cytosolic factor 1 (Ncf1) coding region is associated with genetic susceptibility to RA [21]. The patients with chronic granulomatous disease (CGD) also have amplified susceptibility to RA [21, 22]. Furthermore, a study of NOX2-deficient mice revealed that the absence of ROS averts resistance to autoimmune arthritis. A collagen-induced arthritis model produced by Ncf1 mutation in mice has more acute symptoms, higher anti-CII IgG levels, and stronger T helper type 1 (Th1) responses than wild-type mice. Interestingly, the study also found that T cells from Ncf1-mutated mice reacted more actively to antigen presenting cells (APCs) [23]. Mice with mutated collagen (MMC) have increased resistance to arthritis mediated by a mutated immunodominant epitope in collagen type II that binds to the MHC class II molecule. When these MMC mice are bred with NOX2-deficient mice, their immune tolerance to arthritis disappears, and they display increased autoimmune T cell and higher levels of anti-CII IgG levels [24]. Therefore, the pathophysiology of ROS in RA is an imbalance in homeostasis between pro- and antioxidative and pro and anti-inflammatory conditions, which may lead to the damage of connective tissues, multiple joints and other organs in the body.

4. Conclusion

In ADs, the immune system recognizes and attacks its tissues i.e., self-destruction. Activation, proliferation, and apoptosis of immune cells are dependent on the controlled production of ROS. However, under chronic inflammatory conditions, large amounts of ROS generated are responsible for the pathophysiology of many human disorders. A characteristic feature of ADs is the presence of autoantibodies and inflammatory conditions and cells such as mononuclear phagocytic system, autoreactive T lymphocytes and autoantibody-producing B cells (plasma cells). RA is a systemic autoimmune disease of the joints with underlying synovitis and is also known as inflammatory arthritis. Increased apoptosis and decreased clearance of apoptotic cells observed in systemic autoimmunity may be a be contributing factors in autoimmune disorders such as RA and SLE. Since ROS have been implicated in the initiation and progression of autoimmunity, their role in autoimmunity remains complex. The pathophysiology of ROS in RA though not well understood but could be understood as an imbalance in homeostasis between pro- and antioxidant conditions and pro and anti-inflammatory states, which may become chronic leading to led to damage of connective tissue and multiple joints and other organs in the body. Moreover, it is important to consider the therapeutic development of antioxidants and selective ROS inhibitors as a tool that could be used in the prevention and treatment of a broad range of ADs.

Acknowledgements

HA is thankful to Prof. Fahim H. Khan and Prof. Waseem A. Siddiqui for their encouragement, inspiration and motivation. HA wishes to acknowledge his family members for their endless, everlasting and eternal support in life and wellbeing. RA is extremely grateful to Prof Bassam H. Awary, Prof. Mahdi Abumadni, Dr. M. Madadin and Dr. Kholoud Al Ghamdi for their advice and support.

Conflict of interest

The authors declare that there is no conflict of interest.

Author details

Haseeb Ahsan[1], Mohammad Yusuf Hasan[2] and Rizwan Ahmad[3*]

1 Faculty of Dentistry, Department of Biochemistry, Jamia Millia Islamia, New Delhi, India

2 Department of Biomedical Sciences, School of Biomedical Sciences, Newcastle University, Newcastle upon Tyne, UK

3 College of Medicine, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia

*Address all correspondence to: ahmadriz.biochem@gmail.com; rahassan@iau.edu.sa

References

[1] Ahmad R. In: Ahmad R, editor. Introductory Chapter: Basics of Free Radicals and Antioxidants, Free Radicals, Antioxidants and Diseases. Rijeka: Intech Open; 2018. Available from: https://www.intechopen.com/books/free-radicals-antioxidants-and-diseases/introductory-chapter-basics-of-free-radicals-and-antioxidants

[2] Ahmad R, Ahsan H. Singlet oxygen species and systemic lupus erythematosus: A brief review. Journal of Immunoassay & Immunochemistry. 2019;**40**(4):343-349

[3] Di Dalmazi G, Hirshberg J, Lyle D, Freij JB, Caturegli P. Reactive oxygen species in organ-specific autoimmunity. Auto Immunity Highlights. 2016;7(1):11

[4] Hitchon CA, El-Gabalawy HS. Oxidation in rheumatoid arthritis. Arthritis Research & Therapy. 2004; **6**(6):265-278

[5] Shah D, Mohania D, Mahajan N, Sah S, Paudyal B, Nath SK. Crosstalk between oxidative stress, autophagy and cell death-pathogenesis of autoimmune disease. In: Chatzidionysiou K, editor. Autoimmunity-Pathogenesis, Clinical Aspects and Therapy of Specific Autoimmune Diseases. Rijeka: IntechOpen; 2015. Available from: https://www.intechopen.com/books/autoimmunity-pathogenesis-clinical-aspects-and-therapy-of-specific-autoimmune-diseases/crosstalk-between-oxidative-stress-autophagy-and-cell-death-pathogenesis-of-autoimmune-disease

[6] Ahmad R, Ahsan H. Role of peroxynitrite modified biomolecules in the etiopathogenesis of systemic lupus erythematosus. Clinical and Experimental Medicine. 2014;**14**(1):1-11

[7] Smallwood MJ, Nissim A, Knight AR, Whiteman M, Haigh R, Winyard PG. Oxidative stress in autoimmune rheumatic diseases. Free Radical Biology & Medicine. 2018;**125**: 3-14

[8] Bhatnagar A, Aggarwal A. Oxidative Stress-a major player in the pathophysiology of systemic lupus erythematosus. In: Laher I, editor. Systems Biology of Free Radicals and Antioxidants. Berlin, Heidelberg: Springer; 2014

[9] Lin W, Shen P, Song Y, Huang Y, Tu S. Reactive oxygen species in autoimmune cells: Function, differentiation, and metabolism. Frontiers in Immunology. 2021;**12**:635021

[10] Ahmad R, Hussain A, Ahsan H. Peroxynitrite: Cellular pathology and implications in autoimmunity. Journal of Immunoassay & Immunochemistry. 2019;**40**:123-138

[11] Balogh E, Veale DJ, McGarry T, Orr C, Szekanecz Z, Ng CT, et al. Oxidative stress impairs energy metabolism in primary cells and synovial tissue of patients with rheumatoid arthritis. Arthritis Research & Therapy. 2018;**20**(1):95

[12] Mateen S, Moin S, Zafar A, Khan AQ. Redox signaling in rheumatoid arthritis and the preventive role of polyphenols. Clinica Chimica Acta. 2016; **463**:4-10

[13] da Fonseca LJS, Nunes-Souza V, Goulart MOF, Rabelo LA. Oxidative stress in rheumatoid arthritis: What the future might hold regarding novel biomarkers and add-on therapies. Oxidative Medicine and Cellular Longevity. 2019;**2019**:7536805

[14] Guo Q, Wang Y, Xu D, Nossent J, Pavlos NJ, Xu J. Rheumatoid arthritis: Pathological mechanisms and modern pharmacologic therapies. Bone Research. 2018;**6**:15

[15] Yap HY, Tee SZ, Wong MM, Chow SK, Peh SC, Teow SY. Pathogenic role of immune cells in rheumatoid arthritis: Implications in clinical treatment and biomarker development. Cell. 2018;**7**(10):161

[16] de Brito RS, Baldo DC, Andrade LEC. Clinical and pathophysiologic relevance of autoantibodies in rheumatoid arthritis. Advances in Rheumatology. 2019;**59**:2

[17] van Drongelen V, Holoshitz J. Human leukocyte antigen-disease associations in rheumatoid arthritis. Rheumatic Diseases Clinics of North America. 2017;**43**(3):363-376

[18] Holoshitz J. The rheumatoid arthritis HLA-DRB1 shared epitope. Current Opinion in Rheumatology. 2010;**22**(3): 293-298

[19] Roudier J. Association of MHC and rheumatoid arthritis: Association of RA with HLA-DR4- the role of repertoire selection. Arthritis Research & Therapy. 2000;**2**:217

[20] Kienhöfer D, Boeltz S, Hoffmann MH. Reactive oxygen homeostasis - the balance for preventing autoimmunity. Lupus. 2016;**25**(8):943-954

[21] Zhao J, Ma J, Deng Y, Kelly JA, Kim K, Bang SY, et al. Tsao BP A missense variant in NCF1 is associated with susceptibility to multiple autoimmune diseases. Nature Genetics. 2017;**49**(3):433-437

[22] Battersby AC, Braggins H, Pearce MS, Cale CM, Burns SO, Hackett S, et al. Inflammatory and autoimmune manifestations in X-linked carriers of chronic granulomatous disease in the United Kingdom. The Journal of Allergy and Clinical Immunology. 2017;**140**(2):628-630.e6

[23] Gelderman KA, Hultqvist M, Pizzolla A, Zhao M, Nandakumar KS, Mattsson R, et al. Macrophages suppress T cell responses and arthritis development in mice by producing reactive oxygen species. The Journal of Clinical Investigation. 2007;**117**(10): 3020-3028

[24] Hultqvist M, Bäcklund J, Bauer K, Gelderman KA, Holmdahl R. Lack of reactive oxygen species breaks T cell tolerance to collagen type II and allows development of arthritis in mice. Journal of Immunology. 2007;**179**(3):1431-1437

Chapter 7

Skin Aging: Implications of UV Radiation, Reactive Oxygen Species and Natural Antioxidants

Bristy Ganguly, Manisha Hota and Jyotsnarani Pradhan

Abstract

Skin aging is an inescapable phenomenon that leads to a functional decline of the skin along with emergence of characteristics features such as coarse skin, wrinkles, loss of elasticity and an overall aged appearance. While chronological aging is inevitable occurring with time, photoaging is contributed by Ultraviolet radiation and reactive oxygen species principally which can boost the skin aging process. These processes can however be ameliorated with the help of treatment strategies, one of them being supplementation with antioxidants. This chapter summarizes diverse mechanisms underlying skin aging with regards to Ultraviolet radiation and reactive oxygen species along with role of antioxidants in impeding these processes. Further, it provides a glimpse towards possible future explorations and challenges dominating the field of skin aging.

Keywords: Skin aging, Chronological aging, Photoaging, Ultraviolet Radiation, Reactive Oxygen Species, Antioxidants

1. Introduction

It is common for an individual to aspire a young-looking healthy skin. Nowadays, skin care routines have become an integral part of people's lives belonging to different age groups. People have come to realize it as an essential investment for maintaining a flourishing skin free of aging cues and skin related ailments. But since aging does occur over time, the signs can be coped with by regular care and practicing a conscious approach towards acknowledging skin aging [1].

People grow old with advancing age. As a result of this, the skin also shows aging indications. This phenomenon, where skin aging occurs over time refers to chronological aging. However, these very people are also exposed to external factors that they encounter in the environment. Aging prompted due to these external factors is known as extrinsic aging. If the external factor is Ultraviolet radiation (UVR), then resulting aging process is called photoaging. Thin parched skin and fine wrinkles generally occur during chronological aging. In contrast, photoaged skin is characterized by thickened epidermis and coarse wrinkles [2]. These skins have uneven pigmentation and often show presence of lentigines and freckles [3]. Although underlying routes

for both the aging processes differ, but many overlaps can exist. The fundamental reason behind this is that while people experience certain stage of chronological aging, they also undergo photoaging due to exposure to some amount of UVR on daily basis [4].

The focus of this book chapter is to discuss the importance of studies related to aging especially photoaging, their various underlying mechanism, role of antioxidants in ameliorating the aging signs along with recent advances and challenges associated with skin aging.

2. Significance of photoaging studies

The concept of aging has piqued the curiosity of many people from generation to generation and as a result, huge advances have been made. Among the various scopes of the particular field, recently, the phenomenon of photoaging has grabbed the limelight not only because of limited knowledge about underlying mechanism but also because of connection with associated pathways in other skin related diseases. Possession of a healthy skin has now become of utmost value not only for esthetic reasons but also due to its functional relevance. Simply put, it is like a mirror reflecting our overall health. But for this, the general public needs to have adequate awareness about photoaging, skin cancer and other related skin diseases especially those which are influenced by UVR. In the past, immense amount of research has been performed which have established skin type and UVR exposure as prominent risk factors associated with photoaging and skin cancer development. However, despite such crucial advances, a suitable and effective treatment has been lacking.

3. Role of ultraviolet radiation

The demonization of UVR has occurred due to several investigations conforming its deadly role in eliciting different skin cancers. Consequently, it has been declared as a 'Type I carcinogen' by International Agency for Research on Cancer (IARC). Particularly of concern include UV-A and UV-B radiations. Major contribution of these radiations results in formation of photolesions such as cyclo-butane pyrimidine dimers (CPDs), (6–4) photoproducts (6–4 PPs) together with Dewar isomers through direct or indirect mechanisms [5]. CPDs generally stop advancement of cell cycle by activating the repair machinery whilst 6–4 PPs can prevent apoptosis from occurring [6]. UV-B halts maximum cells in S phase of cell cycle and triggers both p95 and p53. This does not seem to take place for UV-A which halts the cell cycle at S phase only for a limited time. Thus, lesions arising considering UV-A have a higher potential of causing mutation due to lack of active p53 which is vital for stimulating XPC, GADD45 and p48 that are required in turn to induce the Nucleotide excision repair pathway [7, 8].

Moreover, UVR act to dissociate Helicase and DNA polymerase during replication in the S-phase of cell cycle. Consequently, although Helicase keeps on generating ssDNA regions, DNA polymerase stops on encountering a lesion. If a 6–4 PP formation occurs in these ssDNA regions it can both slow repair process while stimulating ATR-Chk-1 pathway. Given the benefits of activating this pathway, one problem still resides which is the elevation of mutation level. Thus, unchecked 6–4 PPs can promote existence of cells portraying higher mutation [9].

Formation of these UV induced photoproducts is not equally distributed throughout the genome, but depends on several factors. Formation of CPDs and 6–4 PPs rely upon nucleosome structure, nucleosomal DNA rotational setting, binding of proteins to transcription factor binding site, chromatin states and particularly certain hypersensitive sites [10]. Telomeres can function as an excellent example of hypersensitive sites prone to UVR. They portray several repeats of TTAGGG sequences and end in the single stranded form at the 3′ end. This end folds and forms a T-loop in assistance with proteins at the ending of a chromosomes thus inhibiting fusion with other chromosomes [11]. Formation and further accumulation of CPDs in such distinct sequences can be high due to near about absence of damage repair [12].

Another factor involves the differential penetration of UVR through the skin layers. As skin embodies numerous cells, so CPD formation could alter with cellular functions. This analysis can be proved as occurrence of 8-oxo dGua and CPD were observed more in fibroblast as compared to keratinocytes under UV-A irradiation. Explanation for this could be as of disparity in endogenous antioxidant activity and distribution of molecules that act as photosensitizers [13, 14]. UVRs particularly UV-B are also held accountable for strand breaks and inception of modified bases such as cytosine photohydrate, thymine glycol 5,6 dihydro thymine and 8-oxoguanine among others [15].

4. Role of reactive oxygen species

An eminent feature of UVR is rise of ROS and reactive nitrogen species. They can either constitute of free radicals or other molecules that are highly toxic or mutagenic to the cell. A free radical is an atom or molecule which contains unpaired electrons in its outer orbit. Due to this property some free radicals are highly unstable and react with other surrounding molecules. They snatch an electron via reacting with another molecule and convert them into a free radical. This free radical generated performs the same process thus initiating a string of events. Among the ROS present, the 'red alert' species include hydroxyl radical, hydrogen peroxide, singlet oxygen, nitric oxide radical and superoxide radical [16].

Main pit for ROS production is the mitochondria. Till now, ten sites have been claimed in mitochondria to form ROS. Multiple sites are either linked to Electron Transport Chain or the Kreb cycle. Complex I has two sites for superoxide formation 1) the Flavin in NADPH oxidizing site 2) the ubiquinone reducing site. Complex III has one site for superoxide production, the quinol oxidizing site. Certain enzymes also take part in ROS generation such as glycerol-3 phosphate dehydrogenase, 2-oxoglutarate dehydrogenase and xanthine oxidase [17]. ROS production inside the cell also results during endoplasmic reticulum (ER) stress. ER is of high priority as it controls protein folding, attachment of additional groups after translation and regulating protein trafficking, to name a few. ROS mediated protein-alterations converts them into displaying an unusual protein structure which can get deposited in the lumen of the organelle. Consequently, this could inhibit translation of mRNA into proteins to limit the damage [18].

ROS concentration is also modulated by pH. Conventionally, the pH in stratum corneum approximates around 4.5 [19]. This value increases and at subsequently deeper layers a pH of about 7.0 is established [20]. Maintenance of this pH gradient is crucial as for its participation in specific skin functions such as defense against

microbes, recovery from injuries and activities of various proteases. This balance is however impaired with aging especially in people eighty years old or above [21]. Imbalance in pH gradient has a detrimental effect on skin from both chronological and photoaging perspective as it influences the photosensitizers contributing to ROS levels. For example, many photosensitizers such as riboflavin, porphyrins, quinones among others comprising of a carbonyl group employ type II mechanism of photosensitization for ROS production wherein energy absorbed by them is transferred directly to a O2 molecule. From this reaction, rise singlet oxygen and superoxide anion [22]. Other sources for ROS generation include 3-hydroxypyridine, N-alkyl 3 hydroxy pyridine and their respective derivatives such as pyridoxine, pyridoxal, pyridoxamine including others. They catalyze reactions by following type I mechanism of photosensitization [23].

Significant contribution towards ROS generation is lent by NADPH oxidase. Irradiation with UV increases the Ca2+ ions concentration in the cell. This increase in the Ca2+ ions level triggers NADPH oxidase to generate ROS species apart from activating Protein kinase C which can phosphorylate Rho GPIα thus permitting Rac to migrate towards the plasma membrane where it could bind to Nox1, NoxO1 and NoxA1 complex. This binding results in stimulating Nox1. Interestingly, UV-A irradiation can produce ceramides in keratinocytes. The underlying mechanisms have not been figured out yet but it is hypothesized that ceramide could activate PI3K which stimulates Rac1 by guanine nucleotide exchange factor β-Pix [24, 25]. Furthermore, leukotriene B4 (LTB4) is also associated with lysophosphatidic acid stimulated ROS level elevation in keratinocytes. LTB4 is synthesized from arachidonic acid by action of several enzymes including 5-lipoxygenase (5-LO). It behaves as a ligand and binds to receptor BLT2 which is expressed ubiquitously in all cells. Moreover, elevation of expression of LTB4 and BLT-2 takes place in keratinocytes under UV-B. BLT-2 triggers ROS levels by activating Nox-1. This event is ensued by stimulation of p38/JNK pathway in keratinocytes [26].

Lipid peroxidation involves lipid molecules reacting with free radicals to produce lipid peroxyl radicals along with hydroperoxides. Other significant outcomes include hexanal, 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA). 4HNE exhibit electrophilic properties and thus react with nucleophiles such as lysine's amino group, imidazole group of histidine and sulfhydryl group of cysteine. Certain metal ions like iron are of significance as lipid hydroperoxides on interacting with transition metals can form lipid peroxyl radicals or lipid alkoxyl radicals [27]. Un-chelated iron produce hydroxyl radical from hydrogen peroxide which can help initiate the lipid peroxidation process [28]. Further irradiation with UV-A has pointed towards elevation of intracellular peroxide levels. Fatty acids, specifically arachidonic acid serve as prime target of these radicals [29, 30]. The iron driven fenton reaction generates hydroxyl radicals along with many lipid peroxides which through the JNK-2 pathway elevate various matrix metalloproteinases (MMPs) [31]. 4 HNE is responsible for elastin modification which could potentially lead to actinic elastosis. 4HNE and acrolein are deposited on elastin fibers which decreases elastin fiber digestion by the elastase released by leukocytes [32].

5. Matrix metalloproteinases: the ECM scissors

Cells are required to maintain a specific collagen turnover. This delicate balance is crucial for remodeling activities while preventing surplus collagen deposition [33].

However, this balance is threatened by numerous agents. Pathways exist for intra as well as extracellular collagen degradation. One of the means of extracellular degradation involve MMP activity.

MMPs come under the Matrixin subfamily which belongs to Zinc metalloprotease family. They play a vital role in tissue reconstruction, cell migration, carcinogenesis, damage recovery and photoaging [34]. Since MMPs function to degrade ECM, their activities are heavily scrutinized by a cell [35]. MMP functioning can be inhibited by α-microglobulin as well as Tissue inhibitor of metalloproteinases (TIMPs). Further, many MMPs are produced as inactivated proenzymes [36]. UV triggered AP-1 expression tends to elevate MMP-1, MMP-3 and MMP-9 levels. Production of activated MMP-9 from human keratinocyte along with MMP-2 can digest type IV collagen found in the basement membrane. Further, they can also digest type V and VII collagen together with elastin [37]. MMP-1 can digest type I collagen which results in cell migration and damage recovery. MMP 14 (membrane bound MMP) can interact with TIMP-2 and pro-MMP-2 resulting in its activation [38]. Certain amount of collagen digestion also occurs due to MMP-8 released from neutrophiles [39]. Human macrophage elastase or MMP-12 acts on elastin fibers after which the degraded products accumulate in the epidermal layer resulting in solar elastosis and keratosis, the distinguishing features of photoaging [40].

6. Signaling routes: manipulation by UVR and ROS

UV induced cell signaling is ligand independent. Therefore, how the cell perceives UVR is still not well defined. A particular study has revealed that Opsin-3 expressed by dermal fibroblast might help sense UVRs. Opsin comes under the GPCR family of receptors. Upon activation they lead to increase in intracellular calcium ion levels which further activate CAMKII. Activated CAMKII can in turn phosphorylate ERK, JNK, p38 and CREB. This event is ensued by rise of MMP-1, 2, 3 and 9 actions [41].

Initially studies conducted concentrated on activation via cytokine or growth factor receptor [42]. Epidermal growth factor receptor belongs to receptor tyrosine kinase (RTK). It is managed by protein tyrosine phosphatase (PTP). PTP bears a cysteine moiety in its active site which is originally employed for catalyzing a phosphohydrolase activity and ceasing the cell signaling cascade. This cysteine moiety can be targeted by ROS which would activate RTK for a prolonged period till ROS are dealt by cellular antioxidants [43]. Activation of receptors leads to recruitment of adaptor proteins for example Src homology-2 [SH2] domains and Src homology 3 domain [SH3]. SH2 interacts with phosphorylated tyrosine residue of the active RTK. SH3 in turn interacts with a phosphorylated proline residue on other targeted proteins. Through this they lead to stimulation of other downstream molecules [44]. Ras near the cell membrane is a guanine nucleotide binding protein that shuttles between GTP/GDP bound form. The GTP bound form interacts with and activates Raf [45]. Raf sequentially activates MAP kinase kinase/MEK via phosphorylation. This is ensued by origination of active ERK [46]. These MAPKs can interact with several proteins and activate them. Interactions are mostly formed with conserved landing sites on the substrate. Jun D has two domains, a D domain and DEF domain. D domain is bound and phosphorylated by JNK under stress while DEF domain is phosphorylated by ERK which is activated by growth factors binding to EGFR receptor [47]. In presence of UVR and/or hydrogen peroxide, overexpression of c-Fos/Jun D or Jun D alone could prevent apoptosis by lowering Caspase-3 levels. Expression of c-Fos plus Jun D

also activates AP-1. Transcription factor AP-1 is a dimer of basic region leucine zipper protein which may constitute of Jun [c-Jun, Jun B and Jun D] and Fos [c-Fos, Fos B, Fra-1 and Fra-2] subfamilies in the cell. This complex is further joined by proteins from ATF and JDP subfamilies [48, 49]. AP-1 and TGFβ/Smad signaling cascade control procollagen synthesis. TGFβ are cytokines that allow pro-collagen formation by binding to their receptors, TβRI and TβRII. Upon meeting of TGFβ with their receptors, Smad 2 and 3 are activated which complex with Smad 4 to form a heteromeric molecule. This complex enters nucleus and helps in transcription of TGFβ regulated genes. In contrast, AP-1 inhibits procollagen formation coupled by destruction of existing collagen fibers by upregulating MMPs [50]. Interestingly, effects of AP-1 are subject to participating dimer proteins. Theoretically, AP-1 can appear as 18 different homo and hetero dimer and this can influence its activity. It is hypothesized that this could be as of differential DNA binding along with different transcriptional activity. Furthermore, the differentiation status of cell also influences the properties of different AP-1 transcription factor forming proteins [51].

Besides this pathway, other cascades such as JNK and p38 MAPK Pathway are also stimulated. c-Jun NH2 terminal kinases [JNK] are activated upon receiving stress signals such as cytokines, ROS, and UVR. A specific stimulus activates the MAP3Ks. The MAP3K catalyze phosphorylation of MAP2K. MAP2K has two isoforms, MKK4 and MKK7. Active MKK4 and MKK7 interact and generate active JNK. Some of its targets comprise of nuclear proteins like AP-1, Elk-1 and c-Jun. p38 MAPK is stimulated under stress [52, 53]. p38 MAPK is switched on by upstream lying MKK3 and MKK6 kinases. If JNK pathway is initially active then MKK4 of the JNK pathway can activate p38 MAPK. Apart from this, p38 can also undergo auto-phosphorylation. p38 MAPK is noteworthy as of its high interactivity with multiple nuclear proteins along with protein kinases [54]. Although many investigations involving UVR and cell fate have taken place, there are still certain gaps. A molecular switch exits between p38 and p53 proteins. If damage due to UV radiation is less, the cell opts to repair and survive. Under this situation p38 and p53 are activated separately. p53 stimulates p21waf1/CIP which stops cell cycle from advancing ahead by regulating Cdc25A/B. This leads to arrest in G1/S and G2/M phase. But if cellular damage is too high then p38 phosphorylates p53 resulting in active p53 that initiates apoptosis [55].

Recently it was shown that p38 MAPK can stimulate HIF when keratinocytes are irradiated with UVR. p38 activates HIF-1 which in turn activates Noxa. Active Noxa catalyzes degradation of Mcl-1. Mcl-1 controls differentiation and survival of keratinocytes. Therefore, its degradation leads to apoptosis. This cascade might be affected by ROS as ROS can stimulate Ask/p38 MAPK pathway. Again, HIF-1 is also responsive towards redox status in the cell [56]. Secretion of cytokines like IL-8 can stimulate the three MAPK pathways. Its activation can turn on ATF-2 nuclear protein which induces elevated secretion of MMP-3 in fibroblast [57].

Since UVR amplifies ROS generation, a prime pathway activated under such conditions is the NFκB pathway. NFκB pathway activation leads to transcription of numerous genes coupled with immunological and inflammatory responses. NFκB family constitutes of 5 proteins that is p65 (Rel A), Rel B, c-Rel, p50 (NFκB 1) and p52 (NFκB 2). Stimulation of suitable receptors upon ligand binding activates IKK (IκB kinase). IKK constitutes of IKKα, IKKβ and IKKγ. The IKK molecule phosphorylates IκBα. After phosphorylation, IκBα is poly-ubiquitinylated at lysine moieties which marks it for degradation by proteasome. The resulting active NFκB is then transported to nucleus for transcription of specific genes. Some of the prime signaling events are illustrated in **Figure 1** [58, 59].

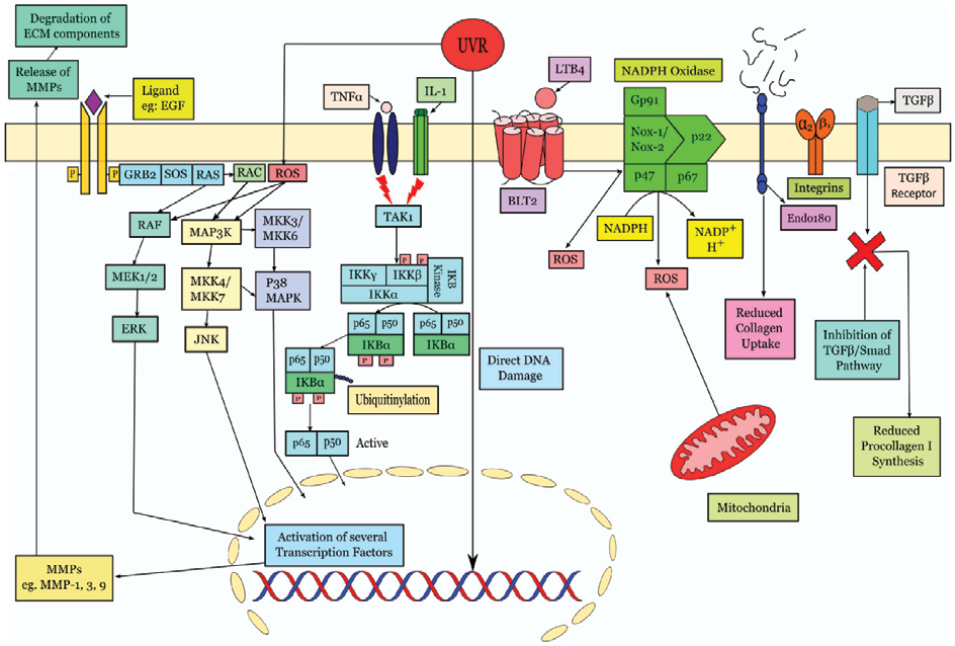


Figure 1.
Schematic diagram showing the important signaling pathways involved in Photoaging.

7. Effects of aging on extracellular matrix

ECM comprises of a network of numerous molecules and primarily functions to provide support to cells. It presents the cells with growth factors which in turn bind their respective receptors to influence response of the cells to various stimulus. Moreover, the matrix present around a cell also protects it from any possible mechanical injury [60, 61].

Depending upon specific function and anatomical position, ECM organization may differ. Exposure to UVR can adversely affect this particular organization. One instance involves the fragmentation of long collagen fibrils into short chains along with accumulation of unstructured elastin containing material which terminates in decreased skin durability [62]. Deposition of fragmented collagen fibrils can further result in decreased formation of type I procollagen [63]. Another effect UVR have is the production of MMPs that function as ECM scissors. Continued exposure to UVR results in elastin fiber injury which can terminate in wrinkle formation. In skin, elastin fibers are classified as oxytalan fibers, elaumin fibers and dermal elastin fibers depending upon their structure and position [64]. They form a network that is prone to elastase secreted from UV exposed fibroblasts and neutrophils. Fibroblast elastase can digest elaumin and oxytalan fibers while neutrophil elastase is able to digest all three elastin fibers which is crucial especially during inflammation and damage recovery. But since the numbers of neutrophils is low in dermis, major damage is contributed by fibroblast elastase which culminate in wrinkles [65, 66].

Another hallmark of photoaging involves decreased proteoglycan content [67]. Hyaluronan degradation in UV irradiated skin occurs due to increased HYBID protein activity. Large hyaluronan molecules bought inside the cell in clathrin coated vesicles

are broken down into smaller components and released into the external surrounding. These hyaluronan components act as inflammatory signals and can additionally lead to wrinkle formation [68].

ECM remodeling generally involves uptake of degraded collagen fibers inside fibroblasts via receptors like integrin α2β1 and Endo180 receptor. Endo180 receptor belongs to type I membrane protein occurring in the plasma membrane. It binds to type I, IV and V collagen to internalize them inside the cell. Few studies regarding endo180 receptor and photoaging have shown that cells exposed to UVR have low endo180 receptor expression which ultimately results in decreased internalization of collagen fragments. Additionally, IL-1α secretion from keratinocytes under UV exposure blocks endo180 receptor expression. Further investigations regarding endo180 receptor expression in photoaged skin are worth undertaking in the future [69, 70].

8. Natural antioxidants: role against aging

Role of antioxidants in quenching ROS has been appreciated for a long time. Recently, a 15 yearlong study showed that ROS formation could be lessened by 10% in people aged 45 or above by simply having an antioxidant rich diet [71].

Antioxidants are compounds that quench the free radicals by providing an electron and generating stable compounds that are less harmful to a cell. In this manner they inhibit the train of events that occur when free radicals oxidize other biomolecules. Antioxidants can be classified into different categories based on enzymatic/non-enzymatic activity, occurrence, solubility although other miscellaneous molecules displaying antioxidant properties have also been reported. Examples of certain regular antioxidants found in a cell involve Superoxide dismutase (SOD), Glutathione peroxidase (GPx) and Catalase (CAT). Non-enzymatic antioxidants include Vitamin E, C, thioredoxin, glutathione and melatonin. Further antioxidants such as minerals (Cu, Zn, Mn, Se), other vitamins, polyphenols and carotenoids are also derived from regular diet [72].

Usually, cells maintain a fine balance between ROS formation and their quenching by resident antioxidants. But, UVR shift the balance towards ROS formation resulting in a phenomenon called 'Oxidative stress', where resident antioxidants become incapable of quenching the high amount of ROS formed [73]. This state can however be circumvented by additional antioxidant supplementation. Drawbacks of synthetic antioxidants such as toxicity, low solubility, cost and a presumed negative outlook towards chemical products have increased the demand for natural antioxidants [74]. These antioxidants are naturally available in numerous sources like vegetables, fruits, spices, herbs, edible mushrooms among others [75]. Furthermore, beverages derived from these sources are also rich in antioxidants and have gained increased populace especially among younger generation.

Till date, several pure phytochemicals and plant-based compounds have been reported for their antioxidant and anti-photoaging activity. We have tried to summarize some important phytochemicals as potential antioxidant and anti-photoaging agents in **Tables 1** and **2**.

8.1 Beverages as source of antioxidants

Various beverages, specifically mixed juices have abundant antioxidants as they are made using various fruits and vegetables [86]. Other beverages like coffee and

Name of antioxidant molecule	Source of radiation	Main observations		Ref.
		Upregulation of	Downregulation of	
Neferine	UV-A UV-B	• SOD and GPx activities	• Wrinkle emergence • collagen destruction in dermis	[76]
Quercetin	Solar UV light system		• collagen breakdown • transcription factors such as AP-1 and NFκB • pERK, pJNK, pAkt and pSTAT3 levels • PKCδ and JAK2 activity	[77]
3,5-dicaffeoyl-epi-quinic acid	UV-B	• SOD-1, HO-1 antioxidant activity with pronounced Nrf-2 level manifestation	• production of ROS • TNF-α, COX-2, IL-6 and IL-1β levels	[78]
Ursolic acid	UV-B	• antioxidant activity of SOD-1, GPx, CAT and GSH • Bcl-2 levels inhibiting apoptosis	• ROS production and lipid peroxidation • Apoptosis by reducing Bax and caspase-3 levels • TNF-α activity and NFκB level • MMP-2 and MMP-9 activity	[79]
Decanal	UV-B	• cAMP and PKA Cα levels • mRNAs for *COL1A1, COL1A2, COL3A1* for collagen production • mRNA for hyaluronic acid synthase 2 required for hyaluronic acid production	• activated MAPKs such as p-ERK, p-JNK and p-P38 • AP-1 dimers that is p-c-Jun and p-c-Fos • mRNAs for MMP-1, MMP-3 and MMP-9	[80]

Table 1.
Pure phytochemicals and their anti-photoaging properties.

tea also include antioxidants and can help alleviate photoaging signs. Green tea is enriched in bioactive compounds like methyl-xanthins, flavon-3-ols and catechins such as (−)-epicatechin (EC), (−)-epicatechin-3-gallate (ECG), (−)-epigallocatechin (EGC) and (−)-epigallocatechin-3-gallate (EGCG). EGCG can inhibit erythema and neutrophil infiltration in the skin induced by UVR [87]. Drinking Green tea can ameliorate other photoaging markers like increased numbers of dermal cysts, sebaceous glands enlargement, vacuole formation and increased epidermal thickness. Green tea polyphenols are also elemental in decreasing MMP-2, 3, 7 and 9 levels along with reduced oxidation and carbonylation of proteins upregulated under UVR [88, 89]. Polyphenols and Chlorogenic acid found in coffee can help avoid free radical associated diseases. Reduction in pigmentation spots induced under UV was seen in Japanese women who consumed coffee at a higher rate [90]. *Coffee Arabica* leaf extracts are rich in caffeic acid, chlorogenic acid and phenols. These bioactive compounds help lower MMP-1, 3 and 9 expressions while elevating type I procollagen production. Further, a decrease p-ERK, p-JNK and p-p38 levels has also been shown thereby effectively blocking UV induced activation of key signaling pathways [91].

Name	Source of radiation	Main observations		Ref.
		Upregulation of	Downregulation of	
Melaleuca leucadendron L	UV-B	• antioxidant levels of SOD, CAT, GPx • number of cells present in G0/G1 phase	• ROS production • activated Caspase-3 action	[81]
Rosa multiflora Thunb	UV-B	• procollagen I production at both mRNA and protein levels	• ROS production • cytokines such as IL-6 and IL-8 • MMP-1 conc. at both mRNA and protein level • levels of p-ERK, p-JNK and p-P65 (NFκB protein)	[82]
Carica papaya	UV-B	• TGF-β cytokine levels required for collagen formation • mRNA levels for procollagen type I	• ROS production up to 60% • activated MAPKs such as p-ERK, p-JNK and p-P38 • AP-1 dimers that is p-c-Jun (44%) and p-c-Fos (89%) • MMP-1 and MMP-3 levels along with IL-6 levels	[83]
Dioscorea alata (purple sweet potato)	UV-B	• antioxidant levels of SOD, CAT and GSH-Px • Of hydroxyproline levels with diminished collagen breakdown	• ROS production and lipid peroxidation • activated MAPKs such as p-ERK, p-JNK, p-P38 levels and NFκB protein levels in nucleus • inflammation markers such as TNF-α and IL-6 • skin tissue associated changes occurring under UV	[84]
Nypa fruticans Wurmb	UV-B	• collagen content following elevated levels of COL1A mRNA levels	• ROS levels • MMP-1, MMP-8 and MMP-13 mRNA levels • p-JNK and p-P38 levels followed by diminished levels of active NFκB and AP-1 protein (p-c-Jun)	[85]

Table 2.
Plant-derived phytocompounds having antioxidant and anti-photoaging activity.

A diterpenoid, Atractyligenin found in Coffee silverskin has been shown to decrease ROS levels by 62% post UV treatment, modulate MMP-1, 2, 3 expressions, inhibit activation of MAPK pathways and increase Endo180 receptor expression [92].

8.2 Animal sources for derivation of antioxidants

Antioxidant peptides derived from *Pinctada fucata* protein have been shown to improve collagen fiber density by increasing hydroxyproline level besides inhibiting

lipid peroxidation [93]. Similar observations have also been observed with Oyster (*Crassostrea gigias*). Additionally, it could modulate AP-1 transcription factor, regulate MAPK signaling and consequently block MMP expression. It could also stimulate TGFβ/SMAD pathway enhancing collagen synthesis [94].

Usually, fish industries generate various waste products such as fish head, internal organs, scales and skin. But several investigations have shown that utilization of these resources for treatment of photoaged skin could be propitious especially in terms of waste management. One such example involves the use of gelatin polypeptides from COD fish (*Gadus microcephalus*) skin. The gelatin polypeptides contain key amino acids that are essential for collagen synthesis. They help elevate hydroxyproline level and collagen synthesis. Beside these, they are able to decrease MDA levels and improve antioxidant activities of SOD, CAT and GPx. In this way they help inhibit photoaging [95]. Similar results have also been observed for antioxidant collagen peptides obtained from Jellyfish umbrella and Silver carp (*Hypophthalmicthys molitrix*) skin [96, 97]. Tilapia (*Oreochromis niloticus*) gelatin peptides have been shown to possess hydroxyl radical scavenging property. This is crucial as hydroxyl radicals are prime ROS species that result in strong oxidative stress [98]. In a study, peptide from Tilapia gelatin hydroxylates was shown to reduce ROS levels and inhibit oxidative damage to DNA besides elevating antioxidant levels (SOD, GSH). More significantly, the peptide inhibited MMP 1 and MMP-9 expression along with regulating MAPK and NFκB pathways [99]. Similar results were also observed in another study where additionally the authors pointed out the role of C-terminus of peptide terminating with GLY-LEU in impeding MMP-1 activity [100].

8.3 Derivation of antioxidants from marine sources

Till now, different algal species have been utilized for numerous investigations aiming towards analysis of molecules effective against photoaging. *Tetraselmis suecica* microalgal extract was found to reduce MDA level while increasing SOD and GSH levels. Further, the extract could improve type I procollagen production and lower MMP-1 expression [101]. Fucoxanthin, a carotenoid found in *Undaria pinnatifida* inhibits wrinkle formation by blocking MMP-13 expression [102].

Recently, another compound known as Mycosporine like amino acid (MAA) has come into limelight. They consist of cyclic rings of aminohexenimine or aminocyclohexenone and can vary among themselves with respect to amino acids and groups attached to them [103]. They are mostly found in algal species belonging to orders Bangiales, Gracilariales, Ceramiales and Gigartinales where their prime function is to provide UV protection [104]. Production of MAA is affected by numerous factors like salt concentration, UV, amount and type of nutrient available among others [105, 106]. Recently, two MAA, Shinorine and Porphyra-334 were reported to prevent photoaging by reducing MDA levels upto ≈56% and restoring antioxidants like SOD, GSH-Px and CAT. Additionally, a reduction in NFκB, IL-1β and IL-6 was also found [107]. Porphyra-334 can further be helpful by increasing procollagen production and blocking UV induced MMP production [108]. In another study where human keratinocytes were treated with UVR, expression profiling along with functional network analysis portrayed that miRNAs regulated by porphyra-334 could modulate various genes associated with UV affected processes such as cell proliferation, apoptosis and translational elongation [109]. Another compound that has attracted the interest of many researchers is Scytonemin. It is found in polysaccharide sheath of cyanobacteria and like MAA functions as an UV protectant [105]. Like MAA, its production

is increased under photo-oxidative stress, elevated temperature osmotic stress and UVR. Recently, scytonemin from *Rivularia sp*. HKAR-4 was tested for analyzing its photoprotective function. Here, scytonemin successfully reduced intracellular ROS production along with thymine dimer formation [110]. In future it is expected that more such molecules will be revealed as research deepens in these areas with time.

9. Other compounds used for treatment of photoaged skin

Apart from use of antioxidants as the treatment strategy for photoaged skin, various other compounds are also used. Among these, Retinoids and alpha-hydroxy acids (AHA) hold a significant track record. Retinoids are Vitamin A derivatives that can be naturally occurring or synthetic. Dietary forms of Vitamin A, Retinol and retinyl esters undergo transformations by enzymes alcohol dehydrogenase and microsomal retinol dehydrogenase to yield Retinaldehyde. Retinaldehyde, in turn, is converted in to Retinoic acid by suitable retinaldehyde dehydrogenases. These retinoic acids show their effects via interacting with suitable intranuclear retinoic acid receptors (RARs) [111]. Among these, all-trans-retinoic acid (Tretinoin) serves as key retinoid for treatment of photoaged skin. However due to tendency of tretinoin to cause skin irritation, there has been a switch to more effective alternatives like Adapalene which is a synthetic retinoid [112]. Adapalene has been shown to be effective against photoaging, solar lentigines and actinic keratosis treatment. A recent study has compared the efficacy of Adapalene and Tretinoin for treatment of cutaneous photoaging. Through various experiments, it was established that Adapalene 0.3% was equally effective as Tretinoin 0.05% and thus could be employed as a future treatment for moderate photoaging [113].

While the first line approach for treatment mostly involves application of topical creams, the second line approach consists of use of chemical peels. This is where AHA play a crucial role. AHAs are a group of naturally available organic acids such as glycolic acid, lactic acid, critic acid, malic acid among others where hydroxyl groups (1 or more) are attached to alpha carbon atom that in turn is bonded to first carbon atom after acid group. AHAs are employed in peels for treatment of melasma, age spots, hyperpigmentation among others. But use of AHAs for treatment of skin photo damage is still controversial. Moreover, it has been reported that AHA function may depend on concentration as well as properties of the compound used [114]. A very recent study tested the efficacy of TCA- Lactic acid chemical peels for treatment of photoaging. It showed that the peels were able to inhibit tyrosinase, collagenase and elastase activity in in-vitro experiments. Further, an increased expression of COL1A, COL3B, elastin and fibronectin genes were shown to occur when peel solution was used for 3D human skin model [115]. Another study compared the efficacy of AHA-ReT cream (double conjugated retinoid cream) with 1% retinol cream and 0.025% Tretinoin cream for treatment of photoaging. Here, they found that AHA-ReT cream was more effective in reducing wrinkles, erythema and increasing hydration coupled with less irritation on application. Thus, such methods are expected to open new avenues in photo-damaged skin treatment [116].

10. Future perspective and challenges

Recently, role of cathepsins with respect to photoaging has been pursued to a greater extent. Cathepsins have important functions in ECM maintenance, apoptosis,

cell proliferation and differentiation among others. Thus, cells maintain a tight regulation of cathepsins that is hampered during the photoaging process. A study reported diminished levels of cathepsins B, D and K whereas cathepsin G was upregulated in the photoaged skin. Cathepsin G in turn could increase various MMP expression [117]. Such imbalance in cathepsin regulation can have strong adverse effects on the autophagy process. Several studies have shown that autophagosomes of photoaged cells display an upregulation of p62, downregulation of cathepsin L, limited degradation of LC3II protein and an unchanged expression of Beclin-1. All these changes hint towards the reduced lysosomal ability towards degradation of unwanted products [118].

Another area that has received abundant attention involves formation of advanced glycation end products (AGE). They are produced when various proteins undergo oxidation followed by glycation and get accumulated in tissues with advancing age, although they are also found in UVR exposed skin. This occurs as a result of decreased cathepsin D levels which is essential for AGE degradation inside the cell [119, 120].

With advancement in molecular field, several investigations have also concentrated on miRNA expression in terms of chronological aging and photoaging [121]. Specifically, sharp changes in miR-34 and miR-29 family regulation have been reported. For instance, increased miR-34b-5p expression in aged dermis has been reported for decreased COL1A1 and elastin expression together with increased MMP-1 expression [122].

These investigations mentioned above, only portray a fraction of the whole story required for understanding the aging process including both intrinsic and extrinsic aging. Various questions yet await their answers regarding aging. Due to the blurred line between photoaging and chronological aging, differentiating characteristics of these two aging types is often approached with confusion. This occurs because variation with respect to skin aging in different individuals is often subject to photoaging locations, natural skin pigmentation and exposure time period among the other factors. Challenges concerning antioxidants have also been proposed by many authors. Antioxidant level in the skin is affected by oxidative stress along with diet of an individual. To alleviate the signs of photoaging, exogenous supplementation of antioxidants can be given either by extrinsic application in the form of topical creams or intrinsic/systemic application in the form of tablets or capsules. Both the forms of supplementation have their own set of drawbacks and advantages [123]. Moreover, in case of plant derived antioxidants, different parts of the plant have different expression levels of a particular bioactive compound that acts as antioxidant. This forms an important factor while extracting particular antioxidants.

Apart from these challenges, a more daunting analysis that can be made is that radiation whether UV, visible or infrared can adversely affect the skin. To combat this problem various sunscreen formulations are used. But till now, most sunscreen formulations provide protection against specific wavelength ranges of UV region only. This shows the need for formulation of sunscreens which provide a broader protection [124].

11. Conclusions

Aging is a part of life. It is a complex process, affected by both intrinsic and extrinsic factors. Further addition of photoaging process induced by UVR makes studying aging and taking protective measures against it more complex. Therefore, alleviating

aging signs is still in requirement of more innovative ideas that are expected to be developed with more knowledge about the various mechanisms involved under aging and important investigations that can further shed light onto this phenomenon.

Acknowledgements

The author is thankful to Department of Biotechnology, Utkal University, Vanivihar, Bhubaneswar-751004, Odisha, India, for their kind support and unconditional help in preparation of this book chapter.

Author details

Bristy Ganguly, Manisha Hota and Jyotsnarani Pradhan*
Department of Biotechnology, Utkal University, Bhubaneswar, Odisha, India

*Address all correspondence to: jyotsna.biotech@utkaluniversity.ac.in

References

[1] Farage MA, Miller KW, Berardesca E, Maibach HI. Psychological and social implications of aging skin: Normal aging and the effects of cutaneous disease. Textbook of Aging Skin. 2010;949-57.

[2] Zhang S, Duan E. Fighting against Skin Aging. Cell Transplant. 2018 May;27(5):729-38.

[3] Rittié L, Fisher GJ. Natural and Sun-Induced Aging of Human Skin. Cold Spring Harb Perspect Med. 2015 Jan;5(1):a015370.

[4] Shirata MMF, Alves G a. D, Campos PMBGM. Photoageing-related skin changes in different age groups: a clinical evaluation by biophysical and imaging techniques. International Journal of Cosmetic Science. 2019;41(3): 265-73.

[5] Cadet J, Douki T. Formation of UV-induced DNA damage contributing to skin cancer development. Photochem Photobiol Sci. 2018 Dec 4;17(12):1816-41.

[6] Lo H-L, Nakajima S, Ma L, Walter B, Yasui A, Ethell DW, et al. Differential biologic effects of CPD and 6-4PP UV-induced DNA damage on the induction of apoptosis and cell-cycle arrest. BMC Cancer. 2005 Oct 19;5:135.

[7] Mouret S, Philippe C, Gracia-Chantegrel J, Banyasz A, Karpati S, Markovitsi D, et al. UVA-induced cyclobutane pyrimidine dimers in DNA: a direct photochemical mechanism? Org Biomol Chem. 2010 Apr 7;8(7):1706-11.

[8] Rünger TM, Farahvash B, Hatvani Z, Rees A. Comparison of DNA damage responses following equimutagenic doses of UVA and UVB: a less effective cell cycle arrest with UVA may render UVA-induced pyrimidine dimers more mutagenic than UVB-induced ones. Photochem Photobiol Sci. 2012 Jan;11(1):207-15.

[9] Hung K-F, Sidorova JM, Nghiem P, Kawasumi M. The 6-4 photoproduct is the trigger of UV-induced replication blockage and ATR activation. Proc Natl Acad Sci U S A. 2020 Jun 9;117(23): 12806-16.

[10] Mao P, Smerdon MJ, Roberts SA, Wyrick JJ. Chromosomal landscape of UV damage formation and repair at single-nucleotide resolution. PNAS. 2016 Aug 9;113(32):9057-62.

[11] Opresko PL, Fan J, Danzy S, Wilson DM, Bohr VA. Oxidative damage in telomeric DNA disrupts recognition by TRF1 and TRF2. Nucleic Acids Res. 2005;33(4):1230-9.

[12] Rochette PJ, Brash DE. Human telomeres are hypersensitive to UV-induced DNA Damage and refractory to repair. PLoS Genet. 2010 Apr 29;6(4): e1000926.

[13] Shindo Y, Witt E, Han D, Epstein W, Packer L. Enzymic and non-enzymic antioxidants in epidermis and dermis of human skin. J Invest Dermatol. 1994 Jan;102(1):122-4.

[14] Courdavault S, Baudouin C, Charveron M, Favier A, Cadet J, Douki T. Larger yield of cyclobutane dimers than 8-oxo-7,8-dihydroguanine in the DNA of UVA-irradiated human skin cells. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis. 2004 Nov 22;556(1):135-42.

[15] Jiang Y, Rabbi M, Kim M, Ke C, Lee W, Clark RL, et al. UVA Generates

Pyrimidine Dimers in DNA Directly. Biophys J. 2009 Feb 4;96(3):1151-8.

[16] Phaniendra A, Jestadi DB, Periyasamy L. Free Radicals: Properties, Sources, Targets, and Their Implication in Various Diseases. Indian J Clin Biochem. 2015 Jan;30(1):11-26.

[17] Quinlan CL, Perevoshchikova IV, Hey-Mogensen M, Orr AL, Brand MD. Sites of reactive oxygen species generation by mitochondria oxidizing different substrates. Redox Biol. 2013;1:304-12.

[18] Farrukh MR, Nissar UA, Afnan Q, Rafiq RA, Sharma L, Amin S, et al. Oxidative stress mediated Ca(2+) release manifests endoplasmic reticulum stress leading to unfolded protein response in UV-B irradiated human skin cells. J Dermatol Sci. 2014 Jul;75(1):24-35.

[19] Park SJ, Choi WW, Kwon OS, Chung JH, Eun HC, Earm YE, et al. Acidic pH-activated Cl Current and Intracellular Ca Response in Human Keratinocytes. Korean J Physiol Pharmacol. 2008 Aug;12(4): 177-83.

[20] Boer M, Duchnik E, Maleszka R, Marchlewicz M. Structural and biophysical characteristics of human skin in maintaining proper epidermal barrier function. Postepy Dermatol Alergol. 2016 Feb;33(1):1-5.

[21] Park G, Oh D-S, Kim Y-U, Park M-K. Acceleration of Collagen Breakdown by Extracellular Basic pH in Human Dermal Fibroblasts. Skin Pharmacol Physiol. 2016;29(4):204-9.

[22] Dalle Carbonare M, Pathak MA. Skin photosensitizing agents and the role of reactive oxygen species in photoaging. J Photochem Photobiol B. 1992 Jun 30;14(1-2):105-24.

[23] Wondrak GT, Roberts MJ, Jacobson MK, Jacobson EL. 3-hydroxypyridine chromophores are endogenous sensitizers of photooxidative stress in human skin cells. J Biol Chem. 2004 Jul 16;279(29):30009-20.

[24] Valencia A, Kochevar IE. Nox1-based NADPH oxidase is the major source of UVA-induced reactive oxygen species in human keratinocytes. J Invest Dermatol. 2008 Jan;128(1):214-22.

[25] Masaki H, Izutsu Y, Yahagi S, Okano Y. Reactive Oxygen Species in HaCaT Keratinocytes After UVB Irradiation Are Triggered by Intracellular Ca2+ Levels. Journal of Investigative Dermatology Symposium Proceedings. 2009 Aug 1;14(1):50-2.

[26] Ryu H-C, Kim C, Kim J-Y, Chung J-H, Kim J-H. UVB Radiation Induces Apoptosis in Keratinocytes by Activating a Pathway Linked to "BLT2-Reactive Oxygen Species." Journal of Investigative Dermatology. 2010 Apr 1;130(4): 1095-106.

[27] Ayala A, Muñoz MF, Argüelles S. Lipid Peroxidation: Production, Metabolism, and Signaling Mechanisms of Malondialdehyde and 4-Hydroxy-2-Nonenal. Oxidative Medicine and Cellular Longevity [Internet]. 2014 [cited 2021 Jul 15];2014. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4066722/

[28] Polte T, Tyrrell RM. Involvement of lipid peroxidation and organic peroxides in UVA-induced matrix metalloproteinase-1 expression. Free Radic Biol Med. 2004 Jun 15;36(12): 1566-74.

[29] Quiec D, Mazière C, Santus R, André P, Redziniak G, Chevy F, et al. Polyunsaturated fatty acid enrichment increases ultraviolet A-induced lipid

peroxidation in NCTC 2544 human keratinocytes. J Invest Dermatol. 1995 Jun;104(6):964-9.

[30] Morlière P, Salmon S, Aubailly M, Risler A, Santus R. Sensitization of skin fibroblasts to UVA by excess iron. Biochimica et Biophysica Acta (BBA) - General Subjects. 1997 Mar 15;1334(2): 283-90.

[31] Brenneisen P, Wenk J, Klotz LO, Wlaschek M, Briviba K, Krieg T, et al. Central role of Ferrous/Ferric iron in the ultraviolet B irradiation-mediated signaling pathway leading to increased interstitial collagenase (matrix-degrading metalloprotease (MMP)-1) and stromelysin-1 (MMP-3) mRNA levels in cultured human dermal fibroblasts. J Biol Chem. 1998 Feb 27;273(9):5279-87.

[32] Larroque-Cardoso P, Camaré C, Nadal-Wollbold F, Grazide M-H, Pucelle M, Garoby-Salom S, et al. Elastin Modification by 4-Hydroxynonenal in Hairless Mice Exposed to UV-A. Role in Photoaging and Actinic Elastosis. Journal of Investigative Dermatology. 2015 Jul 1;135(7):1873-81.

[33] Karsdal MA, Genovese F, Madsen EA, Manon-Jensen T, Schuppan D. Collagen and tissue turnover as a function of age: Implications for fibrosis. J Hepatol. 2016 Jan;64(1):103-9.

[34] Nagase H, Visse R, Murphy G. Structure and function of matrix metalloproteinases and TIMPs. Cardiovasc Res. 2006 Feb 15;69(3):562-73.

[35] Lu P, Takai K, Weaver VM, Werb Z. Extracellular matrix degradation and remodeling in development and disease. Cold Spring Harb Perspect Biol. 2011 Dec 1;3(12):a005058.

[36] Löffek S, Schilling O, Franzke C-W. Biological role of matrix metalloproteinases: a critical balance. European Respiratory Journal. 2011 Jul 1;38(1):191-208.

[37] Pittayapruek P, Meephansan J, Prapapan O, Komine M, Ohtsuki M. Role of Matrix Metalloproteinases in Photoaging and Photocarcinogenesis. Int J Mol Sci. 2016 Jun 2;17(6):E868.

[38] Page-McCaw A, Ewald AJ, Werb Z. Matrix metalloproteinases and the regulation of tissue remodelling. Nat Rev Mol Cell Biol. 2007 Mar;8(3):221-33.

[39] Fisher GJ, Choi HC, Bata-Csorgo Z, Shao Y, Datta S, Wang ZQ, et al. Ultraviolet irradiation increases matrix metalloproteinase-8 protein in human skin in vivo. J Invest Dermatol. 2001 Aug;117(2):219-26.

[40] Chung JH, Seo JY, Lee MK, Eun HC, Lee JH, Kang S, et al. Ultraviolet modulation of human macrophage metalloelastase in human skin in vivo. J Invest Dermatol. 2002 Aug;119(2):507-12.

[41] Lan Y, Wang Y, Lu H. Opsin 3 is a key regulator of ultraviolet A-induced photoageing in human dermal fibroblast cells. Br J Dermatol. 2020 May;182(5):1228-44.

[42] Rosette C, Karin M. Ultraviolet light and osmotic stress: activation of the JNK cascade through multiple growth factor and cytokine receptors. Science. 1996 Nov 15;274(5290):1194-7.

[43] Ostman A, Böhmer FD. Regulation of receptor tyrosine kinase signaling by protein tyrosine phosphatases. Trends Cell Biol. 2001 Jun;11(6):258-66.

[44] Pawson T, Scott JD. Signaling through scaffold, anchoring, and adaptor proteins. Science. 1997 Dec 19;278(5346):2075-80.

[45] Vojtek AB, Hollenberg SM, Cooper JA. Mammalian Ras interacts directly with the serine/threonine kinase Raf. Cell. 1993 Jul 16;74(1):205-14.

[46] Shaul YD, Seger R. The MEK/ERK cascade: from signaling specificity to diverse functions. Biochim Biophys Acta. 2007 Aug;1773(8):1213-26.

[47] Whitmarsh AJ. Regulation of gene transcription by mitogen-activated protein kinase signaling pathways. Biochim Biophys Acta. 2007 Aug;1773(8):1285-98.

[48] Zhou H, Gao J, Lu ZY, Lu L, Dai W, Xu M. Role of c-Fos/JunD in protecting stress-induced cell death. Cell Prolif. 2007 Jun;40(3):431-44.

[49] Hess J, Angel P, Schorpp-Kistner M. AP-1 subunits: quarrel and harmony among siblings. J Cell Sci. 2004 Dec 1;117(Pt 25):5965-73.

[50] Kang S, Fisher GJ, Voorhees JJ. Photoaging: pathogenesis, prevention, and treatment. Clin Geriatr Med. 2001 Nov;17(4):643-59, v–vi.

[51] Eckert RL, Adhikary G, Young CA, Jans R, Crish JF, Xu W, et al. AP1 Transcription Factors in Epidermal Differentiation and Skin Cancer. Journal of Skin Cancer. 2013 May 23;2013:e537028.

[52] Weston CR, Davis RJ. The JNK signal transduction pathway. Curr Opin Cell Biol. 2007 Apr;19(2):142-9.

[53] Hammouda MB, Ford AE, Liu Y, Zhang JY. The JNK Signaling Pathway in Inflammatory Skin Disorders and Cancer. Cells. 2020 Apr;9(4):857.

[54] Cuenda A, Rousseau S. p38 MAP-kinases pathway regulation, function and role in human diseases. Biochim Biophys Acta. 2007 Aug;1773(8):1358-75.

[55] Gong X, Liu A, Ming X, Deng P, Jiang Y. UV-induced interaction between p38 MAPK and p53 serves as a molecular switch in determining cell fate. FEBS Letters. 2010;584(23):4711-6.

[56] Nys K, Van Laethem A, Michiels C, Rubio N, Piette JG, Garmyn M, et al. A p38MAPK/HIF-1 Pathway Initiated by UVB Irradiation Is Required to Induce Noxa and Apoptosis of Human Keratinocytes. Journal of Investigative Dermatology. 2010 Sep 1;130(9): 2269-76.

[57] Kitanaka N, Nakano R, Sakai M, Kitanaka T, Namba S, Konno T, et al. ERK1/ATF-2 signaling axis contributes to interleukin-1β-induced MMP-3 expression in dermal fibroblasts. PLOS ONE. 2019 Sep 19;14(9):e0222869.

[58] Liu T, Zhang L, Joo D, Sun S-C. NF-κB signaling in inflammation. Sig Transduct Target Ther. 2017 Jul 14;2(1):1-9.

[59] Cooper SJ, Bowden GT. Ultraviolet B regulation of transcription factor families: roles of nuclear factor-kappa B (NF-kappaB) and activator protein-1 (AP-1) in UVB-induced skin carcinogenesis. Curr Cancer Drug Targets. 2007 Jun;7(4):325-34.

[60] Gelse K, Pöschl E, Aigner T. Collagens--structure, function, and biosynthesis. Adv Drug Deliv Rev. 2003 Nov 28;55(12):1531-46.

[61] Kular JK, Basu S, Sharma RI. The extracellular matrix: Structure, composition, age-related differences, tools for analysis and applications for tissue engineering. J Tissue Eng. 2014;5:2041731414557112.

[62] Fisher GJ, Datta S, Wang Z, Li XY, Quan T, Chung JH, et al. c-Jun-dependent inhibition of cutaneous

procollagen transcription following ultraviolet irradiation is reversed by all-trans retinoic acid. J Clin Invest. 2000 Sep;106(5):663-70.

[63] Varani J, Warner RL, Gharaee-Kermani M, Phan SH, Kang S, Chung JH, et al. Vitamin A antagonizes decreased cell growth and elevated collagen-degrading matrix metalloproteinases and stimulates collagen accumulation in naturally aged human skin. J Invest Dermatol. 2000 Mar;114(3):480-6.

[64] Cotta-Pereira G, Guerra Rodrigo F, Bittencourt-Sampaio S. Oxytalan, elaunin, and elastic fibers in the human skin. J Invest Dermatol. 1976 Mar;66(3): 143-8.

[65] Takeuchi H, Gomi T, Shishido M, Watanabe H, Suenobu N. Neutrophil elastase contributes to extracellular matrix damage induced by chronic low-dose UV irradiation in a hairless mouse photoaging model. J Dermatol Sci. 2010 Dec;60(3):151-8.

[66] Tsuji N, Moriwaki S, Suzuki Y, Takema Y, Imokawa G. The role of elastases secreted by fibroblasts in wrinkle formation: implication through selective inhibition of elastase activity. Photochem Photobiol. 2001 Aug;74(2): 283-90.

[67] McCabe MC, Hill RC, Calderone K, Cui Y, Yan Y, Quan T, et al. Alterations in extracellular matrix composition during aging and photoaging of the skin. Matrix Biol Plus. 2020 Nov;8:100041.

[68] Yoshida H, Okada Y. Role of HYBID (Hyaluronan Binding Protein Involved in Hyaluronan Depolymerization), Alias KIAA1199/CEMIP, in Hyaluronan Degradation in Normal and Photoaged Skin. Int J Mol Sci. 2019 Nov 19;20(22):5804.

[69] Tang S, Lucius R, Wenck H, Gallinat S, Weise JM. UV-mediated downregulation of the endocytic collagen receptor, Endo180, contributes to accumulation of extracellular collagen fragments in photoaged skin. J Dermatol Sci. 2013 Apr;70(1):42-8.

[70] Iwahashi H, Kawashima Y, Masaki H. Interleukin-1 alpha derived from ultraviolet B-exposed keratinocytes is associated with a decrease of endocytic collagen receptor Endo180. Photodermatol Photoimmunol Photomed. 2020 Jan;36(1):34-41.

[71] Hughes MCB, Williams GM, Pageon H, Fourtanier A, Green AC. Dietary Antioxidant Capacity and Skin Photoaging: A 15-Year Longitudinal Study. J Invest Dermatol. 2021 Apr;141(4S):1111-1118.e2.

[72] Kumar S, Sharma S, Vasudeva N. Review on antioxidants and evaluation procedures. Chin J Integr Med. 2017 Oct 6;

[73] Wenk J, Brenneisen P, Meewes C, Wlaschek M, Peters T, Blaudschun R, et al. UV-induced oxidative stress and photoaging. Curr Probl Dermatol. 2001;29:83-94.

[74] J Mbah C, Orabueze I, H Okorie N. Antioxidants Properties of Natural and Synthetic Chemical Compounds: Therapeutic Effects on Biological System. Act Scie Pharma. 2019 May 3;3(6):28-42.

[75] Anwar H, Hussain G, Mustafa I. Antioxidants from Natural Sources [Internet]. Antioxidants in Foods and Its Applications. IntechOpen; 2018 [cited 2021 Jul 15]. Available from: https://www.intechopen.com/books/antioxidants-in-foods-and-its-applications/antioxidants-from-natural-sources

[76] Khan A, Bai H, Khan A, Bai Z. Neferine prevents ultraviolet radiation-induced skin photoaging. Exp Ther Med. 2020 May;19(5):3189-96.

[77] Shin EJ, Lee JS, Hong S, Lim T-G, Byun S. Quercetin Directly Targets JAK2 and PKCδ and Prevents UV-Induced Photoaging in Human Skin. Int J Mol Sci. 2019 Oct 23;20(21):E5262.

[78] Oh JH, Karadeniz F, Lee JI, Seo Y, Kong C-S. Protective effect of 3,5-dicaffeoyl-epi-quinic acid against UVB-induced photoaging in human HaCaT keratinocytes. Mol Med Rep. 2019 Jul;20(1):763-70.

[79] Samivel R, Nagarajan RP, Subramanian U, Khan AA, Masmali A, Almubrad T, et al. Inhibitory Effect of Ursolic Acid on Ultraviolet B Radiation-Induced Oxidative Stress and Proinflammatory Response-Mediated Senescence in Human Skin Dermal Fibroblasts. Oxidative Medicine and Cellular Longevity. 2020 Jun 15;2020:e1246510.

[80] Kang W, Choi D, Park T. Decanal Protects against UVB-Induced Photoaging in Human Dermal Fibroblasts via the cAMP Pathway. Nutrients. 2020 May;12(5):1214.

[81] Bianchini Silva LS, Perasoli FB, Carvalho KV, Vieira KM, Paz Lopes MT, Bianco de Souza GH, et al. *Melaleuca leucadendron* (L.) L. flower extract exhibits antioxidant and photoprotective activities in human keratinocytes exposed to ultraviolet B radiation. Free Radic Biol Med. 2020 Nov 1;159:54-65.

[82] Kwak CS, Yang J, Shin C-Y, Chung JH. Rosa multiflora Thunb Flower Extract Attenuates Ultraviolet-Induced Photoaging in Skin Cells and Hairless Mice. J Med Food. 2020 Sep;23(9): 988-97.

[83] Seo S, Ngo H, Hwang E, Park B, Yi T-H. Protective effects of *Carica papaya* leaf against skin photodamage by blocking production of matrix metalloproteinases and collagen degradation in UVB-irradiated normal human dermal fibroblasts. South African Journal of Botany. 2020 Jul 1;131: 398-405.

[84] Zhi Q, Lei L, Li F, Zhao J, Yin R, Ming J. The anthocyanin extracts from purple-fleshed sweet potato exhibited anti-photoaging effects on ultraviolent B-irradiated BALB/c-nu mouse skin. Journal of Functional Foods. 2020 Jan 1;64:103640.

[85] Choi H-J, Alam MB, Baek M-E, Kwon Y-G, Lim J-Y, Lee S-H. Protection against UVB-Induced Photoaging by *Nypa fruticans* via Inhibition of MAPK/AP-1/MMP-1 Signaling. Oxidative Medicine and Cellular Longevity. 2020 Jun 23;2020:e2905362.

[86] Islam J, Kabir Y. 5 - Effects and Mechanisms of Antioxidant-Rich Functional Beverages on Disease Prevention. In: Grumezescu AM, Holban AM, editors. Functional and Medicinal Beverages [Internet]. Academic Press; 2019 [cited 2021 Jul 15]. p. 157-98. Available from: https://www.sciencedirect.com/science/article/pii/B9780128163979000054

[87] Katiyar SK, Matsui MS, Elmets CA, Mukhtar H. Polyphenolic antioxidant (−)-epigallocatechin-3-gallate from green tea reduces UVB-induced inflammatory responses and infiltration of leukocytes in human skin. Photochem Photobiol. 1999 Feb;69(2):148-53.

[88] Hassan SMA, Hussein AJ, Saeed AK. Role of Green Tea in Reducing Epidermal Thickness upon Ultraviolet Light-B Injury in BALB/c Mice. Advances in Biology. 2015 Oct 1;2015:e890632.

[89] Vayalil PK, Mittal A, Hara Y, Elmets CA, Katiyar SK. Green tea polyphenols prevent ultraviolet light-induced oxidative damage and matrix metalloproteinases expression in mouse skin. J Invest Dermatol. 2004 Jun;122(6):1480-7.

[90] Fukushima Y, Takahashi Y, Hori Y, Kishimoto Y, Shiga K, Tanaka Y, et al. Skin photoprotection and consumption of coffee and polyphenols in healthy middle-aged Japanese females. Int J Dermatol. 2015 Apr;54(4):410-8.

[91] Chiang H-M, Lin T-J, Chiu C-Y, Chang C-W, Hsu K-C, Fan P-C, et al. *Coffea arabica* extract and its constituents prevent photoaging by suppressing MMPs expression and MAP kinase pathway. Food Chem Toxicol. 2011 Jan;49(1):309-18.

[92] Xuan SH, Lee NH, Park SN. Atractyligenin, a terpenoid isolated from coffee silverskin, inhibits cutaneous photoaging. J Photochem Photobiol B. 2019 May;194:166-73.

[93] Wu Y, Tian Q, Li L, Khan M, Yang X, Zhang Z, et al. Inhibitory effect of antioxidant peptides derived from Pinctada fucata protein on ultraviolet-induced photoaging in mice. Journal of Functional Foods. 2013 Feb 19;5:527-38.

[94] Bang JS, Choung S-Y. Inhibitory effect of oyster hydrolysate on wrinkle formation against UVB irradiation in human dermal fibroblast via MAPK/AP-1 and TGFβ/Smad pathway. Journal of Photochemistry and Photobiology B: Biology. 2020 Aug 1;209:111946.

[95] Hou H, Li B, Zhao X, Zhuang Y, Ren G, Yan M, et al. The effect of Pacific cod (*Gadus macrocephalus*) skin gelatin polypeptides on UV radiation induced skin photoaging in ICR mice. Food Chemistry. 2009 Aug 1;115:945-50.

[96] Fan J, Zhuang Y, Li B. Effects of collagen and collagen hydrolysate from jellyfish umbrella on histological and immunity changes of mice photoaging. Nutrients. 2013 Jan 17;5(1):223-33.

[97] Zhang L, Zheng Y, Cheng X, Meng M, Luo Y, Li B. The anti-photoaging effect of antioxidant collagen peptides from silver carp (*Hypophthalmichthys molitrix*) skin is preferable to tea polyphenols and casein peptides. Food Funct. 2017 Apr 19;8(4):1698-707.

[98] Sun L, Zhang Y, Zhuang Y. Antiphotoaging effect and purification of an antioxidant peptide from tilapia (*Oreochromis niloticus*) gelatin peptides. Journal of Functional Foods. 2013 Jan 31;5:154-62.

[99] Xiao Z, Liang P, Chen J, Chen M-F, Gong F, Li C, et al. A Peptide YGDEY from Tilapia Gelatin Hydrolysates Inhibits UVB-mediated Skin Photoaging by Regulating MMP-1 and MMP-9 Expression in HaCaT Cells. Photochem Photobiol. 2019 Nov;95(6):1424-32.

[100] Liping S, Qiuming L, Jian F, Xiao L, Yongliang Z. Purification and Characterization of Peptides Inhibiting MMP-1 Activity with C Terminate of Gly-Leu from Simulated Gastrointestinal Digestion Hydrolysates of Tilapia (*Oreochromis niloticus*) Skin Gelatin. J Agric Food Chem. 2018 Jan 24;66(3): 593-601.

[101] Jo WS, Yang KM, Park HS, Kim GY, Nam BH, Jeong MH, et al. Effect of Microalgal Extracts of Tetraselmis suecica against UVB-Induced Photoaging in Human Skin Fibroblasts. Toxicol Res. 2012 Dec;28(4):241-8.

[102] Urikura I, Sugawara T, Hirata T. Protective effect of Fucoxanthin against UVB-induced skin photoaging in hairless

mice. Biosci Biotechnol Biochem. 2011;75(4):757-60.

[103] Conde FR, Churio MS, Previtali CM. The photoprotector mechanism of mycosporine-like amino acids. Excited-state properties and photostability of porphyra-334 in aqueous solution. J Photochem Photobiol B. 2000 Jul;56(2-3):139-44.

[104] Sun Y, Zhang N, Zhou J, Dong S, Zhang X, Guo L, et al. Distribution, Contents, and Types of Mycosporine-Like Amino Acids (MAAs) in Marine Macroalgae and a Database for MAAs Based on These Characteristics. Marine Drugs. 2020 Jan;18(1):43.

[105] Fuentes-Tristan S, Parra-Saldivar R, Iqbal HMN, Carrillo-Nieves D. Bioinspired biomolecules: Mycosporine-like amino acids and scytonemin from Lyngbya sp. with UV-protection potentialities. Journal of Photochemistry and Photobiology B: Biology. 2019 Dec 1;201:111684.

[106] Gharib R, Tabarzad M, Hosseinabadi T. Effect of High Salinity on Mycosporine-Like Amino Acid Production in Desmodesmus sp. Trends in Peptide and Protein Sciences. 2020 Jun 24;5.

[107] Ying R, Zhang Z, Zhu H, Li B, Hou H. The Protective Effect of Mycosporine-Like Amino Acids (MAAs) from *Porphyra yezoensis* in a Mouse Model of UV Irradiation-Induced Photoaging. Mar Drugs. 2019 Aug 14;17(8):E470.

[108] Ryu J, Park S-J, Kim I-H, Choi YH, Nam T-J. Protective effect of porphyra-334 on UVA-induced photoaging in human skin fibroblasts. Int J Mol Med. 2014 Sep;34(3):796-803.

[109] Suh S-S, Lee SG, Youn UJ, Han SJ, Kim I-C, Kim S. Comprehensive Expression Profiling and Functional Network Analysis of Porphyra-334, One Mycosporine-Like Amino Acid (MAA), in Human Keratinocyte Exposed with UV-radiation. Mar Drugs. 2017 Jun 24;15(7):E196.

[110] Rastogi RP, Sinha RP, Incharoensakdi A. Partial characterization, UV-induction and photoprotective function of sunscreen pigment, scytonemin from Rivularia sp. HKAR-4. Chemosphere. 2013 Nov;93(9):1874-8.

[111] Szymański Ł, Skopek R, Palusińska M, Schenk T, Stengel S, Lewicki S, et al. Retinoic Acid and Its Derivatives in Skin. Cells. 2020 Dec 11;9(12):E2660.

[112] Ascenso A, Ribeiro H, Marques HC, Oliveira H, Santos C, Simões S. Is tretinoin still a key agent for photoaging management? Mini Rev Med Chem. 2014;14(8):629-41.

[113] Bagatin E, Gonçalves H de S, Sato M, Almeida LMC, Miot HA. Comparable efficacy of adapalene 0.3% gel and tretinoin 0.05% cream as treatment for cutaneous photoaging. Eur J Dermatol. 2018 Jun 1;28(3):343-50.

[114] Tang S-C, Yang J-H. Dual Effects of Alpha-Hydroxy Acids on the Skin. Molecules. 2018 Apr 10;23(4):863.

[115] Bhardwaj V, Sharma K, Maksimovic S, Fan A, Adams-Woodford A, Mao J. Professional-Grade TCA-Lactic Acid Chemical Peel: Elucidating Mode of Action to Treat Photoaging and Hyperpigmentation. Front Med (Lausanne). 2021;8:617068.

[116] McDaniel DH, Mazur C, Wortzman MS, Nelson DB. Efficacy and tolerability of a double-conjugated retinoid cream vs 1.0% retinol cream or

0.025% tretinoin cream in subjects with mild to severe photoaging. J Cosmet Dermatol. 2017 Dec;16(4):542-8.

[117] Y Z, W L, M W, Hi M. Expression of cathepsins in human skin photoaging. Skin pharmacology and physiology [Internet]. 2011 [cited 2021 Jul 15];24(1). Available from: https://pubmed.ncbi.nlm.nih.gov/20588086/

[118] Huang Y, Li Y, Qu Y, Zheng Y, Ouyang M, Zhang Y, et al. UVA-induced photoaging inhibits autophagic degradation by impairing lysosomal function in dermal fibroblasts. Biochem Biophys Res Commun. 2019 Oct 22;518(4):611-8.

[119] Crisan M, Taulescu M, Crisan D, Cosgarea R, Parvu A, Cãtoi C, et al. Expression of Advanced Glycation End-Products on Sun-Exposed and Non-Exposed Cutaneous Sites during the Ageing Process in Humans. PLOS ONE. 2013 Oct 7;8(10):e75003.

[120] Xu X, Zheng Y, Huang Y, Chen J, Gong Z, Li Y, et al. Cathepsin D contributes to the accumulation of advanced glycation end products during photoaging. J Dermatol Sci. 2018 Jun;90(3):263-75.

[121] Blackstone BN, Wilgus TA, Roy S, Wulff BC, Powell HM. Skin Biomechanics and miRNA Expression Following Chronic UVB Irradiation. Adv Wound Care (New Rochelle). 2020 Mar 1;9(3):79-89.

[122] Li T, Yan X, Jiang M, Xiang L. The comparison of microRNA profile of the dermis between the young and elderly. J Dermatol Sci. 2016 May;82(2):75-83.

[123] Darvin ME, Fluhr JW, Schanzer S, Richter H, Patzelt A, Meinke MC, et al. Dermal carotenoid level and kinetics after topical and systemic administration of antioxidants: enrichment strategies in a controlled in vivo study. J Dermatol Sci. 2011 Oct;64(1):53-8.

[124] Battie C, Jitsukawa S, Bernerd F, Del Bino S, Marionnet C, Verschoore M. New insights in photoaging, UVA induced damage and skin types. Exp Dermatol. 2014 Oct;23 Suppl 1:7-12.

Chapter 8

Epigenetic Modifications Involved in Ageing Process: The Role of Histone Methylation of SET-Domain

Pambu Lelo Aaron, Zakuani Luzinga Nadege, Fabrice Ndayisenga and Bongo Ngiala Gedeon

Abstract

Ageing is characterized by the maintaining deterioration of homeostatic processes over time, leading to functional decline and increased risk of disease and death. Several distinct mechanisms underlying ageing have been reported and mounting shreds of evidence have shown that histone methylation, an epigenetic marker, regulates gene expression during ageing. Recently, SET-domain genes have gained attentions and have been identified as histone methyltransferase involved in ageing process. Deletion of these genes extends lifespan and increased oxidative stress resistance in *Caenorhabditis elegans* depends on the *daf-16* activity in the insulin/IGF pathway. In this chapter, we propose to investigate the role of histone methylation in the process of ageing and oxidative stress with an emphasis on the role of *set-18* gene in ageing process.

Keywords: SET-domain, ageing, oxidative stress, *set-18*

1. Introduction

Ageing is considered as a complex and multi-factorial biological process driven by diverse molecular pathways and biochemical events shared by all living organisms [1]. It is characterized by the deterioration in the maintenance of homeostatic processes over time, which leads to functional decline and the increased risks of diseases and death [2]. It is also known as a general and complex biological process that predisposes humans to many complex diseases, including neurodegenerative diseases, type 2 diabetes, and various types of cancers.

Numerous studies have focused on the decipherment of the hallmarks of ageing in order to identify potential therapeutic targets to mitigate the ageing process. These hallmarks include stem cell exhaustion, altered intercellular communication, senescence, genomic instability, and recently epigenetic deregulation [3]. The end result of epigenetic changes alters the local accessibility to the genetic material, leading to aberrant gene expression, reactivation of transposable elements, and genomic instability. DNA accessibility is a determinant of genetic expression in the human genome.

Strikingly, certain types of epigenetic information can function in a transgenerational manner to influence the lifespan.

In eukaryotes, the physiological and cellular mechanisms of ageing are conserved. However, various molecular mechanisms of ageing come from a variety of eukaryotic models, like *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, *Drosophila melanogaster*, *Mus musculus*, mammalian tissue culture and human premature ageing [4]. Several distinct mechanisms underlying this process have been reported. Among them, the genetic screening and naturally occurring mutations have identified hundreds of genes involved in different pathways that affect ageing, of which insulin-like growth factor 1 (IGF-1) signalling, target of Rampamicyn (TOR) signalling, autophagy pathway, mitochondrial respiration signalling pathway, and hypoxia-inducible factor 1 (HIF-1) pathways [5].

Recently, researchers have tended to hold a comprehensive view to explain the complex interaction of genetic and environmental factors. Epigenetics, which can be defined as the study of stable genetic modifications that result in changes in gene expression and function without a corresponding alteration in the DNA sequence [6], has been found to be a necessary component to establish the overall understanding of ageing. Evidences have shown that epigenetic alterations can be regarded as a trigger of ageing pathway mechanisms, namely [7] histone modifications, which are supposed to be the essential component of epigenetic regulation and broadly studied. Nowadays, histone methylation occupies a crucial role during the development of organisms. Regulators of histone methylation have been mainly associated with ageing in worms and flies [8]. By RNAi screening of *C. elegans* genes, histone methyltransferase and demethylase genes have been identified as the key modulator of lifespan. These modifications are involved both in gene silencing, generally associated with transcriptional repression (H3K27me3), and in gene activation, associated with gene expression (H3K4me3) [9, 10], among them are SET-domain genes.

Since their discovery, SET-domain genes have attracted significant interest in multiple areas of biology and medicine, including endocrinology, growth, metabolism, nutrition, ageing, and oncology. The signalling pathways elicited by SET-domain genes have been extensively characterized in biochemical and molecular terms over the past years. However, fundamental questions regarding basic differences between the mechanisms of action of SET-domain genes and the closely related role in ageing are yet to be resolved. More recently, a study carried out by Su et al. 2018 [11] observed that a SET-domain protein, *set-18*, which is presented as a novel H3K36 dimethyltransferase in *C. elegans*, is specifically expressed in muscle, and its expression level is gradually increased during ageing. The mutation on this gene extended *C. elegans* lifespan and increased its oxidative stress resistance. However, the mechanism in which this gene is implicated remains unknown [4].

This chapter will provide a collection of information dealing with the role of epigenetic modifications involved in ageing process and highlights the significant role of histone methylation of SET-domain with an emphasis on the *set-18* gene. Among all the epigenetics modifications affecting organism lifespan, the histone methylation and demethylation stand out as a highly conserved and critical mechanism.

2. The effect of histone modification in ageing regulation

Although many studies have shown the molecular mechanism of ageing and the regulatory pathways, it appears that just in the past decade scientists have begun to understand that dynamic histone modification may be involved in the regulation of

the ageing process of organisms. Histone proteins, in contrast to DNA, are subject to a huge number of modifications that contain methylation, acetylation, ubiquitination, and phosphorylation [8]. Researchers have observed that histone modifications undergo alterations during the ageing process. Although these alterations are the causes or consequences of ageing are still debatable, it is widely accepted that there is a certain connection between them as shown in **Figure 1**.

Recent findings revealed that epigenetic factors that regulate histone methylation, a type of chromatin modification, can affect the lifespan of organisms. While acetylation of histone tails is largely ephemeral in nature. Histone methylation is widely observed to be a mark that confers long-standing epigenetic memory [12, 13]. This histone modification is accomplished by the catalysis of histone methyltransferase (HMT). According to the different methylation sites, it is mainly divided into histone lysine modification and arginine modification. Histone lysine methylation occurs at three different levels: monomethylation modification, dimethylation modification, and trimethylation modification. This modification is highly conserved between single-celled organisms and different species of mammals [4].

Most of the known targeted lysine of histone methyltransferase occurs on histone H3, which thereby serves as a conduit of epigenetic regulation. Mostly, lysine methylation at histone H3, lysine 9(H3K9), H3K27 or H4K20 act as gene silencing, whereas H3K4, H3K36 or H3K79 are associated with the actively transcribed genes [14]. Histone methylations, especially histone 3 lysine 4 trimethylation (H3K4me3) activation and H3K27me repressing, are epigenetic modifications with close ties to transcription and have been directly linked to lifespan regulation in many organisms [15]. These alterations are not only hallmarks to monitor and evaluate the course of ageing, but also the potential targets of anti-ageing treatments.

The age-dependent variations of histone marks increase the instability of the genome and influence the expression of corresponding genes, and the accumulated genomic instability in old cells can lead to oxidative stress and lifespan reduction.

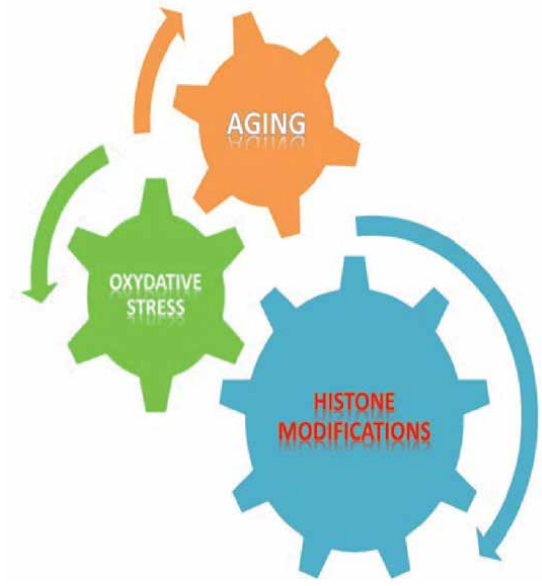


Figure 1.
The interplay between histone modifications, oxidative stress, and ageing.

Mounting evidence has reported the correlation between oxidative stress, ageing and epigenetic modifications. To date, histone methylation is a classic epigenetic mark recognized to be involved in gene expression and has key functions in ageing control [16–19]. By RNAi screening of *C. elegans*, histone methyltransferases and demethylases have been identified as key modulators of lifespan. These include H3K27 demethylase UTX-1. H3K9 trimethyltransferase *set-26* and H3K4 trimethylation complex *set-2*/ASH2/WDR-5 [11] are SET-domain proteins.

3. Oxidative stress and ageing

Free radicals are highly considered as reactive atoms or molecules having one or more unpaired electron(s) in their external shell. These radicals are produced by losing or accepting a single electron, therefore acting as oxidants or reductants [20]. The terms reactive oxygen species (ROS) refer to reactive radical and non-radical derivatives of oxygen. They are produced by all aerobic cells in the mitochondria and play an important role in cell immunity, in ageing as well as in age-related diseases [21] and they are also known to cause oxidative damage to cells and molecules. This, in turn, is widely recognized as a determinant of both lifespan and health span.

Ageing, over time, is considered as a progressive loss of tissue and organ function. It is generally regarded as an endogenous, irreversible and deleterious process poorly understood biologically [22]. However, in spite of considerable research efforts, the endogenous causes of ageing remain elusive. More recently, the free radical theory of ageing, later termed as oxidative stress theory of ageing, has been postulated. This theory is based on the structural damage-based hypothesis that age-associated functional losses are the result of the accumulation of oxidative injury to macromolecules such as lipids, DNA, and proteins by ROS [22]. The theory was later refined by Harman himself to emphasise the role of mitochondrial ROS, as the majority of free radical oxygen species (ROS) production originates in the mitochondria of mammalian cells, and was termed as the mitochondrial theory of ageing (**Figure 2**).

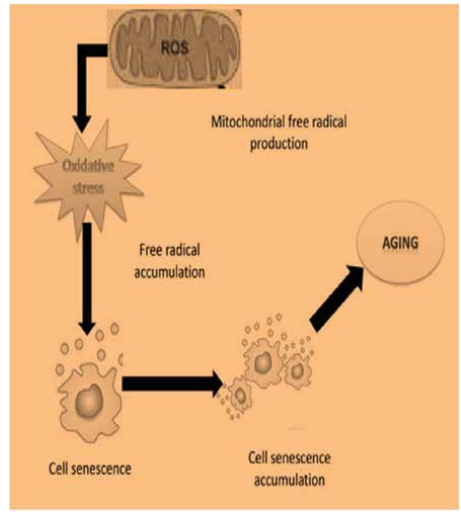


Figure 2.
Oxidative stress implication in ageing.

Even though the exact mechanism of oxidative stress-induced ageing has remained unclear, however, scientists are agreed that the increased ROS levels lead to cellular senescence, a physiological mechanism that stops cellular proliferation in response to damages that occur during replication [23].

4. SET-domain protein implication in ageing

SET-domain is a short name for a highly conserved 130 to 140 amino acid motif characterising a group of proteins known to methylate histones on lysine. The function of SET-domain proteins is to transfer a methyl group from S-adenosyl-L-methionine (AdoMet) to the amino group of a lysine residue on the histone or other protein [24].

Initially, the SET-domain proteins were associated exclusively with the regulation of developmental genes in metazoan. However, the finding of SET-domain genes in the unicellular yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* suggested that SET-domain proteins regulate a much broader variety of biological programmes [25]. By performing a targeted RNAi screen in fertile worms with selected genes that encode known worm methyltransferases, proteins containing the enzymatic domain of methyltransferases (SET-domain), or orthologues of regulators of histone methylation, the results depicted that *set-9* and *set-15* knock-down extended lifespan and also *set-2* and *set-4* knock-down extended fertile worm lifespan as previously reported by many investigators [8].

The authors also investigated whether the known histone lysine methyltransferase regulates lifespan and screened genes encoding the SET domain in *C. elegans* by RT-qPCR, and the results revealed that the mRNA levels of *set-2* and *set-15* increased significantly on day 11; both genes had been reported to promote ageing. In addition to these two genes, they found that the mRNA levels of *set-10*, *set-18*, *set-32* and F54F7.7 were also upregulated in nematodes, and these genes have not yet been reported to be involved in lifespan regulation [11].

Focusing on *set-18*, results revealed that this gene expression increases according to animal age and is highly expressed in muscle. The mutation on this gene revealed a lifespan extension and oxidative stress resistance compared to the wild-type worms. It is important to note that, when there is increased exposure to reactive oxygen species (ROS), the cell enters the state of chronic oxidative stress. The more a cell is growing, the more oxidative stress damages are increasing; thus, this has an influence on longevity [3].

Studies have shown that there is a close relationship between the survivals of the mitochondrial and that of the cells due to the central role of mitochondria in programmed cell death (apoptosis) as well as the important involvement of ROS produced at 90% in mitochondria. High levels of ROS and calcium, acting together, can trigger the mechanism of cell death *via* apoptosis or necrosis. For several years, plenty of researches aimed to understand the adverse effects of ageing and were conducted out on a wide range of model organisms, and nine general hallmarks of ageing in living organisms have been identified. These hallmarks affect the organism at different scales. Some occur at the molecular level within cells, while others impact tissues (muscles) and even beyond at the level of an organ or the entire organism [3]. These mechanisms have been revealed to influence longevity partially dependent on *daf-16*, a prominent longevity gene that encodes the worm orthologue of the highly conserved forkhead transcription factor forkhead box O (FOXO).

Sequence analysis showed that *set-18* has high homology to the mammalian histone methyltransferase SMYD family, which contains a SET-domain split into two segments by a myeloid, nervy, and DEAF-1 (MYND) domain [11]. *set-18* contains a conserved SET-domain that encodes proteins homologous to human SMYD1, SMYD2, and SMYD3 [26, 27]. The human homologue SMYD1, SMYD2, and SMYD3 of *set-18* have been reported to have the activity of histone methyltransferase. The SMYD family encloses five discrete proteins, of which SMYD1-5, with reported functions in both normal and pathologic conditions (ageing diseases). The key feature of all SMYD family members has been established to be the methylation of H3K4. For many SMYD family members, the SET-domain contains two sections: 1) the S-sequence, which may work as a cofactor binder as well as for protein–protein interactions and 2) the core SET-domain, which functions as the primary catalytic location [28, 29]. In close relation to the SET domain are two other domains: 1) the post-SET and 2) the SET-I, which assist in cofactor binding, substrate binding, and protein stabilization [30, 31].

5. *set-18* gene involvement in ageing and lifespan regulation

C. elegans set-18 gene was identified as a histone H3K36 dimethyltransferase. The deletion of *set-18* increased lifespan and oxidative stress resistance depending on the *daf-16* activity in the insulin/IGF pathway. Muscle-specific expression of *set-18* increased in aged worms, resulting in elevation of global H3K36me2 and inhibition of *daf-16a* expression and, consequently, decreased longevity. These results suggest that H3K36me2 and H3K36me3 modification have distinct functions in regulating ageing [11].

The FOXO family of transcription factors are highly conserved key converging points of several longevity pathways. *C. elegans* has a single FOXO orthologue, *daf-16*, and mutants with *daf-16* deficiency exhibit shortened lifespan compared with wild-type animals. Very recent studies showed that *daf-16* functions downstream of a histone modifier to influence lifespan. *daf-16* lifespan epistasis analyses suggest that the *set-18* gene works through *daf-16* to modulate lifespan. As *daf-16* transcript levels are not affected by *set-18* status in worms and that *set-18* regulates global histone modifications, an interesting possibility is that *set-18* acts as a cofactor of *daf-16* in target gene regulation *via* changes in local chromatin accessibility.

However, it is also possible that like UTX-1, *set-18* modulates the lifespan by regulating the expression of components of daf-16-dependent longevity pathways. Several well-known longevity pathways impinge on *daf-16*, including the *daf-2*/insulin pathway (insulin and IGF-1 signalling), the germline pathway, and the energetic metabolism pathway. Lifespan and oxidative stress tolerance are promoted by the FOXO *daf-16* and suppressed by its upstream IIS pathway genes such as the exclusive insulin-like growth factor receptor *daf-2* [32, 33].

The insulin and IGF-1 signalling (IIS) pathway are so highly conserved to modulate ageing and longevity. The IIS pathway is a signal transduction cascade that consists of insulin-like peptides (ILPs), an insulin/IGF-1 receptor (*daf-2*), a phosphoinositide 3-kinase (AGE-1/AAP-1/PI3K), serine/threonine kinases (PDK-1, AKT-1 and AKT-2) and the pivotal downstream forkhead box O transcription factor (*daf-16*) in *C. elegans*. This cascade in turn phosphorylates the FOXO/*daf-16* and prevents it from entering the nucleus to trigger anti-ageing genes, such as the genes conferring resistance to heat, oxidative stress resistance and DNA damage [24–27] as presented in **Figure 3**. *daf-16*/FOXO receives phosphorylation from the direct upstream AKT

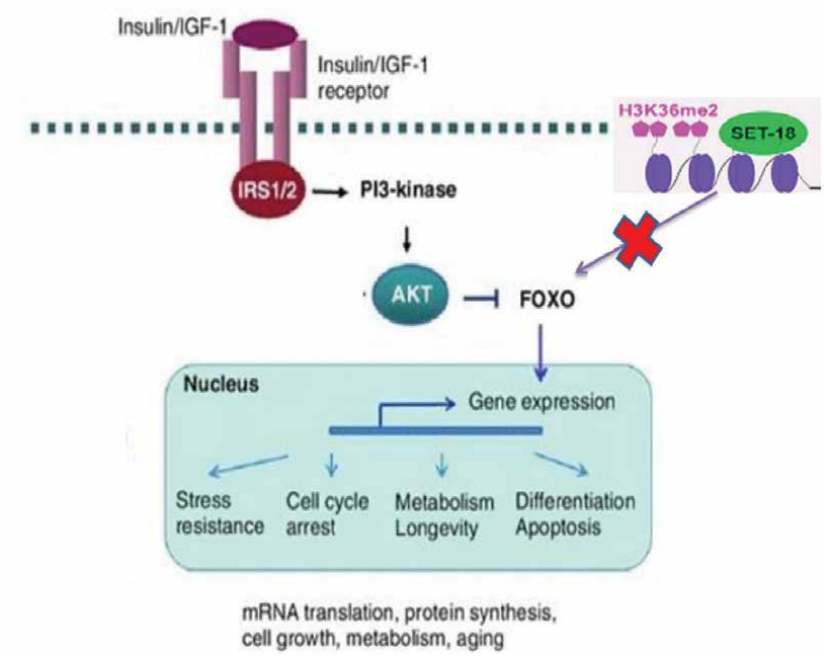


Figure 3.
Possible action of set-18.

kinases mediated signal transduction response to insulin or IGF and is subsequently sequestered in the cytoplasm by 14-3-3 proteins [34–36], which antagonises FOXO and negatively regulates the longevity [27, 32].

6. Conclusion

As ageing is inevitably a biological process associated with the deterioration of functional activities and resistance to hazards of individuals, researchers are always interested in investigating the relevant factors affecting the ageing process. The continued combination of functional studies and molecular analyses in different age groups, different organisms, and different tissue types will hopefully provide the details necessary to comprehend this evolutionarily conserved fundamental process and to facilitate the development of therapeutic interventions to counteract age-induced complications or to explore the direction of anti-ageing or rejuvenation. Our chapter revealed the role of epigenetic modification in ageing. Changes in epigenetic status have been shown to associate with ageing in many organisms. We focused on the role played by various histone modifications especially on histone methylation to lifespan modulation. Much more attention was on the SET-domain containing genes, *set-18*; further, we have shown that this gene regulates lifespan through *daf-16*, which is implicated in various mechanisms underlying ageing and oxidative stress. We demonstrated that *set-18* as an H3K36 dimethyltransferase is expressed specifically in muscle. Hence, their exact role in ageing and its underlying mechanism has yet to be explored, thus providing more directions and strategies for the rejuvenation of ageing and the recovering of age-related diseases.

Acknowledgements

The authors thank Professor Li Xiaoxue, Professor Mbemba Fundu, and Professor Iteku Bekomo Jeff for their advices and contribution.

Author details

Pambu Lelo Aaron[1,2,3*], Zakuani Luzinga Nadege[1], Fabrice Ndayisenga[4] and Bongo Ngiala Gedeon[1,2]

1 Laboratory of analysis and research on Food and nutrition, Department of Biology, Faculty of science, University of Kinshasa, Kinshasa, D.R. Congo

2 Unit of Complex Morbidity, Department of Parasitology, National Institute of Biomedical Research, Kinshasa, D.R. Congo

3 School of Microecology, Dalian Medical University, Dalian, Liaoning, China

4 College of Resources and Environment, University of Chinese Academy of Sciences, Beijing, P.R. China

*Address all correspondence to: aaron.pambu@unikin.ac.cd; aaronpambu@yahoo.fr

IntechOpen

References

[1] Slagboom PE, Beekman M, Passtoors WM, Deelen J, Vaarhorst AA, Boer JM, et al. Genomics of human longevity. Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences. 2011;**366**(1561): 35-42

[2] Nir B, Huffman DM, Muzumdar RH, Bartke A. The critical role of metabolic pathways in ageing. Diabetes. 2012; **61**(6):1315-1322

[3] Lopez-Otin C, Blasco MA, Partridge L, et al. The hallmarks of ageing. Cell. 2013;**153**(6):1194-1217

[4] Kim SK. Common ageing pathways in worms, flies, mice and humans. The Journal of Experimental Biology. 2007;**210**:1607-1612

[5] Kenyon C, Chang J, Gensch E, et al. A *C. elegans* mutant that lives twice as long as wild type. Nature. 1993;**366**(6454): 461-464

[6] Probst AV, Dunleavy E, Almouzni G. Epigenetic inheritance during the cell cycle. Nature Reviews. Molecular Cell Biology. 2009;**10**(3):192-206

[7] Huidobro C, Fernandez AF, Fraga MF. Ageing epigenetics: Causes and consequences. Molecular Aspects of Medicine. 2013;**34**(4):765-781

[8] Greer EL, Beese-Sims SE, Brookes E, Spadafora R, Zhu Y, Rothbart SB, et al. A histone methylation network regulates transgenerational epigenetic memory in *C. elegans*. Cell Reports. 2014;**7**(1):113-126

[9] Jin C, Li J, Green CD, Yu X, Tang X, et al. Histone demethylase UTX-1 regulates *C. elegans* life span by targeting the insulin/IGF-1 signalling pathway. Cell Metabolism. 2011;**14**(2):161-172

[10] Maures TJ, Greer EL, Hauswirth AG, Brunet A. The H3K27 demethylase UTX-1 regulates *C. elegans* lifespan in a germeline-independent, insulin-dependent manner. Aging Cell. 2011;**10**(6):980-990

[11] Su LP, Hongyuan L, Cheng H, Tingting Z, Yongjun Z, Xuequing B, et al. Muscle-specific histone H3K36 dimethyltransferase set-18 shortens lifespan of *C. elegans* by repressing daf-16 expression. Cell Reports. 2018;**22**: 2716-2729

[12] Shaye DD, Greenwald I. OrthoList: A compendium of *C. elegans* genes with human orthologs. PLoS One. 2011;**6**: e20085

[13] Tomoya S, Yuhei K, Ashida H. Glucosamine extends the lifespan of *Caenorhabditis elegans* via autophagy induction. Journal of Applied Glycoscience. 2018;**65**:37-43

[14] Sims RJ, Nishioka K, Reinberg D. Histone lysine methylation: A signature for chromatin function. Trends in Genetics. 2003;**19**(11):629-639

[15] Sen P, Shah PP, Nativio R, Berger SL. Epigenetic mechanisms of longevity and ageing. Cell. 2016;**166**(4):822-839

[16] Berger SL. The complex language of chromatin regulation during transcription. Nature. 2007;**447**:407-412

[17] Klose RJ, Zhang Y. Regulation of histone methylation by demethylimination and demethylation. Nature Reviews. Molecular Cell Biology. 2007;**8**:307-318

[18] Li B, Carey M, Workman JL. The role of chromatin during transcription. Cell. 2007;**128**:707-719

[19] Yu H, Zhu S, Zhou B, Xue H, Han JD. Inferring causal relationships among different histone modifications and gene expression. Genome Research. 2008;**18**:1314-1324

[20] Chandrasekaran A, Idelchik MDPS, Melendez JA. Redox control of senescence and age-related disease. Redox Biology. 2017;**11**:91-102

[21] Venkataraman K, Khurana S, Tai TC. Oxidative stress in ageing-matters of the heart and mind. International Journal of Molecular Sciences. 2013;**14**(9): 17897-17925

[22] Beckman KB, Ames BN. The free radical theory of ageing matures. Physiological Reviews. 1998;**78**(2): 547-581

[23] Pole A, Dimri M, Dimri G. Oxidative stress, cellular senescence and ageing. AIMS Molecular Science. 2016;**3**(3): 300-324

[24] Lee S-J, Murphy CT, Kenyon C. Glucose shortens the lifespan of *Caenorhabditis elegans* by down-regulating aquaporin gene expression. Cell Metabolism. 2009;**10**(5):379-391

[25] Veerappan CS, Avramova Z, Moriyama EN. Evolution of SET-domain protein families in the unicellular and multi-cellular Ascomycota fungi. BMC Evolutionary Biology. 2008;**8**(1):190

[26] Leinhart K, Brown M. SET/MYND lysine methyltransferases regulate gene transcription and protein activity. Genes. 2011;**2**(1):210-218

[27] Uddin GM et al. Head to head comparison of short-term treatment with the NAD(+) precursor nicotinamide mono-nucleotide (NMN) and 6 weeks of exercise in obese female mice. Frontiers in Pharmacology. 2016;**7**:258

[28] Tracy C, Warren J, Szulik M, Wang L, Garcia J, Makaju A, et al. The Smyd family of methyltransferases: Role in cardiac and skeletal muscle physiology and pathology. Current Opinion in Physiology. 2018;**1**:140-152. DOI: 10.1016/j.cophys.2017.10.001

[29] Foreman K, Brown M, Park F, Emtage S, Harriss J, Das C, et al. Structural and functional profiling of the human histone methyltransferase SMYD3. PLoS One. 2011;**6**(7):e22290. DOI: 10.1371/journal.pone.0022290

[30] Brown M, Foreman K, Harriss J, Das C, Zhu L, Edwards M, et al. C-terminal domain of SMYD3 serves as a unique HSP90-regulated motif in oncogenesis. Oncotarget. 2015;**6**(6): 4005-4019. DOI: 10.18632/oncotarget.2970

[31] Wu J, Cheung T, Grande C, Ferguson A, Zhu X, Theriault K, et al. Biochemical characterization of human SET and MYND domain-containing protein 2 methyltransferase. Biochemistry. 2011;**50**(29):6488-6497. DOI: 10.1021/bi200725p

[32] Hsu AL, Coleen TM, Cynthia K. Regulation of ageing and age-related disease by DAF-16 and heat-shock factor. Science. 2003;**300**(5622): 1142-1145

[33] Jia X et al. Mec1 and Rad53 inhibit formation of single-stranded DNA at telomeres of Saccharomyces cerevisiae cdc13-1 mutants. Genetics. 2004;**166**(2): 753-764

[34] Lamitina ST, Strange K. Transcriptional targets of daf-16 insulin signalling pathway protect *C. elegans* from extreme hypertonic stress. American Journal of Physiology. Cell Physiology. 2005;**288**:C467-C474

[35] Libina N, Berman JR, Kenyon C. Tissue-specific activities of *C. elegans* DAF-16 in the regulation of lifespan. Cell. 2003;**115**(4):489-502

[36] Sun X, Chen W-D, Wang Y-D. DAF-16/FOXO transcription factor in ageing and longevity. Frontiers in Pharmacology. 2017;**8**:548. DOI: 10.3389/fphar.2017.00548

Chapter 9

Reactive Oxygen Species in Neurodegenerative Diseases: Implications in Pathogenesis and Treatment Strategies

Johnson Olaleye Oladele, Adenike T. Oladiji, Oluwaseun Titilope Oladele and Oyedotun M. Oyeleke

Abstract

Neurodegenerative diseases are debilitating disorders which compromise motor or cognitive functions and are rapidly becoming a global communal disorder with over 46.8 million people suffering dementia worldwide. Aetiological studies have showed that people who are exposed to agricultural, occupational and environmental toxic chemicals that can interfere and degenerate dopaminergic neurons are prone to developing neurodegenerative diseases such as Parkinson Disease. The complex pathogenesis of the neurodegenerative diseases remains largely unknown; however, mounting evidence suggests that oxidative stress, neuroinflammation, protein misfolding, and apoptosis are the hallmarks of the diseases. Reactive oxygen species (ROS) are chemically reactive molecules that have been implicated in the pathogenesis of neurodegenerative diseases. ROS play a critical role as high levels of oxidative stress are commonly observed in the brain of patients with neurodegenerative disorders. This chapter focus on the sources of ROS in the brain, its involvement in the pathogenesis of neurodegenerative diseases and possible ways to mitigate its damaging effects in the affected brain.

Keywords: oxidative neuronal damage, neuroinflammation, oxidative stress

1. Introduction

Neurodegenerative diseases are debilitating disorders which compromise motor or cognitive functions and are rapidly becoming a global communal disorder with over 46.8 million people suffering dementia worldwide. They are characterised by progressive damage in neural cells and neuronal loss. Common neurodegenerative diseases include amyotrophic lateral sclerosis, Parkinson's disease, Alzheimer's disease, Huntington's disease, and spinocerebellar ataxia [1]. These diseases represent major health challenges especially in the ageing population [2]. For instance, PD is the second

most prevalent neurodegenerative disease affecting 1 to 2% of the population above age of 65 while AD is ranked the top 6 leading causes of death in the United States [3, 4].

It is estimated that more than 10 million individuals with the disease will be domiciled in the top 10 most populous nation in the world by 2030. In Nigeria, the most populous nation in Africa, neurodegenerative disease related cases have a significant impact on the overall hospital frequency of neurological cases reported [5]. Some of the characterised clinical features of these diseases include bradykinesia, rigidity, postural instability, resting tremor, prolonged reaction times, and freezing of gait, which may degenerate to tightened facial expression and unconscious facial movement [6, 7]. Aetiological reports have documented that individual who are exposed to industrial, occupational and environmental toxic chemicals that can interfere with the functions of the central nervous system and degenerate dopaminergic neurons are prone to developing neurodegenerative diseases such as Alzheimer's disease, Parkinson disease [8, 9].

The complex pathogenesis of the neurodegenerative diseases remains largely unknown; however, mounting evidence suggests that oxidative stress, neuroinflammation, protein misfolding, and apoptosis are the hallmarks of the diseases (**Figure 1**). ROS may play a critical role as high levels of oxidative stress are commonly observed in the brain of patients with neurodegenerative conditions [10]. Reactive oxygen species (ROS) are chemically reactive molecules that have been implicated in the pathogenesis of neurodegenerative diseases. They are naturally generated within the biological system, playing significant functions in mediating cellular activities including stressor responses, cell survival, and inflammation. They also play pivotal role in the pathogenesis of many diseases such as cancer, allergy, muscle dysfunction, and cardiovascular disorders [11, 12]. Due to their reactivity, Presence of ROS in high quantity may lead to oxidative stress and ultimately cell death if left uncontrolled or treated. Oxidative stress is defined as the disruption of balance between pro-oxidant and antioxidant levels in biological systems [11].

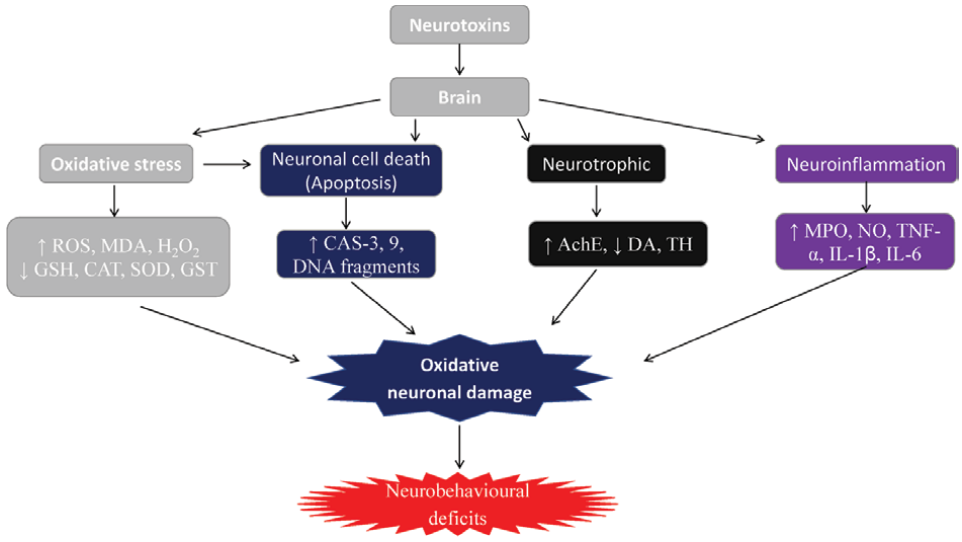


Figure 1.
Possible involvement of oxidative stress, apoptosis, and neuroinflammation in pathogenesis of neurodegenerative diseases.

A number of experimental studies have been carried out to elucidate the significances of oxidative stress in neurodegenerative diseases [13, 14]. ROS may not be sufficient itself to induce neurodegenerative diseases but they appear to exacerbate the diseases' progression through oxidative macromolecule damage and interaction with mitochondria [10]. Interestingly, neuronal cells have been identified to be vulnerable to oxidative damage due to their high oxygen consumption, high polyunsaturated fatty acid content in membranes, and weak antioxidant defence [15]. Under basal or unstressed physiological conditions, free radicals and ROS generated from mitochondria, NADPH oxidase (Nox), and xanthine oxidase are kept at relatively low levels by endogenous antioxidants [11]. Nevertheless, abnormal mitochondrial function and/or neuro-inflammation can alter the redox status and interrupt the balance [15]. Accumulation of misfolded proteins is part of the hallmark of pathogenesis of some neurodegenerative diseases such as Alzheimer disease and Parkinson disease (**Figure 2**). The aggregation of these misfolded or modified proteins can in turn triggers inflammatory response in the brain, which induces marked ROS release and subsequent oxidative stress [16]. Mitochondrial dysfunction with concomitant aberrant ROS secretion is strongly associated with neurodegenerative disorders [17]. For instance, mutant huntingtin (mHTT) in HD may directly interact with mitochondria causing compromised and alteration in energy supply and increased production of ROS [18].

Another key player in the pathogenesis of neurodegenerative diseases is neuroinflammation. The existence of neuroinflammatory processes in human brain has also been confirmed during autopsy on a molecular basis. Mogi and colleagues reported an increase in concentrations of TNFα, β2-microglobulin, epidermal growth factor (EGF), transforming growth factor α (TGFα), TGFβ1, and interleukins 1β, 6, and 2 in the striatum of patients with Parkinson's disease [19–22]. TNFα, interleukin 1β, and interferon γ were also detected in the effects indirectly. Proinflammatory cytokines, such as TNFα, interleukin 1β, and interferon γ, can induce the expression of the inducible form of nitric oxide synthase (iNOS) [23, 24] or cyclooxygenase 2 (COX2) [25]. These enzymes produce toxic reactive species. Other enzymes involved in

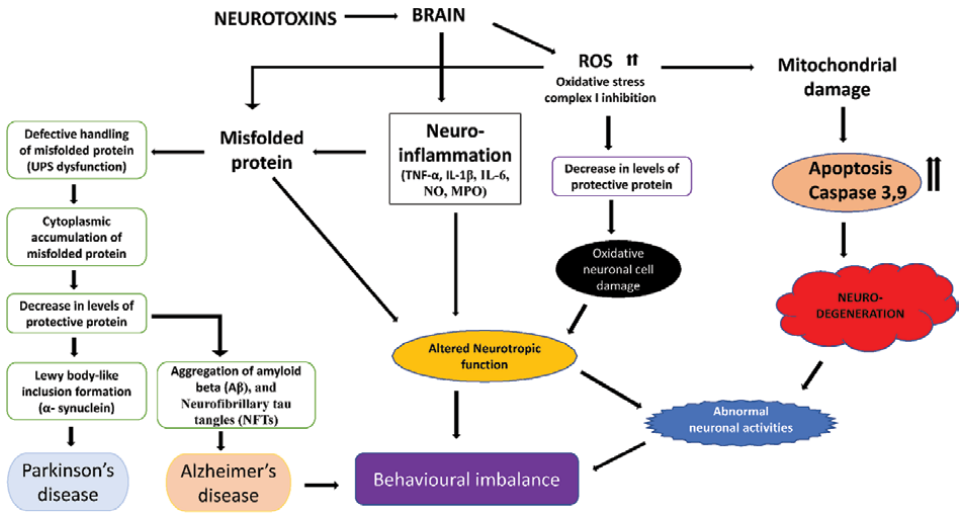


Figure 2.
Molecular mechanisms underlying pathogenesis of Parkinson's disease and Alzheimer's disease.

neuroinflammatory processes mediated by oxidative stress such as myeloperoxidase, NADPH oxidase, and COX2, also have increased concentrations in neurodegenerative diseases [26].

Apoptosis has been implicated as the major pathway involved in the progressive neuronal cell death/loss observed in neurodegenerative diseases. Degeneration of one or more nerve cell populations is a major feature in many acute and chronic neurological diseases. Many criteria for apoptotic cell death are also fulfilled during the course of chronic neurodegenerative diseases. Therefore, the development of new therapeutic strategies for the treatment of neurodegenerative diseases requires an understanding of the molecular mechanisms underlying neuronal apoptosis. Extrinsic and intrinsic apoptosis pathways and several possible avenues for crosstalk between them can be distinguished. Whereas the extrinsic pathway is initiated by cell surface activation of cytokine receptors of the tumour necrosis factor (TNF) family, the intrinsic pathway depends on the integrity and function of mitochondria within the cell [27].

Various evidences from biochemical, genetic, cellular, and neuropathological studies have shown that protein misfolding, oligomerization, and accumulation in the brain are the main events triggering pathological abnormalities responsible for neurodegenerative diseases [28, 29]. The proteins most commonly implicated in the accumulation of cerebral misfolded aggregates in neurodegenerative diseases include: amyloid-beta (Aβ) in Alzheimer disease; tau in Alzheimer disease, frontotemporal dementia, corticobasal degeneration, progressive supranuclear palsy, argyrophilic grain disease, and chronic traumatic encephalopathy; alpha-synuclein (α-Syn) in PD, multiple system atrophy, and dementia with Lewy bodies; TAR DNA-binding protein 43 (TDP-43) in amyotrophic lateral sclerosis and frontotemporal frontotemporal dementia; and prion proteins in PrDs (i.e., Creutzfeldt–Jakob disease (CJD), bovine spongiform encephalopathy, chronic wasting disease, and scrapie). Despite the fact that the protein aggregates involved in distinct neurodegenerative diseases are different, the process of protein misfolding, its intermediates, end-products, and main features are remarkably similar [30].

Considering the pivotal roles of oxidative stress, neuroinflammation, protein misfolding, and apoptosis in neurodegenerative diseases (**Figure 1**), the manipulation of major key players in each of the pathological mechanisms may represent a promising treatment option to slow down neurodegeneration and alleviate associated symptoms. This chapter examine the role of reactive oxygen species (ROS) and oxidative stress in the pathogenesis and progression of neurodegenerative diseases. This chapter focus on the sources of ROS in the brain, its involvement in the pathogenesis of neurodegenerative diseases and possible ways to mitigate its damaging effects in the brain.

2. Role of oxidative stress in pathogenesis of Parkinson's disease (PD)

PD is the second most common neurodegenerative disorder, characterised by the degeneration of dopaminergic neurons in the brain's substantia nigra pars compacta [31]. PD affects around 1–2 percent of the population over the age of 65, and the prevalence rises to 4% in people over the age of 85 [32]. Overabundance of ROS or other free radicals has been linked to the pathological mechanism underlying dopaminergic neuron degeneration. Mitochondrial dysfunction or inflammation may both cause excessive ROS production [10]. The proper role of redox-sensitive signalling proteins in neuron cells, as well as neuronal survival, is dependent on maintaining

redox homeostasis [33]. Mitochondria in neurons and glia are the main sources of ROS in the brain [10]. The production of these free radicals is exacerbated in PD due to neuroinflammation, dopamine degradation, mitochondrial dysfunction, ageing, GSH depletion, and high levels of iron or Ca^{2+} [10].

Consequently, when people with PD are exposed to environmental factors including pesticides, neurotoxins, and dopamine, ROS deposition may be exacerbated [34]. This is supported by a strong link between pesticide exposure and an increased risk of Parkinson's disease [34]. ROS have been shown to contribute significantly to dopaminergic neuronal loss [10]. Other research has indicated that the loss of dopaminergic neurons is linked to the existence of neuromelanin, since highly pigmented neurons are more vulnerable to damage [35]. The formation of neuromelanin appears to be related to dopamine auto-oxidation, a process induced by ROS overproduction [35].

Neurodegeneration produces reactive oxygen species (ROS), which can destroy key cellular proteins and disrupt lipid membranes, leading in oxidative stress. Mitochondrial dysfunction increases free radical generation in the respiratory chain [10]. Parkinson's disease has been linked to deficiencies in mitochondrial complex I in particular. Certainly, a significant portion of the unfavourable neuronal apoptosis seen in Parkinson's disease is due to a complex I deficiency [36]. A mutation in the PTEN-induced putative kinase 1 gene is associated to this impairment (PINK1). PINK1 is a protein found in all human tissues that plays a key role in keeping mitochondrial membrane potential and preventing oxidative stress [36]. The PINK1 mutation is linked to the onset of Parkinson's disease [36]. Mutations of leucine-rich repeat kinase 2 (LRRK2), parkin, alpha-synuclein, and DJ-1 have all been linked to the pathogenesis of Parkinson's disease. These mutations may impair mitochondrial function, resulting in an increase in reactive oxygen species (ROS) production and oxidative stress vulnerability. Mutant parkin may play key roles in the development of autosomal recessive PD due to its involvement in lowering ROS and limiting the production of neurotoxic proteins produced by ubiquitination [36]. Additionally, alpha synuclein aggregation has been demonstrated to disrupt mitochondrial complex I activities, causing ATP production impairment and mitochondrial malfunction [37]. Proteasomal dysfunction which is exacerbated by dopamine-derived ROS, has been linked to neurodegeneration in Parkinson's disease [37].

Currently, there is no effective cure for the treatment of Parkinson's disease, however, deeper insights into the role of ROS in the disease pathogenesis (initiation and progression) should lead to more effective treatments for PD symptoms. Many neuroprotective approaches have been discovered to minimise mitochondrial oxidative stress in dopaminergic neurons. Free radicals damage has been proven to be reduced by antioxidants [38]. GSH, ascorbic acid and tocopherol are essential antioxidants that the antioxidant lipoic acid can recycle. Secretion of GSH which enhance reduction of lipid peroxide is one of the mechanisms by which lipoic acid offered beneficial effects against oxidative damage in oxidative stress-induced mitochondrial dysfunction [39]. In an animal study, it was discovered that treatment with lipoic acid enhanced motor coordination and ATP efficiency resulting in neuroprotection [40]. Furthermore, treatment of lipoic acid in a rotenone rats' model of parkinsonian rats showed enhanced motor performance and marked reduction in neuronal lipid peroxide in the brain [40]. Neuroprotective ability of phytochemicals and antioxidant substances including polyphenols, Ginko biloba, docosahexaenoic acid (DHA), tocopherol, ascorbic acid, and coenzyme Q10, and have all been studied in animal experiments with remarkable findings [41–46]. However, no convincing evidence of their neuroprotective benefits has been found in human [47]. Failures of such

antioxidant medications should provide future recommendations for treating PD patients with combination therapies aimed at limiting ROS production in the brain and improving mitochondrial function [48].

3. Role of oxidative stress in pathogenesis of Alzheimer's disease (AD)

Alzheimer's disease (AD) is the most common neurodegenerative disease, characterised by gradual declines in memory, behaviour, and functionality that severely limit day-to-day activities [49]. The pathophysiology of Alzheimer's disease is primarily linked to the formation of extracellular amyloid beta (Aβ) plaques and intracellular tau neurofibrillary tangles (NFT) [50]. Plaques in the endoplasmic reticulum (ER) can deplete calcium ions (Ca^{2+}) storage, resulting in cytosolic Ca^{2+} overload. Endogenous GSH levels are reduced in response to an increase in cytosolic Ca^{2+}, and ROS will accumulate within the cells [51]. ROS-induced ROS overproduction is believed to play a critical role in the aggregation and secretion of Aβ in AD, and oxidative stress is emerging as a significant factor in the pathogenesis of AD [52]. Mitochondrial dysfunction can result in increased production of reactive oxygen species (ROS), decreased ATP production, altered Ca^{2+} homeostasis, and excitotoxicity. All these alterations may be implicated in the development of AD [53].

Overactivation of N-methyl-D-aspartate-type glutamate receptors (NMDARs) can cause severe oxidative stress in Alzheimer's patients. NMDAR activation has been showed to trigger excessive Ca^{2+} influx by increasing cell permeability and resulting in the production of neurotoxic levels of reactive oxygen and nitrogen species (RNS) [54, 55]. JNK/stress-activated protein kinase pathways can be mediated by reactive oxygen species (ROS). The hyperphosphorylation of tau proteins and Aβ-induced cell death have both been linked to the activation of these cascades [56]. Furthermore, Aβ proteins can directly cause formation of free radicals by inducing NADPH oxidase [57]. The activation of p38 mitogen activated protein kinase (p38 MAPK) by Aβ-induced ROS overproduction modifies cellular signalling pathways and initiates tau hyperphosphorylation. Intracellular NFT formation may be caused by an abnormal aggregation of hyperphosphorylated tau proteins [58, 59]. Consequently, Aβ has been shown to play a key role in the induction of cellular apoptosis [60]. Aβ may boost the activity of calcineurin, which then activates the Bcl-2-associated death promoter, causing mitochondrial cytochrome c release [61]. Aβ can also interact directly with caspases, resulting in neuron apoptosis [61].

Environmental stress, ageing, inflammation, and certain dietary factors (e.g., redox-active metals) may all trigger an increase in Aβ output by inducing additional oxidative stress [62]. Oxidative stress is more common in the elderly, which helps to explain why older people are more susceptible to Alzheimer's disease [62]. Increased expression of cytokines, ROS levels, and cellular toxicity are all caused by inflammation, which accelerates the development of Alzheimer's disease [63]. Aβ deposition results in microglial activation [64]. It's becoming clear that sustained activation of microglia results in the release of pro-inflammatory cytokines, triggering a pro-inflammatory cascade and leading to neuronal loss and damage [65]. Environmental factors such as toxins, chemicals, and radiation may cause oxidative stress [66]. The production of reactive oxygen species (ROS) increases, where there are excess iron deposits [66]. Aβ itself can interact with metal ions to generate free radicals, therefore methionine 35 plays an important role in these reactions [67]. Cu^{2+}/Zn^{2+}-bound Aβ has been showed to have a structure identical to superoxide dismutase (SOD),

suggesting that it could have antioxidant properties [68]. As a result, Cu^{2+} and Zn^{2+} supplementation has been considered as a novel strategy to reduce Aβ-induced ROS generation and metal catalysed Aβ deposition [68].

Drugs for Alzheimer's disease are aimed at lowering Aβ oligomers and phosphorylated tau levels, lowering oxidative stress, and regulating epigenetic changes [69]. The majority of Alzheimer's disease therapies depend on compounds with neuroprotective, anti-inflammatory, and antioxidant properties [70]. Medications that target ROS-mediated cascades like JNK and NF-B (e.g., tocopherol, resveratrol, and rutin) have demonstrated some promising results *in vitro* and *in vivo* [49]. When using antioxidants, significant factors including reaction kinetics and bioavailability (permeability, retention in the targeted region, distribution, and transport) must be taken into account [70]. Several ROS-related neuroprotective therapeutic techniques have shown great promise in the treatment of Alzheimer's disease. The antioxidant response element (ARE) pathway regulated by nuclear factor erythroid 2-related factor 2 (Nrf2) is known to be an important conditioned response against oxidative stress [71]. The binding of Nrf2 to ARE activates the expression of several antioxidant genes in a synchronised manner that can work together for oxidative detoxification. Weakened Nrf2-ARE pathways were observed in the brains of transgenic mice with AD symptoms, while the enhancement of Nrf2-ARE cascades using adenoviral Nrf2 gene transfer has shown protective effects against the toxicity of Aβ deposition [71]. As a result, transcriptional modulation of endogenous antioxidants could hold great promise in the treatment of Alzheimer's disease symptoms [71].

4. Role of oxidative stress in pathogenesis of spinocerebellar ataxia disease

Spinocerebellar ataxia is a progressive neurodegenerative illness caused by an autosomal dominant gene. Cognitive impairments, dysarthria, osculomotor abnormalities, and ataxic gait are all well-known signs of spinocerebellar ataxia, which can lead to mortality. Based on genetic descriptions, about 20 forms of spinocerebellar ataxia have been identified [72, 73]. The main pathogenic mutation in spinocerebellar ataxia has been linked to the expansion of repeated CAG trinucleotides [74]. The mutant ataxin 1 (ATXN1) protein, which has an enlarged polyglutamine, is overexpressed as a result of the mutation from expansion of repeated CAG trinucleotides. RAR-related orphan receptor alpha, which plays a key role in Purkinje cell activities, can be affected by mutant ataxin 1. Reduced RAR-related orphan receptor alpha gene expression has been linked to cerebellar hypoplasia and ataxia [75].

Majority of spinocerebellar ataxia are thought to be genetic disorders linked to ATXN mutations, however, different pathogenic pathways involving mitochondrial malfunction have been hypothesised [75]. Hakonen et al. [76] reported mitochondrial DNA depletion and respiratory complex I deficiency in the brain of infantile-onset spinocerebellar ataxia patients. Small concentration of ROS has been documented to be beneficial for cellular activities including cell signalling, nonetheless, higher concentration is dangerous to the brain being neurotoxic and have been established to cause neurodegeneration [49]. A study conducted by Stucki et al. have reported marked mitochondrial alterations and excessive accumulation of oxidative stress in the Purkinje cells of Spinocerebellar ataxia 1. It was suggested that there exists a connection between oxidative stress mediated mitochondrial impairments and the progression of spinocerebellar ataxia [75]. Similarly, the study evaluated the possible

neuroprotective roles of MitoQ (a mitochondrial antioxidant) in a spinocerebellar ataxia mouse model. The result revealed long-term treatment of MitoQ markedly improved mitochondrial morphology and enhanced its functions in Purkinje cells resulting in amelioration of spinocerebellar ataxia 1-related symptoms including motor incoordination [75]. This report demonstrated the neuroprotective potential of mitochondria-targeted antioxidants as a potential treatment for spinocerebellar ataxia 1.

Similar to previous neurodegenerative diseases discussed, pathogenesis of spinocerebellar ataxia is associated with mitochondrial dysfunction [77]. For instance, Friedreich ataxia, is characterised by the absence of frataxin, an iron transporter protein located on the mitochondrial inner membrane. Decrease in the level of frataxin, leads to increase in concentration of iron in the mitochondrial matrix, thus stimulating the Fenton reaction which convert of H_2O_2 to $^{\cdot}OH$. The highly reactive $^{\cdot}OH$ molecules can compromise the efficiency of energy production in neuron cells by causing oxidative damage to mitochondria [77]. Therefore, antioxidant supplementation, such as coenzyme Q10 and tocopherol, has been proven to increase energy production in many Friedreich ataxia patients by decreasing oxidative stress and restoring mitochondrial activity [78].

Because the brain contains so many mitochondria, mitochondrial malfunction can have a considerable deleterious impact on the nervous system. ROS are created spontaneously by the mitochondrial respiratory chain and are vital for sustaining mitochondrial function as well as brain cell resilience. However, there has been little study done to determine the potential involvement of ROS in spinocerebellar ataxia illnesses and establish optimum therapy options. More research is required to better understand the redox mechanisms driving various forms of spinocerebellar ataxias, with an emphasis on ROS-targeted therapy.

5. Role of oxidative stress in pathogenesis of Huntington's disease (HD)

Huntington's Disease, a neurological disorder is associated with unstable amplification of cytosine, adenine, and guanine (CAG) repeats in the HTT gene [79]. Development of CAG repeats within exon 1 of the huntingtin (HTT) gene results in a mutation that causes the polyglutamine tract to elongate, resulting in an HTT protein product that is prone to aggregation [79]. The mutant huntingtin (mHTT) aggregates are accrued throughout the brain of the affected persons, which can disturb transcription process and protein quality control. Those alterations are potentially responsible for the impaired cognitive functions and aberrant motor observed in HD are caused by mutant huntingtin aggregations and concomitant alterations om transcription process and protein quality control [79]. Currently available meditations for HD is palliative as it only inhibits the degree of severeness of symptoms. No meditation/remedy has effectively treated or markedly reversed or arrested the progression of the disease [79]. The mutant huntingtin has been demonstrated to suppress the expression of peroxisome proliferator-activated receptor-coactivator-1 and reduce the concentration of striatal mitochondrial [79, 80]. Similarly, mutant huntingtin has been documented as mutant of HD which has been implicated in the development of neuronal nuclear inclusion in HD as a result of excessive accumulation of cytoplasmic plaque [81]. Notwithstanding the well-proven connection between HD and OS, researches focused at providing treatment for the disease using antioxidant approach have not been successful [82].

A number of studies have documented that there exists link between irreversible neuronal damage and elevated oxidative markers [83]. The concentrations of well-established indicators of oxidative damage in HD such as neuron-specific enolase (NSE) and 8-hydroxy-2-deoxyguanosine (8-OHdG) have been monitored in one study to determine the benefits of neuro rehabilitation exercise [84]. Furthermore, Cu/Zn-SOD (SOD1) was documented as a possible peripheral indicator of neuronal oxidative damage, with levels considerably higher in HD patients compared to controls, implying a compensatory response to increasing oxidative levels in HD patients [84]. Nevertheless, consideration of SOD1 as an oxidative biomarker in HD remains undecided due to varied results obtained displaying different activity and concentration levels of SOD in HD [85]. After the end of the three weeks regimen neurorehabilitation exercise program, significant reduction in the levels of 8-OHdG and NSE were documented while SOD1 level remained high, indicating the possible neuroprotective role of SOD1 as an antioxidant enzyme mitigating against oxidative stress and scavenging free radicals [84]. Taken together, physical exercise was suggested for HD patients as it may possibly inhibit the disease progression and enhance redox homeostasis [86].

The consequence of HD on brain energy levels has stimulated researchers' interest. In HD patients, reduced glucose consumption and higher lactate levels have been observed, supporting the theory that HD reduces energy levels [81]. According to new researches, oxidative damage is connected to reduced expression of the glucose transporter (GLUT)-3, which consequences lead to lactate build-up and glucose uptake inhibition [87]. Most of ATP synthesis take place via the production of proton motive force through processes of the electron transport chain [88]. mHTT has been demonstrated to perform a crucial function in mitochondrial dysfunction. Panov et al. [89] used electron microscopy to detect that the interaction between mitochondrial membranes and the N-terminal of mHTT leads to mitochondrial calcium abnormalities. Furthermore, mHTT inhibits respiratory complex II in a direct manner [90]. This alteration of the mitochondrial electron transport could lead to over production ROS with concomitant reduction in production of ATP [90].

According to a new mechanism hypothesised in 2015 for mitochondrial damage in HD, oxidative stress could incapacitate glyceraldehyde-3-phosphate dehydrogenase catalytic activities. The incapacitated glyceraldehyde-3-phosphate dehydrogenase is linked with impaired mitochondria which serve as a signalling molecule to initiate the damaged mitochondria towards lysosome engulfment through selective degradation. However, in the existence of mHTT, incapacitate glyceraldehyde-3-phosphate dehydrogenase can react unusually with the long polyglutamine of mHTT at the mitochondrial outer membrane, which result in the inhibition of degradation pathway mediated by incapacitate glyceraldehyde-3-phosphate dehydrogenase. As a result, impaired mitochondria are unable to be engulfed by lysosomes resulting into excessive accumulation of mHTT-expressing cells, thus, facilitating cell death [91]. ROS and mitochondrial alterations can both encourage the positive feedback loops, exacerbating neuronal loss in the cortex and striatum and increases oxidative stress [79]. Excessive generation of ROS and mitochondrial alterations have been implicated in the pathogenesis of HD, however, the event that occurred first remain elusive [92].

3-nitrotyrosine, thiobarbituric acid reactive substances (TBARS), and protein carbonyls are some of the other oxidative biomarkers often used in HD models [93]. Likewise, elevated levels of F_2-isoprostane have been reported in the cerebrospinal fluid and brain tissue of Alzheimer's disease and HD patients. As a result, measuring F_2-isoprostane could be a useful way to assess the relevance of oxidative stress in HD

patients. It's worth noting that F_2-isoprostane levels between the HD and control groups may overlap in the early stages of HD development [94]. Thus, interpretation of modifications of oxidative biomarkers in HD should be done with caution due to involvement of oxidative stress in other pathological conditions such as ageing, cancer, and soon. Additionally, oxidative biomarkers alterations levels may not reveal adequate evidence on whether the oxidative alterations perform a significant role on the neuronal cell death or disease pathogenesis [94]. The use of more sensitive and specific indicators or biomarkers would be essential to give detailed information and elucidate the specific functions performed by free radical and oxidative stress in pathogenesis of neurodegenerative diseases, which will provide a mechanistic approach to finding a suitable drug candidate for the effective treatment of HD.

6. Role of oxidative stress in pathogenesis of amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis is a disease in which motor neurons in the anterior horn of the spinal cord gradually diminish [95]. Depending on whether there is a strongly outlined inherited genetic factor, amyotrophic lateral sclerosis is characterised as familial or sporadic. Sporadic amyotrophic lateral sclerosis usually appears between the ages of 50 and 60 [96]. Because the cause of sporadic amyotrophic lateral sclerosis is unknown, finding causal genes and environmental variables has been difficult. About 20% of instances of familial amyotrophic lateral sclerosis were caused by mutations in the SOD1 gene [97]. SOD1 has many activities, including posttranslational modification, energy consumption, controlling cellular respiration, and scavenging superoxide radicals ($O_2^{\bullet-}$) [98]. Despite the fact that SOD malfunction results in a loss of antioxidant capacity, research suggests that genetic ablation of SOD1 in mice does not result in neurodegenerative diseases [14]. In divergence, the gain-of-function of mutant SOD1 protein has been markedly documented in the motor neuron diseases [14]. For example, a study has exhibited that mutant SOD1 can altered the amino acid biosynthesis of cells in a yeast model and induced cellular destruction, responsible for the neural degeneration in amyotrophic lateral sclerosis [99].

Rac1 is directly regulated by SOD1 via endosome connection, which then activates Nox. Redoxosomes which as Nox-containing endosomes play an essential role in NF-kB-mediated regulation of proinflammatory signals. Nox converts molecular oxygen into $O_2^{\bullet-}$, which has vital functions in antibacterial activity, enzyme control, and cell signalling (Li et al., 2011). The ratio of reactive oxygen species to antioxidative molecules is balanced under normal physiological conditions. On the other hand, during pathological conditions, there is always rapid fluctuations in ROS levels and disturbances in antioxidant function, which result in elevated level of apoptosis, lipid peroxidation, and DNA damage during disease states [49]. SOD1 is an enzyme which convert $O_2^{\bullet-}$ into hydrogen peroxide (H_2O_2) and molecular oxygen. SOD1 mutants increase Nox2-dependent ROS generation, which is assumed to be the cause of motor neuron death in amyotrophic lateral sclerosis [100]. SOD1 that has been oxidised or misfolded has been found to cause mitochondrial dysfunction, which has been linked to the aetiology of sporadic amyotrophic lateral sclerosis [101].

Mutant SOD1 may enhance the progression of familial amyotrophic lateral sclerosis via the alterations of signal transduction pathways in motor neurons and in the activity of supportive glial cells [100]. SOD1, for instance, is regarded to be a key cell-signalling molecule with neuromodulatory functions. SOD1 is secreted via the microvesicular secretory pathway, according to studies *in vitro* and in transgenic mice

models. SOD1 secreted into the environment binds to muscanaric receptors on nearby neurons, increasing intracellular Ca^{2+} concentration and ERK/AKT signalling [102]. SOD1 preserves motor neuron integrity by activating ERK/AKT signalling, and it has been demonstrated that SOD1 secretion can be enhanced in neurons under oxidative stress conditions [103]. Propofol conditioning treatment was demonstrated to protect the spinal cord against ischemia–reperfusion injury in rats by boosting PI3K/AKT signalling, which could be mediated by enhanced SOD1 activity [104]. Furthermore, oxidative stress can cause neuron cell death by blocking the neuroprotective IGF-I/AKT pathway, implying that the role of AKT signalling in neurodegeneration should be investigated further [105].

In conclusion, over secretion of ROS in the brain leads to oxidative stress which if not suppressed or inhibited could lead to oxidative damage of essential components of the central nervous system. This can also initiate or enhance some reactions which may have detrimental effects on the physiological functions and health of the brain. These reactions such as neuroinflammation, progressive neuronal cell loss via apoptosis if not abated can exacerbate protein misfolding and formation of protein aggregates resulting into neurodegeneration and associated neurobehavioural incompetence. Considering the pivotal roles of oxidative stress, neuroinflammation, protein misfolding, and apoptosis in neurodegenerative diseases (**Figure 1**), the manipulation of these major players in each of the pathological mechanisms may represent a promising treatment option to slow down neurodegeneration and alleviate associated symptoms.

Author details

Johnson Olaleye Oladele[1,2*], Adenike T. Oladiji[1], Oluwaseun Titilope Oladele[3] and Oyedotun M. Oyeleke[2]

1 Department of Biochemistry, University of Ilorin, Ilorin, Nigeria

2 Biochemistry Unit, Department of Chemical Sciences, Kings University, Ode-Omu, Osun State, Nigeria

3 Phytomedicine and Molecular Toxicology Research Laboratories, Department of Biochemistry, Osun State University, Osogbo, Nigeria

*Address all correspondence to: oladelejohn2007@gmail.com

References

[1] Matilla-Duenas, T. Ashizawa, A. Brice et al. consensus paper: Pathological mechanisms underlying neurodegeneration in spinocerebellar ataxias, Cerebellum, vol. 13, pp. 269-302, 2014.

[2] M. Hamer and Y. Chida, Physical activity and risk of neurodegenerative disease: A systematic review of prospective evidence, Psychological Medicine, vol. 39, pp. 3-11, 2009.

[3] L. M. Bekris, I. F. Mata, and C. P. Zabetian, The genetics of Parkinson disease, Journal of Geriatric Psychiatry and Neurology, vol. 23, pp. 228-242, 2010.

[4] Alzheimer's Association, 2016 Alzheimer's disease facts and figures, Alzheimer's and Dementia, vol. 12, pp. 459-509, 2016.

[5] Okubadejo NU, Ojo OO, Oshinaike OO, 2010, Clinical profile of parkinsonism and parkinson's disease in Lagos, South Western Nigeria, BMC Neurol, 10, 1.

[6] Blandini F, 2013, Neural and immune mechanisms in the pathogenesis of Parkinson's disease, J. Neuroimmune Pharmacol., 8, 189-201.

[7] Chaudhuri KR, Schapira AH, 2009, Non-motor symptoms of Parkinson's disease: Dopaminergic pathophysiology and treatment, Lancet Neurol., 8, 464-474.

[8] Costello S, Cockburn M, Bronstein J, Zhang X, Ritz B, 2009, Parkinson's disease and residential exposure to maneb and paraquat from agricultural applications in the central valley of California, Am. J. Epidemiol., 169, 919-926.

[9] Gao, H.M., Zhang, F., Zhou, H., Kam, W., Wilson, B., Hong, J.S. (2011). Neuroinflammation and alpha-synuclein dysfunction potentiate each other, driving chronic progression of neurodegeneration in a mouse model of Parkinson's disease. Environ. Health Perspect. 119 (6), 807-814.

[10] V. Dias, E. Junn, and M. M. Mouradian, The role of oxidative stress in Parkinson's disease, Journal of Parkinson's Disease, vol. 3, pp. 461-491, 2013.

[11] L. Zuo, T. Zhou, B. K. Pannell, A. C. Ziegler, and T. M. Best, Biological and physiological role of reactive oxygen species – The good, the bad and the ugly, Acta Physiologica, vol. 214, pp. 329-348, 2015.

[12] F. He and L. Zuo, Redox roles of reactive oxygen species in cardiovascular diseases, International Journal of Molecular Sciences, vol. 16, pp. 27770-27780, 2015.

[13] J. St-Pierre, S. Drori, M. Uldry et al., Suppression of reactive oxygen species and neurodegeneration by the PGC-1 transcriptional coactivators, Cell, vol. 127, pp. 397-408, 2006.

[14] K. Hensley, M. Mhatre, S. Mou et al., On the relation of oxidative stress to neuroinflammation: Lessons learned from the G93A-SOD1 mouse model of amyotrophic lateral sclerosis, Antioxidants and Redox Signaling, vol. 8, pp. 2075-2087, 2006.

[15] A. C. Rego and C. R. Oliveira, Mitochondrial dysfunction and reactive oxygen species in excitotoxicity and apoptosis: Implications for the pathogenesis of neurodegenerative

diseases, Neurochemical Research, vol. 28, pp. 1563-1574, 2003.

[16] T. Wyss-Coray and L. Mucke, Inflammation in neurodegenerative disease – A double-edged sword, Neuron, vol. 35, pp. 419-432, 2002.

[17] M. T. Lin and M. F. Beal, Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases, Nature, vol. 443, pp. 787-795, 2006.

[18] A. Ross and S. J. Tabrizi, Huntington's disease: From molecular pathogenesis to clinical treatment, Lancet Neurology, vol. 10, pp. 83-98, 2011.

[19] Mogi M, Harada M, Kondo J. (1994). Interleukin-1 beta, interleukin-6, epidermal growth factor and transforming growth factor-alpha are elevated in the brain from parkinsonian patients. Neurosci Lett; 180: 147-150.

[20] Mogi M, Harada M, Kondo T, Narabayashi H, Riederer P, Nagatsu T. (1995). Transforming growth factor-beta 1 levels are elevated in the striatum and in ventricular cerebrospinal fl uid in Parkinson's disease. Neurosci Lett; 193: 129-132.

[21] Mogi M, Harada M, Kondo T, Riederer P, Nagatsu T. (1995). Brain beta 2-microglobulin levels are elevated in the striatum in Parkinson's disease. J Neural Transm Park Dis Dement Sect; 9: 87-92.

[22] Mogi M, Harada M, Riederer P, Narabayashi H, Fujita K, Nagatsu T. (1994). Tumor necrosis factor-alpha (TNF-alpha) increases both in the brain and in the cerebrospinal fluid from parkinsonian patients. Neurosci Lett; 165: 208-210.

[23] Chao CC, Hu S, Molitor TW, Shaskan EG, Peterson PK. (1992). Activated microglia mediate neuronal cell injury via a nitric oxide mechanism. J Immunol 149: 2736-2741.

[24] Boje KM, Arora PK. (1992). Microglial-produced nitric oxide and reactive nitrogen oxides mediate neuronal cell death. Brain Res; 587: 250-256.

[25] Vane JR, Bakhle YS, Botting RM. (2000). Cyclooxygenases 1 and 2. Annu Rev Pharmacol Toxicol; 38: 97-120.

[26] Knott C, Stern G, Wilkin GP. (2000). Inflammatory regulators in Parkinson's disease: iNOS, lipocortin-1, and cyclooxygenases-1 and -2. Mol Cell Neurosci; 16: 724-739.

[27] Reed JC: Mechanisms of apoptosis. Am J Pathol 2000;157:1415-1430.

[28] Ross, C. A. and Poirier, M. A. Protein aggregation and neurodegenerative disease. Nat. Med. 10(Suppl), S10–S17 (2004).

[29] Goedert, M. Neurodegeneration. Alzheimer's and Parkinson's diseases: The prion concept in relation to assembled Aβ, tau, and α-synuclein. Science 349, 1255555 (2015).

[30] Soto, C. Unfolding the role of protein misfolding in neurodegenerative diseases. Nat. Rev. Neurosci. 4, 49-60 (2003).

[31] A. L. McCormack, M. Thiruchelvam, A. B. Manning-Bog et al., Environmental risk factors and Parkinson's disease: Selective degeneration of nigral dopaminergic neurons caused by the herbicide paraquat, Neurobiology of Disease, vol. 10, pp. 119-127, 2002.

[32] M. J. Farrer, Genetics of Parkinson disease: Paradigm shifts and future prospects, Nature Reviews Genetics, vol. 7, pp. 306-318, 2006.

[33] S. J. Chinta and J. K. Andersen, Redox imbalance in Parkinson's disease, Biochimica et Biophysica Acta, vol. 1780, pp. 1362-1367, 2008.

[34] S. Gangemi, E. Gofita, C. Costa et al., Occupational and environmental exposure to pesticides and cytokine pathways in chronic diseases (review), International Journal of Molecular Medicine, vol. 38, pp. 1012-1020, 2016.

[35] R. Perfeito, T. Cunha-Oliveira, and A. C. Rego, Revisiting oxidative stress and mitochondrial dysfunction in the pathogenesis of Parkinson disease – Resemblance to the effect of amphetamine drugs of abuse, Free Radical Biology and Medicine, vol. 53, pp. 1791-1806, 2012.

[36] L. Zuo and M. S. Motherwell, The impact of reactive oxygen species and genetic mitochondrial mutations in Parkinson's disease, Gene, vol. 532, pp. 18-23, 2013.

[37] G. Ganguly, S. Chakrabarti, U. Chatterjee, and L. Saso, Proteinopathy, oxidative stress and mitochondrial dysfunction: Cross talk in Alzheimer's disease and Parkinson's disease, drug design, Development and Therapy, vol. 11, pp. 797-810, 2017.

[38] N. A. Mazo, V. Echeverria, R. Cabezas et al., Medicinal plants as protective strategies against Parkinson's disease, Current Pharmaceutical Design, vol. 23, 2017.

[39] P. I. Moreira, X. Zhu, X. Wang et al., Mitochondria: A therapeutic target in neurodegeneration, Biochimica et Biophysica Acta, vol. 1802, pp. 212-220, 2010.

[40] S. A. Zaitone, D. M. Abo-Elmatty, and A. A. Shaalan, Acetyl-L-carnitine and alpha-lipoic acid affect rotenone induced damage in nigral dopaminergic neurons of rat brain, implication for Parkinson's disease therapy, Pharmacology, Biochemistry, and Behavior, vol. 100, pp. 347-360, 2012.

[41] Oladele JO., Oladele OT. Oyeleke OM., Oladiji AT. (2021). Neurological complications in COVID-19: Implications on international health security and possible interventions of phytochemicals. Contemporary developments and perspectives in international health security – Volume 2. DOI:10.5772/intechopen.96039

[42] Oladele JO., Oyeleke OM., Oladele OT. Olaniyan MD. (2020). Neuroprotective mechanism of *Vernonia amygdalina* in a rat model of neurodegenerative diseases. Toxicology report. 7: 1223-1232. DOI:10.1016/j.toxrep.2020.09.005

[43] Oyewole O.I., Olabiyi B.F. and Oladele J.O. (2017). Antioxidative potential of *Ricinus communis* leaf extract on cadmium-induced liver and brain toxicity in rats. UNIOSUN Journal of Sciences. 2, 2. 84-90.

[44] Oladele, J.O., Oyewole, O.I., Bello, O.K. and Oladele, O.T. (2017). Assessment of protective potentials of *Ficus exasperata* leaf on arsenate-mediated Dyslipidemia and oxidative damage in Rat's brain. J. basic appl. Res. 3(3): 77-82.

[45] Oladele, JO., Adewale, OO., Oyeleke, OM., Oyewole IO., Salami, MO., Owoade G. (2020). *Annona muricata* protects against cadmium mediated oxidative damage in brain and liver of rats. Acta facultatis medicae Naissensis. 37 (3): 252-260. DOI:10.5937/afmnai2003252O.

[46] Oladele JO, Ajayi EIO, Oyeleke OM, Oladele OT, Olowookere BD,

Adeniyi BM, Oyewole OI, Oladiji AT. (2020). A systematic review on COVID-19 pandemic with special emphasis on curative potentials of medicinal plants. Heliyon. 6: 1-17. DOI:10.1016/j.heliyon.2020.e04897

[47] M. Etminan, S. S. Gill, and A. Samii, Intake of vitamin E, vitamin C, and carotenoids and the risk of Parkinson's disease: A meta-analysis, Lancet Neurology, vol. 4, pp. 362-365, 2005.

[48] M. H. Yan, X. Wang, and X. Zhu, Mitochondrial defects and oxidative stress in Alzheimer disease and Parkinson disease, Free Radical Biology and Medicine, vol. 62, pp. 90-101, 2013.

[49] L. Zuo, B. T. Hemmelgarn, C. C. Chuang, and T. M. Best, The role of oxidative stress-induced epigenetic alterations in amyloid-beta production in Alzheimer's disease, Oxidative Medicine and Cellular Longevity, vol. 2015, Article ID 604658, 13 pages, 2015.

[50] A. Butterfield, The 2013 SFRBM discovery award: Selected discoveries from the Butterfield laboratory of oxidative stress and its sequela in brain in cognitive disorders exemplified by Alzheimer disease and chemotherapy induced cognitive impairment, Free Radical Biology and Medicine, vol. 74, pp. 157-174, 2014.

[51] Ferreiro, C. R. Oliveira, and C. M. Pereira, The release of calcium from the endoplasmic reticulum induced by amyloid-beta and prion peptides activates the mitochondrial apoptotic pathway, Neurobiology of Disease, vol. 30, pp. 331-342, 2008.

[52] J. Bonda, X. Wang, G. Perry et al., Oxidative stress in Alzheimer disease: A possibility for prevention, Neuro-pharmacology, vol. 59, pp. 290-294, 2010.

[53] W. J. Huang, X. Zhang, and W. W. Chen, Role of oxidative stress in Alzheimer's disease, Biomedical Reports, vol. 4, pp. 519-522, 2016.

[54] T. Nakamura and S. A. Lipton, Redox modulation by S-nitrosylation contributes to protein misfolding, mitochondrial dynamics, and neuronal synaptic damage in neurodegenerative diseases, Cell Death and Differentiation, vol. 18, pp. 1478-1486, 2011.

[55] T. Nakamura and S. A. Lipton, Preventing Ca2+—mediated nitrosative stress in neurodegenerative diseases: Possible pharmacological strategies, Cell Calcium, vol. 47, pp. 190-197, 2010.

[56] D. A. Patten, M. Germain, M. A. Kelly, and R. S. Slack, Reactive oxygen species: stuck in the middle of neurodegeneration, Journal of Alzheimer's Disease, vol. 20, Supplement 2, pp. S357–S367, 2010.

[57] P. B. Shelat, M. Chalimoniuk, J. H. Wang et al., Amyloid beta peptide and NMDA induce ROS from NADPH oxidase and AA release from cytosolic phospholipase A2 in cortical neurons, Journal of Neurochemistry, vol. 106, pp. 45-55, 2008.

[58] Giraldo, A. Lloret, T. Fuchsberger, and J. Vina, Abeta and tau toxicities in Alzheimer's are linked via oxidative stress induced p 38 activation: Protective role of vitamin E, Redox Biology, vol. 2, pp. 873-877, 2014.

[59] N. Bulat and C. Widmann, Caspase substrates and neurodegenerative diseases, Brain Research Bulletin, vol. 80, pp. 251-267, 2009.

[60] P. Agostinho, J. P. Lopes, Z. Velez, and C. R. Oliveira, Overactivation of calcineurin induced by amyloid-beta and prion proteins, Neurochemistry International, vol. 52, pp. 1226-1233, 2008.

[61] A. Awasthi, Y. Matsunaga, and T. Yamada, Amyloid-beta causes apoptosis of neuronal cells via caspase cascade, which can be prevented by amyloid-beta-derived short peptides, Experimental Neurology, vol. 196, pp. 282-289, 2005.

[62] Hamilton and C. Holscher, The effect of ageing on neurogenesis and oxidative stress in the APPswe/PS1deltaE9 mouse model of Alzheimer's disease, Brain Research, vol. 1449, pp. 83-93, 2012.

[63] Holmes, C. Cunningham, E. Zotova et al., systemic inflammation and disease progression in Alzheimer disease, Neurology, vol. 73, pp. 768-774, 2009.

[64] T. J. Seabrook, L. Y. Jiang, M. Maier, and C. A. Lemere, Minocycline affects microglia activation, a beta deposition, and behavior in APP-tg mice, Glia, vol. 53, pp. 776-782, 2006.

[65] W. Y. Wang, M. S. Tan, J. T. Yu, and L. Tan, Role of pro-inflammatory cytokines released from microglia in Alzheimer's disease, Annals of Translational Medicine, vol. 3, p. 136, 2015.

[66] M. Nizzari, S. Thellung, A. Corsaro et al., Neurodegeneration in Alzheimer disease: role of amyloid precursor protein and presenilin 1 intracellular signaling, Journal of Toxicology, vol. 2012, Article ID 187297, 13 pages, 2012.

[67] A. Butterfield and D. Boyd-Kimball, The critical role of methionine 35 in Alzheimer's amyloid beta-peptide (1-42)-induced oxidative stress and neurotoxicity, Biochimica et Biophysica Acta, vol. 1703, pp. 149-156, 2005.

[68] C. Curtain, F. Ali, I. Volitakis et al., Alzheimer's disease amyloid-beta binds copper and zinc to generate an allosterically ordered membrane-penetrating structure containing superoxide dismutase-like subunits, The Journal of Biological Chemistry, vol. 276, pp. 20466-20473, 2001.

[69] M. W. Dysken, P. D. Guarino, J. E. Vertrees et al., Vitamin E and memantine in Alzheimer's disease: Clinical trial methods and baseline data, Alzheimer's and Dementia, vol. 10, pp. 36-44, 2014.

[70] M. Dumont and M. F. Beal, Neuroprotective strategies involving ROS in Alzheimer disease, Free Radical Biology and Medicine, vol. 51, pp. 1014-1026, 2011.

[71] K. Kanninen, T. M. Malm, H. K. Jyrkkanen et al., Nuclear factor erythroid 2-related factor 2 protects against beta amyloid, Molecular and Cellular Neurosciences, vol. 39, pp. 302-313, 2008.

[72] M. Rossi, S. Perez-Lloret, L. Doldan et al., Autosomal dominant cerebellar ataxias: A systematic review of clinical features, European Journal of Neurology, vol. 21, pp. 607-615, 2014.

[73] Y. M. Sun, C. Lu, and Z. Y. Wu, Spinocerebellar ataxia: Relationship between phenotype and genotype – A review, Clinical Genetics, vol. 90, pp. 305-314, 2016.

[74] M. U. Manto, The wide spectrum of spinocerebellar ataxias (SCAs), Cerebellum, vol. 4, pp. 2-6, 2005.

[75] M. Stucki, C. Ruegsegger, S. Steiner et al., Mitochondrial impairments contribute to spinocerebellar ataxia type 1 progression and can be ameliorated by the mitochondria targeted antioxidant MitoQ, Free Radical Biology and Medicine, vol. 97, pp. 427-440, 2016.

[76] A. H. Hakonen, S. Goffart, S. Marjavaara et al., Infantileonset spinocerebellar ataxia and mitochondrial recessive ataxia syndrome are associated

with neuronal complex I defect and mtDNA depletion, Human Molecular Genetics, vol. 17, pp. 3822-3835, 2008.

[77] V. Campuzano, L. Montermini, Y. Lutz et al., Frataxin is reduced in Friedreich ataxia patients and is associated with mitochondrial membranes, Human Molecular Genetics, vol. 6, pp. 1771-1780, 1997.

[78] R. Lodi, P. E. Hart, B. Rajagopalan et al., Antioxidant treatment improves in vivo cardiac and skeletal muscle bioenergetics in patients with Friedreich's ataxia, Annals of Neurology, vol. 49, pp. 590-596, 2001.

[79] J. Gil-Mohapel, P. S. Brocardo, and B. R. Christie, The role of oxidative stress in Huntington's disease: Are antioxidants good therapeutic candidates?, Current Drug Targets, vol. 15, pp. 454-468, 2014.

[80] P. Weydt, V. V. Pineda, A. E. Torrence et al., Thermoregulatory and metabolic defects in Huntington's disease transgenic mice implicate PGC-1alpha in Huntington's disease neurodegeneration, Cell Metabolism, vol. 4, pp. 349-362, 2006.

[81] Mochel and R. G. Haller, Energy deficit in Huntington disease: why it matters, The Journal of Clinical Investigation, vol. 121, pp. 493-499, 2011.

[82] A. Kumar and R. R. Ratan, Oxidative stress and Huntington's disease: The good, the bad, and the ugly, Journal of Huntington's Disease, vol. 5, pp. 217-237, 2016.

[83] Tunez, F. Sanchez-Lopez, E. Aguera, R. Fernandez-Bolanos, F. M. Sanchez, and I. Tasset-Cuevas, important role of oxidative stress biomarkers in Huntington's disease, Journal of Medicinal Chemistry, vol. 54, pp. 5602-5606, 2011.

[84] Ciancarelli, D. AmicisDe, C. MassimoDi et al., influence of intensive multifunctional neurorehabilitation on neuronal oxidative damage in patients with Huntington's disease, Functional Neurology, vol. 30, pp. 47-52, 2015.

[85] M. A. Sorolla, G. Reverter-Branchat, J. Tamarit, I. Ferrer, J. Q. Ros, and E. Cabiscol, Proteomic and oxidative stress analysis in human brain samples of Huntington disease, Free Radical Biology and Medicine, vol. 45, pp. 667-678, 2008.

[86] R. M. Arida, F. A. Scorza, and E. A. Cavalheiro, Role of physical exercise as complementary treatment for epilepsy and other brain disorders, Current Pharmaceutical Design, vol. 19, pp. 6720-6725, 2013.

[87] A. Covarrubias-Pinto, P. Moll, M. Solis-Maldonado et al., Beyond the redox imbalance: Oxidative stress contributes to an impaired GLUT3 modulation in Huntington's disease, Free Radical Biology and Medicine, vol. 89, pp. 1085-1096, 2015.

[88] M. Bonora, M. R. Wieckowsk, C. Chinopoulos et al., Molecular mechanisms of cell death: Central implication of ATP synthase in mitochondrial permeability transition, Oncogene, vol. 34, p. 1608, 2015.

[89] A. V. Panov, C. A. Gutekunst, B. R. Leavitt et al., Early mitochondrial calcium defects in Huntington's disease are a direct effect of polyglutamines, Nature Neuroscience, vol. 5, pp. 731-736, 2002.

[90] E. Bossy-Wetzel, A. Petrilli, and A. B. Knott, Mutant huntingtin and mitochondrial dysfunction, Trends in Neurosciences, vol. 31, pp. 609-616, 2008.

[91] Liot, J. Valette, J. Pepin, J. Flament, and E. Brouillet, Energy defects in

Huntington's disease: why "in vivo" evidence matters, Biochemical and Biophysical Research Communications, vol. 483, no. 4, pp. 1084-1095, 2017.

[92] C. Zuccato, M. Valenza, and E. Cattaneo, Molecular mechanisms and potential therapeutical targets in Huntington's disease, Physiological Reviews, vol. 90, pp. 905-981, 2010.

[93] M. C. Polidori, P. Mecocci, S. E. Browne, U. Senin, and M. F. Beal, Oxidative damage to mitochondrial DNA in Huntington's disease parietal cortex, Neuroscience Letters, vol. 272, pp. 53-56, 1999.

[94] E. Miller, A. Morel, L. Saso, and J. Saluk, Isoprostanes and neuroprostanes as biomarkers of oxidative stress in neurodegenerative diseases, Oxidative Medicine and Cellular Longevity, vol. 2014, Article ID 572491, 10 pages, 2014.

[95] P. Taylor, R. H. Brown Jr., and D. W. Cleveland, Decoding ALS: From genes to mechanism, Nature, vol. 539, pp. 197-206, 2016.

[96] C. Ingre, P. M. Roos, F. Piehl, F. Kamel, and F. Fang, Risk factors for amyotrophic lateral sclerosis, Clinical Epidemiology, vol. 7, pp. 181-193, 2015.

[97] Gamez, M. Corbera-Bellalta, G. Nogales et al., mutational analysis of the cu/Zn superoxide dismutase gene in a Catalan ALS population: Should all sporadic ALS cases also be screened for SOD1?, Journal of the Neurological Sciences, vol. 247, pp. 21-28, 2006.

[98] R. A. Saccon, R. K. Bunton-Stasyshyn, E. M. Fisher, and P. Fratta, Is SOD1 loss of function involved in amyotrophic lateral sclerosis?, Brain, vol. 136, pp. 2342-2358, 2013.

[99] E. L. Bastow, A. R. Peswani, D. S. J. Tarrant et al., New links between SOD1 and metabolic dysfunction from a yeast model of amyotrophic lateral sclerosis, Journal of Cell Science, vol. 129, pp. 4118-4129, 2016.

[100] Q. Li, N. Y. Spencer, N. J. Pantazis, and J. F. Engelhardt, Alsin and SOD1(G93A) proteins regulate endosomal reactive oxygen species production by glial cells and proinflammatory pathways responsible for neurotoxicity, The Journal of Biological Chemistry, vol. 286, pp. 40151-40162, 2011.

[101] E. D'Amico, P. Factor-Litvak, R. M. Santella, and H. Mitsumoto, Clinical perspective on oxidative stress in sporadic amyotrophic lateral sclerosis, Free Radical Biology and Medicine, vol. 65, pp. 509-527, 2013.

[102] P. Mondola, S. Damiano, A. Sasso, and M. Santillo, The cu, Zn superoxide dismutase: Not only a dismutase enzyme, Frontiers in Physiology, vol. 7, p. 594, 2016.

[103] S. Damiano, T. Petrozziello, V. Ucci, S. Amente, M. Santillo, and P. Mondola, Cu-Zn superoxide dismutase activates muscarinic acetylcholine M1 receptor pathway in neuroblastoma cells, Molecular and Cellular Neurosciences, vol. 52, pp. 31-37, 2013.

[104] Q. J. Yu and Y. Yang, Function of SOD1, SOD2, and PI3K/AKT signaling pathways in the protection of propofol on spinal cord ischemic reperfusion injury in a rabbit model, Life Sciences, vol. 148, pp. 86-92, 2016.

[105] D. Davila and I. Torres-Aleman, Neuronal death by oxidative stress involves activation of FOXO3 through a two-arm pathway that activates stress kinases and attenuates insulin like growth factor I signaling, Molecular Biology of the Cell, vol. 19, pp. 2014-2025, 2008.

Chapter 10

Metal Ions-Mediated Oxidative Stress in Alzheimer's Disease and Chelation Therapy

Dongjin Yeo, Tae Gyu Choi and Sung Soo Kim

Abstract

Alzheimer's disease (AD), ranked as the seventh leading cause of death worldwide, is one of the most incidental neurodegenerative disorders. AD patients experience irreparable damages to the brain, indicated as progressive, insidious, and degenerative. Past research has discovered that the *amyloid cascade hypothesis* best describes the pathophysiological etiology of AD, designating amyloid-β plaques and neurofibrillary tangles as the 'hallmarks' of AD pathology. Furthermore, accumulating evidence show that the oxidative stress state, the imbalance between reactive oxygen species (ROS) production and antioxidation, contributes to AD development. This chapter describes the oxidative stress process in AD. It mainly tackles the correlation of metal-catalyzed ROS production with amyloid-β and how it oxidatively damages both the amyloid-β itself and the surrounding molecules, potentially leading to AD. Additionally, both the role of metal chelation therapy as a treatment for AD and its challenges will be mentioned as well. This chapter specially focuses on how metal ions imbalance induces oxidative stress and how it affects AD pathology.

Keywords: Alzheimer's disease, Amyloid-β, Reactive oxygen species, Oxidative stress process, Metal ions, Metal chelation therapy

1. Introduction

Past research has tried to find the pathogenesis and etiologies regarding Alzheimer's disease (AD). Recent studies show that reactive oxygen species (ROS) are linked with the progression and development of AD, especially superoxide anion, hydrogen peroxide, and hydroxyl radical. Reactive oxygen species have been found as the by-products of metal-catalyzed oxidation associated with amyloid-$\boldsymbol{\beta}$. These findings are crucial for the treatment of AD, as they provide the underlying mechanism for metal chelation therapy, which involves the use of metal chelators for metal removal.

This chapter discusses both past and current research with regards to AD pathology and treatment in the following order: Alzheimer's disease, reactive oxygen species, oxidative stress and Alzheimer's disease, metal chelation therapy, and challenges of metal chelation therapy.

2. Oxidative stress and Alzheimer's disease

2.1 Alzheimer's disease

Alzheimer's disease (AD) is one of the most common neurodegenerative diseases characterized as insidious, progressive, and degenerative. It accounts for 70% of all dementia cases in people aged 65 years and older [1]. The World Health Organization revealed that AD ranked as the seventh leading cause of death worldwide from 2000 to 2019. Although it is assumed that AD is triggered from genetics, environment, and dietary factors, the exact causes of AD are still not fully understood [2].

Patients with AD experience irreversible damage to the brain which leads to cognitive and behavioral deterioration, shrinkage of brain tissue, and progressive memory loss [3]. $A\beta$ and NFTs are considered as the two key factors in the neurodegeneration of AD patients. The forebrain cholinergic neurons are damaged due to neurofibrillary tangles (NFTs) of P-tau and the accumulation of senile plaques composed of amyloid-β($A\beta$) in the hippocampus, neocortex, amygdala, and basal nucleus of Meynert [4].

Although still debated, the amyloid cascade hypothesis best explains the pathology of AD. The $A\beta$ protein, a 36 to 43 residue polypeptide (in several studies, 39 to 43 residues/38 to 43 residues), is generated in the process of amyloid precursor protein (APP) enzymatic proteolysis, a transmembrane protein responsible for neuron growth and repair. Among the two main pathways for disposal of APP, a non-amyloidogenicα -secretase-mediated pathway and an amyloidogenic β-and γ -secretase-mediated pathway, the neuropathology of AD derives from the latter, in which $A\beta$ peptide is produced [5].

APP consists of both a cytoplasmic C-terminus and an extracellular glycosylated N-terminus. In the amyloidogenic pathway, APP is initially cleaved by a β-secretase creating a membrane bound 99-amino-acid C-terminal fragment. The C-terminal fragment, now acting as a substrate, is serially cleaved by a γ-secretase, resulting in a full length $A\beta$, mainly the 40-amino-acid $A\beta$40 and the 42-amino-acid $A\beta$42 [6, 7].

Due to the insolubility in AD patients, the $A\beta$ monomers abnormally aggregate into higher order assemblies, oligomers, protofibrils, and fibrils, which ultimately deposit into senile plaques. Amyloid senile plaques spread throughout the brain, eventuating in the interference of intercellular communication and the activation of immune cells which provoke inflammation. Neurological brain damage induced from amyloid plaques are commonly detected in the neocortex of AD patients [7, 8].

NFTs, another factor regarded as a key contributor of AD, is linked with $A\beta$ as well. Microtubules (MTs) in neurons work as directional highways between the axon and dendrites for organelle transport such as nutrients, neurotransmitters, motor proteins. The MT arrays also act as architectural elements that stabilize the structure and shape of the neuron [9]. The firmness of MTs depends on tau, a microtubule-associated protein (MAP), which plays a vital role in regulating the dynamic network and assembly of MTs [10].

There is accumulating evidence that $A\beta$ peptide induces tau hyperphosphorylation, which reduces the MT-tau affinity. Tau, no longer able to bind to MTs, start to aggregate forming tau clumps. Consequently, due to the decreased stability, MTs start to disintegrate. Separated tau cluster into tau oligomers, which eventually develop into neurofibrillary tangles (NFTs) [11]. With the breakdown of the MT system, neurons are incompetent to transmit organelles, resulting in the neurodegeneration of nerve cells which explains the memory loss and cognitive and behavioral decline of AD patients.

2.2 Reactive oxygen species

Reactive oxygen species (ROS) are unstable, highly reactive molecules and radicals which are derived from molecular oxygen. ROS production takes place in aerobic organisms that utilize mitochondrial electron transport for respiration or undergo oxidation catalyzed by metals and intracellular enzymes [12]. In normal settings, ROS play a crucial role for cell signaling such as cell cycle regulation, enzyme activation and apoptosis. Yet, under oxidative stress conditions, the immoderate production of ROS has detrimental effects on cells causing protein, DNA, and lipids damage and eventually, cell death [1].

When a molecular oxygen goes through a monovalent reduction, superoxide anion radical ($O_2^{\cdot-}$), a precursor compound of ROS, is formed [13]. $O_2^{\cdot-}$, due to its unstable state, react with other radicals such as nitric oxide (NO), forming highly reactive peroxynitrite ($ONNO^-$). $0_2^{\cdot-}$ also propagates further oxidative chain reactions, producing hydrogen peroxide (H_2O_2) with the help of superoxide dismutase (SOD). H_2O_2 are sequentially reduced either to hydroxyl radical ($OH^{\cdot}$), one of the most reactive oxidants, or fully reduced to water [14, 15].

ROS generation, mainly in forms of $O_2^{\cdot-}$, H_2O_2, $OH^{\cdot}$, are induced by both endogenous and exogenous pathways. The endogenously produced ROS are mainly byproducts of mitochondrial respiratory chain and phagocytic nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, circumstances in which the reduction of oxygen is enabled (**Figures 1** and **2**). Transition metals and numerous intracellular enzymes such as, Xanthine oxidase (XO), Lipoxygenases (LXO), and Cyclooxygenase (COX) are also principal endogenous ROS generators (**Figures 3–5**) [16].

ROS are produced in response to exogenous or environmental factors as well, such as radiation, air pollutants, diet, tobacco smoke, drugs and xenobiotics, chemotherapy, and pesticides [16, 17]. Exposure to UVR from solar radiation develops high concentrations of ROS, which causes an imbalance between ROS and cellular antioxidants, thus provoking oxidative stress [17]. Tobacco smoke, another notable factor of ROS production, consists of 10^{14}-10^{16} free radicals per puff which can potentially produce H_2O_2 and $OH^{\cdot}$ [16].

The right duration, quantity, and location of ROS production is required for normal physiological processes. In cases where the appropriate conditions are not met,

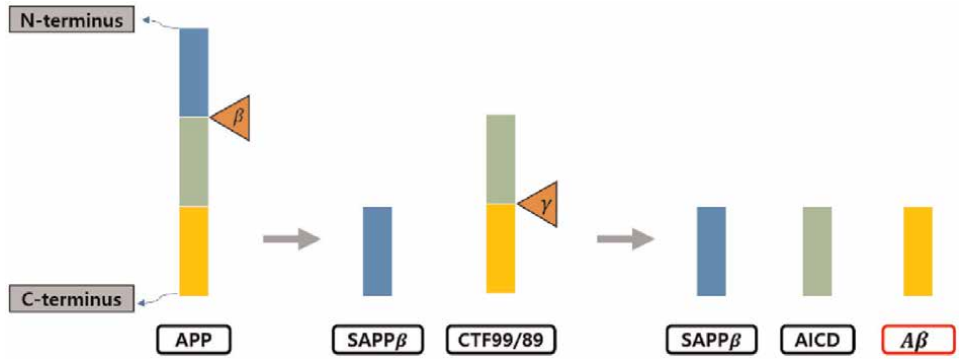


Figure 1.
Amyloidogenicβ -and γ-secretase-mediated pathway of APP disposal In the amyloidogenic pathway of APP disposal, APP is first cleaved by a β-secretase yielding SAPPβ and CTF99/89. Subsequently, CTF99/89 is cleaved by a γ-secretase creating AICD and Aβ.

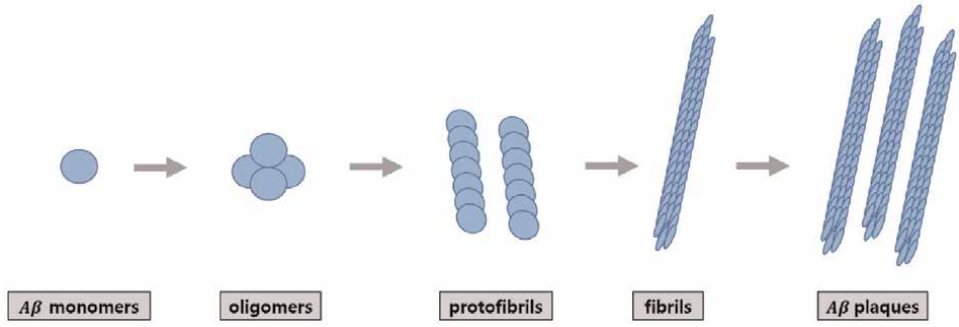


Figure 2.
Aβ plaques formation abnormal aggregation ofAβ monomers into oligomers, protofibrils, fibrils, and ultimately plaques can be seen in AD patients.

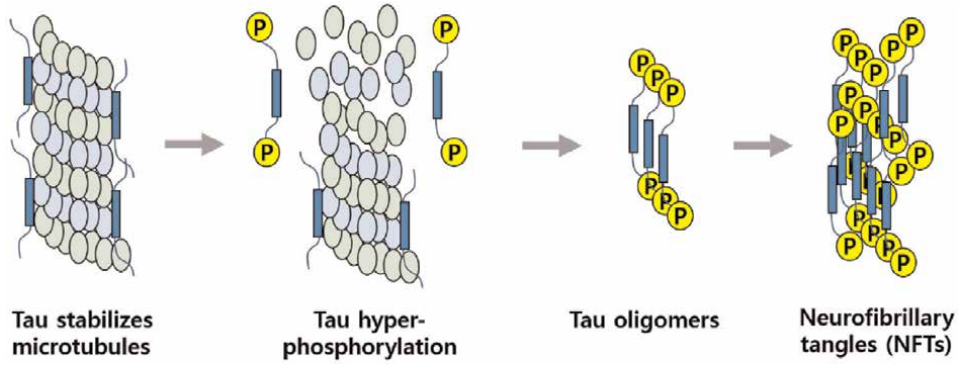


Figure 3.
NFTs formation Aβ peptide induces tau hyperphosphorylation, which reduces MT-tau affinity. Separated tau develop into oligomers and eventually NFTs.

both insufficient and excessive ROS production, ROS-related diseases can arise [15]. Such medical conditions include glucolipotoxicity, insulin resistance, diabetes mellitus, mitochondrial dysfunction, cancer, autoimmune disorders, cardiovascular, neurological, and psychiatric disease [15, 18].

Antioxidants work as the defense mechanism against ROS induced damage. Its role is to maintain the effective functions of ROS while at the same time, regulate its level. Oxidative stress is attenuated by both endogenous antioxidant system and the exogenous intake of antioxidants [19, 20]. The former includes enzymes such as SOD, glutathione (GSH), catalase and glutathione peroxidase (GPx) [19]. Meanwhile, the essential exogenous antioxidants are absorbed through vegetables, whole grains, fruits, and omega-3 fatty acid containing diet. Vitamin C, vitamin E, β-carotene, selenium, carotenoids, and polyphenols represent exogenous antioxidants [19, 20].

2.3 Oxidative stress and Alzheimer's disease

Majority of current research show that oxidative stress, the imbalanced state of ROS production level and antioxidative level, is related to the pathogenesis of neurodegenerative diseases, representatively AD [21]. This chapter approaches mainly the

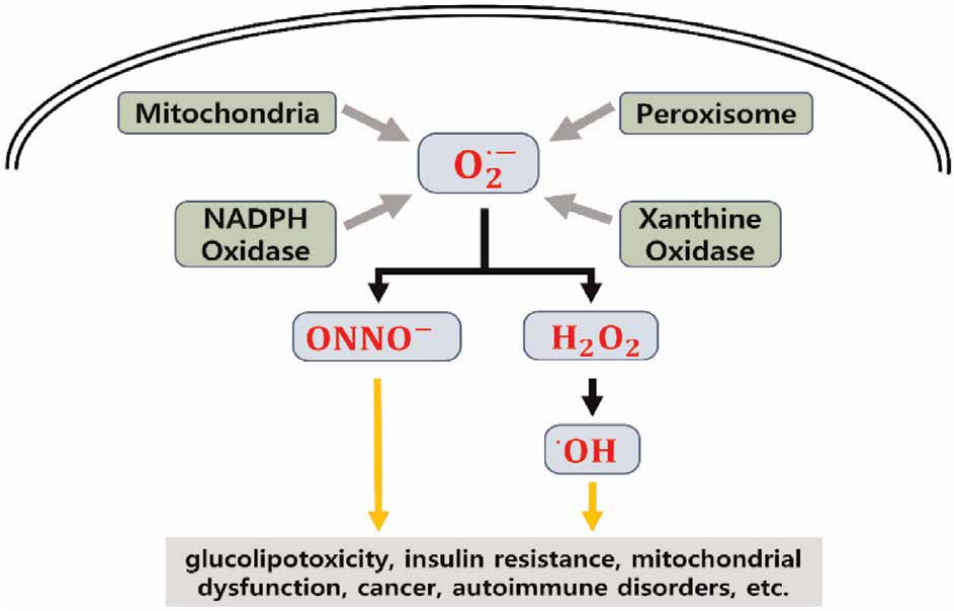


Figure 4.
ROS production pathways endogenous and exogenous pathways of ROS production include mitochondrial production, NADPH oxidase, peroxisome, and xanthine oxidase. Through such pathways, $O_2^{\cdot-}$, $ONNO^-$, H_2O_2, and $OH^{\cdot}$ are yielded.

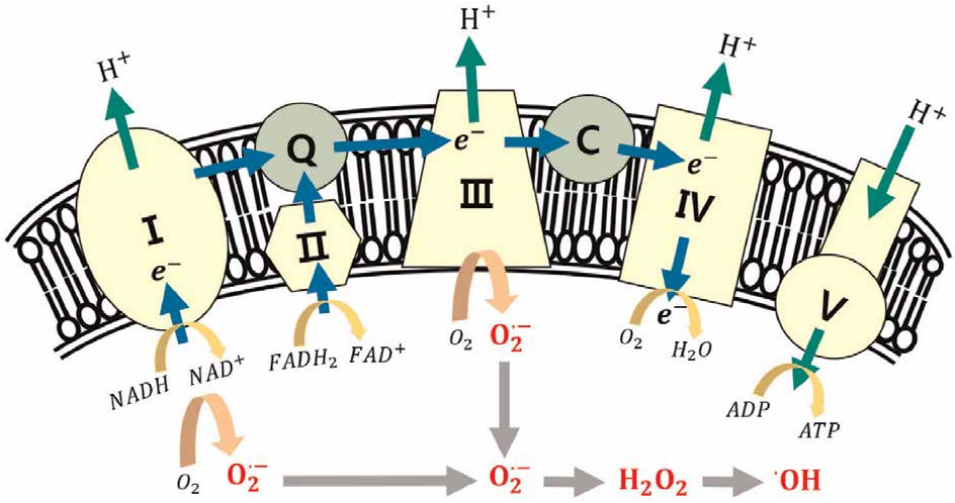


Figure 5.
Mitochondrial ROS production complex Iand complex III of the inner mitochondrial membrane create $O_2^{\cdot-}$ through oxidative phosphorylation. $O_2^{\cdot-}$, through further reactions, can also yield H_2O_2, and $OH^{\cdot}$.

association of oxidative stress with AD, mostly regarding the correlation between *Aβ* and ROS production and how it affects the neighboring neural molecules.

As previously stated above in the *Alzheimer's Disease* section, amyloid plaques and NFTs are regarded as the 'hallmarks' of AD. Overwhelming evidence show that amyloid plaques are highly concentrated in metal ions, such as copper(Cu), iron(Fe), zinc(Zn)

and calcium(Ca), which are present in the synaptic areas. Such metal ions are interconnected with the amyloid cascade reaction and NFT formation [22].

Metal ions imbalance induces oxidative stress which triggers ROS production. Increased production of ROS leads to secretases imbalance and phosphatases imbalance, each interconnected with the formation of $A\beta$ and P-tau. Accordingly, $A\beta$ and P-tau production increases, which eventually leads to neurodegenerative diseases including AD [23]. Thus, the $A\beta$ toxicity, NFTs, oxidative stress, and ultimately neuronal cell death depend on the existence of redox metals [24]. This chapter mainly discusses the correlation of metal-catalyzed ROS production with Aβ (**Figure 6**).

2.3.1 Copper

Among the metal ions, copper is considered the most redox reactive. The association of copper ions with $A\beta$ can be described as a three-step process. First, endogenous reductants bind with the copper, followed by the reduction of Cu(II) to Cu(I). The reductive state of copper triggers the reduction of molecular oxygen as well, producing ROS [25]. Copper directly interacts with $A\beta$, promoting increased aggregation of $A\beta$ and the toxicity of amyloid oligomers and plaques [22, 26].

Histidine ($His6, His13, His14$) and Tyrosine (Tyr10) amino acid residues modulate the binding of copper to $A\beta.Cu$(II) is reduced to Cu(I), after its chemically binding to $A\beta$(higher affinity to $A\beta$1- 42 compared to $A\beta$1- 40), generating hydrogen peroxide as a byproduct which has high potential to be reduced to hydroxyl radical. Accordingly, the complexation of copper in $A\beta$ elevates the neurotoxicity, now endowed with enlarged

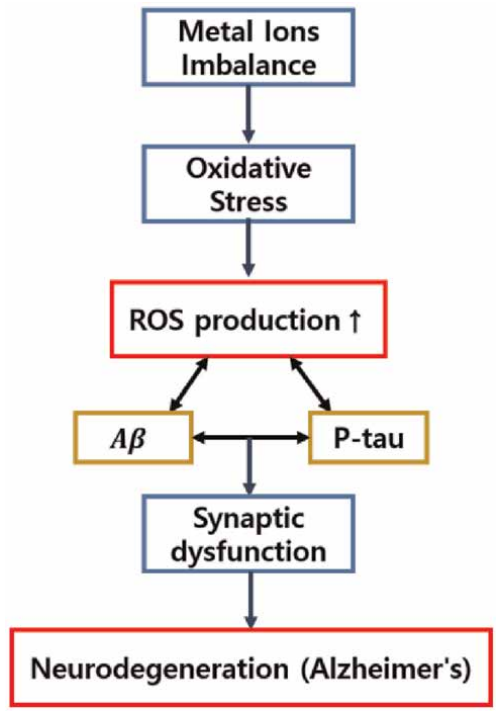


Figure 6.
Metal ions imbalance, increased ROS production, and neurodegeneration imbalance of metal ions, such as copper, iron, zinc, and calcium, creates oxidative stress condition. This is followed by increased production of ROS, and consequently Aβ and NFTs, which eventually provokes neurodegenerative diseases including AD.

reduction potential [24]. The *Cu- Aβ* couple correspondingly assists the further process of ROS production. The copper- *Aβ*-mediated oxidation of reductant species such as ascorbate, which are abundant in the brain, induces generation of ROS: hydrogen peroxide, hydroxyl radical and superoxide anion [25].

2.3.2 Iron

Iron, as a redox active metal, is also significantly linked with AD pathology. However, unlike copper, iron ions do not directly interact with and bind to *Aβ* [27]. Iron exists in both in redox-inactive forms (Fe^{3+}) and redox-active forms (Fe^{2+}) within the brain. They are also found in zero-oxidation-state (Fe^{0}) or as ionic compounds such as magnetite(Fe_3O_4) as well. All forms are possible inducers for *Aβ* aggregation, prompting the iron redox cycle and ROS production [28, 29]. Iron concentration and increased free radical production had been noticed in the cerebellum and glia cells of AD patients [23].

After iron's indirect interaction with *Aβ*, the redox cycle of Haber-Wiess and Fenton reaction is triggered, yielding ROS in forms of hydrogen peroxide, hydroxyl radical and superoxide anion, as in the process of copper-mediated oxidation. The resulting ROS effects *Aβ* aggregation and other oxidative damages in local organelles as well. Research results based on high-resolution transmission electron microscopy (HR-TEM) and synchrotron-based X-ray absorption studies support the storage of iron within *Aβ* and the iron-catalyzed ROS production [27, 29].

During the process of the metal-catalyzed ROS production in correlation with *Aβ*, both the *Aβ* peptide itself and the surrounding molecules undergo oxidative damages. The amino acid residues of *Aβ*, cysteine, methionine, arginine, histidine, lysine, phenylalanine, tryptophan, and tyrosine, are oxidated as well, chemically changed, and impaired. The ROS produced through metal-mediated oxidation also cause protein carbonylation and nitration, lipid peroxidation, and protein modification. The mitochondria of nearby cells also experience oxidation, leading to increased mitochondrial and nuclear DNA &RNA damages which all potentially lead to the etiology of AD [30].

2.3.3 Zinc

The impact of zinc(Zn^{2+}) in AD is rather controversial [23]. Some research suggests irregularly high concentration of Zn^{2+} have been investigated in AD patients' brains, inferring the linkage between imbalance of Zn^{2+} homeostasis with AD pathogenesis [31]. One study indicated that Zn^{2+} promotes both *Aβ*40 and *Aβ*42 aggregation, but only at the early stage [32]. In another study, high concentration of Zn^{2+} was shown to induce NADPH-oxidase reaction and ROS production (especially mitochondrial ROS production) in AD pathological state. Excessive zinc therefore prompted *Aβ* cascade reaction [23]. On the contrary, other research analysis show significant decrease of Zn^{2+} in AD patients [33].

2.3.4 Calcium

Calcium(Cu^{2+}) elevation also significantly contributes to *Aβ* production in AD patients. Sequentially, increased *Aβ* level in turn promotes an increase in Cu^{2+} level by triggering the opening of voltage-dependent Cu^{2+} channels. Moreover, high degree of Cu^{2+}provokes further influx of Cu^{2+} by enabling overexpression of L-type calcium

channel subtype (Cav1.2). Excessive Cu^{2+} consecutively stimulate $A\beta$ production and aggregation [23].

2.4 Metal chelation therapy, a potential treatment for Alzheimer's disease

Based on the thesis that AD pathology relates to the interplay between metal ions and $A\beta$, treatments for AD have been proposed established on this characteristic. Metal chelation therapy has been raised as a method to agitate metal- $A\beta$ interactions to treat AD in a lot of research [27]. Metal chelation therapy is initiated by injection of chelators (chelating agents) into the bloodstream which bind to the targeted metals and excrete them [34].

Studies show that metal chelating agents must satisfy the following conditions to manipulate as prospective treatments for AD.

1. Low molecular weight
2. Target certain: must be able to selectively attach to targeted metal ions bound to $A\beta$
3. Free or poor charge: must be able to cross blood brain barrier (BBB)
4. Low toxicity
5. Low possibility of side effects

Metal chelators content with above properties will successfully affix to aimed metal ions associated with $A\beta$, engendering their break-up and removal [27].

Among the various chelator drugs, only a few are suitable for AD; drugs that fulfill the properties stated above. The common chelator drugs adopted for AD treatments that have shown favorable results include desferrioxamine (DFO), bathophenanthroline, bathocuproine (BC), trientine, penincillamine, bis (thiosemicarbazone), tetrathiomolybdate (TTM) [35–37].

In one clinical trial in 48 patients with AD, DFO has shown its positive effects. Using trace-metal analysis, the research team confirmed that DFO decreased the aluminum level in neocortical brains of AD patients dosed with DFO;125 mg per injection, twice a day, five days a week [38]. Although it showed outcomes regarding aluminum, one research insisted that, considering the affinity DFO has for iron, the result might have also been due to the elimination of iron [39]. In addition to iron, DFO also shows binding affinity towards copper [40].

Penincillamine, bathophenanthroline, bathocuproine (BC), and trientine have also been proven to be effective copper chelators. In one research test, these agents showed interaction with Cu- $A\beta$ couple, deleting copper and improving $A\beta$ solubility. Furthermore, BC has been proved to be the most efficacious, showing constant results across the broad range of AD brain tissue samples [37].

It has been suggested that the bis(thiosemicarbazone) compounds can regulate the concentration of copper in $A\beta$ as well [41]. In one study, chemical compounds of the bis (thiosemicarbazone) metal complex family have shown successful treatment for animal models with AD [42]. Similar results have been noticed in another study using APP/PS1 transgenic AD mice model as well. Bis(thiosemicarbazone) enhanced the soluble $A\beta$ level by deleting copper and led to the restoration of cognitive activity [43].

The effect of tetrathiomolybdate (TTM) as a copper chelator has been demonstrated as well. In one experiment, TTM was applied to Tg2576 transgenic mice model for five months. Positive effects were derived, showing that TTM lowered both the level of *Aβ* and *Aβ* plaques present in the brain [44].

2.5 Challenges of metal chelation therapy

Although the above-stated metal chelating agents have shown positive effects in reducing *Aβ* levels in AD patients, there are still challenges surrounding the metal chelation therapy.

First, in addition to the originally aimed effects, metal chelating agents can induce undesirable outcomes as well. One study revealed that the application of divalent chelators, such as *Cu*, *Fe* and *Zn*, to severe AD patients lessened the requisite divalent metals that were already in their appropriate levels, as well as the targeted metal ions. Accordingly, the depletion of essential metals aggravated rather than treated AD pathology [45].

Furthermore, as stated in *Metal Chelation Therapy, a Potential Treatment for Alzheimer's Disease*, metal chelating agents have been proved to lower*Aβ* level through solubilization. However, it is still rather controversial whether metal chelators can not only *solubilize* but *reverse* the *Aβ* plaques to any forms of intermediates such as monomers, oligomers, protofibrils, short fibrils, or extended fibrils [27, 45].

Finally, there are remaining questions concerning the efficacy of certain metal chelating agents. For instance, clioquinol (CQ), a*Cu - Zn* chelator capable of agitating *Aβ* aggregation has been used in numerous clinical trials. However, the clinical and experimental results show that the effectiveness of CQ is yet contentious [45, 46]. In one experiment, the utilization of CQ perturbed *Cu* and *Zn* homeostasis which elevated metal ion concentrations, which is contradictory to the predicted results. CQ also showed side effects, arising astrogliosis, spongiosis, and brain edema to the mice model [47].

For further development of metal chelation therapy, such disadvantages should be improved.

3. Conclusion

Aβ and amyloid plaques are determining symbols of AD. Metal ions, especially copper and iron, interact with *Aβ* in AD patient's brain which generates ROS, such as superoxide anion, hydrogen peroxide, and hydroxyl radical. This process promotes the aggregation *Aβ* and increases the toxicity of *Aβ* plaques. ROS induces damages to both *Aβ* itself and the surrounding molecules leading to protein, lipid, DNA, and RNA impairment. Metal chelation therapy has been proposed as method to agitate metal-*Aβ* interactions for AD treatment. Metal chelators injected into the bloodstream will target metals associated with *Aβ* and eliminate them, cutting off the activity of causative substances. The metal chelating agents that have shown positive effects towards AD so far include desferrioxamine (DFO), bathophenanthroline, bathocuproine (BC), bis(thiosemicarbazone), tetrathiomolybdate (TTM), trientine, and penicillamine. However, there are ongoing challenges facing the metal chelation therapy. The remaining questions regarding the efficacy of chelating agents and the precise mechanism of chelation therapy should be solved.

Acknowledgements

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korean government (MEST) (NRF-2020R1I1A1A01069013 and NRF-2018R1A6A1A03025124).

Conflict of interest

The authors declare no conflict of interest.

Appendices and nomenclature

Aβ	amyloid-*β*
AD	Alzheimer's disease
APP	amyloid Precursor Protein
BBB	blood brain barrier
Cav1.2	L-type calcium channel subtype
COX	cyclooxygenase
CQ	clioquinol
DOS	desferrioxamine
GPx	glutathione peroxidase
GSH	glutathione
HR-TEM	high-resolution transmission electron microscopy
LXO	lipoxygenases
MAP	microtubule-associated protein
MT	microtubules
NADPH	phagocytic nicotinamide adenine dinucleotide phosphate
NFT	neurofibrillary tangle
ROS	reactive oxygen species
SOD	superoxide dismutase
TTM	tetrathiomolybdate
XO	Xanthine oxidase

Author details

Dongjin Yeo, Tae Gyu Choi* and Sung Soo Kim*
Department of Biochemistry and Molecular Biology, School of Medicine, Kyung Hee University, Seoul, Republic of Korea

*Address all correspondence to: aske@daum.net; sgskim@khu.ac.kr

References

[1] Manoharan S, Guillemin G, Abiramasundari R, Essa M, Akbar M, Akbar M. The Role of Reactive Oxygen Species in the Pathogenesis of Alzheimer's Disease, Parkinson's Disease, and Huntington's Disease: A Mini Review. Oxidative Medicine and Cellular Longevity. 2016;2016:1-15.

[2] Dosunmu R, Wu J, Basha M, Zawia N. Environmental and dietary risk factors in Alzheimer's disease. Expert Review of Neurotherapeutics. 2007;7(7):887-900.

[3] Gugliandolo A, Bramanti P, Mazzon E. Role of Vitamin E in the Treatment of Alzheimer's Disease: Evidence from Animal Models. International Journal of Molecular Sciences. 2017;18(12):2504.

[4] Wenk, Gary L. "Neuropathologic changes in Alzheimer's disease." *The Journal of clinical psychiatry* vol. 64 Suppl 9 (2003): 7-10.

[5] Silva M, Loures C, Alves L, de Souza L, Borges K, Carvalho M. Alzheimer's disease: risk factors and potentially protective measures. Journal of Biomedical Science. 2019;26(1).

[6] Smith D, Cappai R, Barnham K. The redox chemistry of the Alzheimer's disease amyloid β peptide. Biochimica et Biophysica Acta (BBA) - Biomembranes. 2007;1768(8):1976-1990.

[7] Chen G, Xu T, Yan Y, Zhou Y, Jiang Y, Melcher K et al. Amyloid beta: structure, biology and structure-based therapeutic development. Acta Pharmacologica Sinica. 2017;38(9): 1205-1235.

[8] DaRocha-Souto B, Scotton T, Coma M, Serrano-Pozo A, Hashimoto T, Serenó L et al. Brain Oligomeric β-Amyloid but Not Total Amyloid Plaque Burden Correlates With Neuronal Loss and Astrocyte Inflammatory Response in Amyloid Precursor Protein/Tau Transgenic Mice. Journal of Neuropathology & Experimental Neurology. 2011;70(5):360-376.

[9] Baas P, Rao A, Matamoros A, Leo L. Stability properties of neuronal microtubules. Cytoskeleton. 2016;73(9): 442-460.

[10] Barbier P, Zejneli O, Martinho M, Lasorsa A, Belle V, Smet-Nocca C et al. Role of Tau as a Microtubule-Associated Protein: Structural and Functional Aspects. Frontiers in Aging Neuroscience. 2019;11.

[11] Lee H, Perry G, Moreira P, Garrett M, Liu Q, Zhu X et al. Tau phosphorylation in Alzheimer's disease: pathogen or protector?. Trends in Molecular Medicine. 2005;11(4): 164-169.

[12] An Introduction to Reactive Oxygen Species - Measurement of ROS in Cells | April 7, 2021 [Internet]. Biotek.com. 2021 [cited 12 April 2021]. Available from: https://www.biotek.com/resources/white-papers/an-introduction-to-reactive-oxygen-species-measurement-of-ros-in-cells/

[13] Hayyan M, Hashim M, AlNashef I. Superoxide Ion: Generation and Chemical Implications. Chemical Reviews. 2016;116(5):3029-3085.

[14] Turrens J. Mitochondrial formation of reactive oxygen species. The Journal of Physiology. 2003;552(2):335-344.

[15] Brieger K, Schiavone S, Miller J, Krause K. Reactive oxygen species: from

health to disease. Swiss Medical Weekly. 2012;142:w13659.

[16] Bhattacharyya A, Chattopadhyay R, Mitra S, Crowe S. Oxidative Stress: An Essential Factor in the Pathogenesis of Gastrointestinal Mucosal Diseases. Physiological Reviews. 2014;94(2): 329-354.

[17] Naidoo K, Birch-Machin M. Oxidative Stress and Ageing: The Influence of Environmental Pollution, Sunlight and Diet on Skin. Cosmetics. 2017;4(1):4.

[18] Alfadda A, Sallam R. Reactive Oxygen Species in Health and Disease. Journal of Biomedicine and Biotechnology. 2012;2012:1-14.

[19] Poljsak B, Šuput D, Milisav I. Achieving the Balance between ROS and Antioxidants: When to Use the Synthetic Antioxidants. Oxidative Medicine and Cellular Longevity. 2013;2013:1-11.

[20] Poljšak B, Fink R. The Protective Role of Antioxidants in the Defence against ROS/RNS-Mediated Environmental Pollution. Oxidative Medicine and Cellular Longevity. 2014; 2014:1-22.

[21] Gella A, Durany N. Oxidative stress in Alzheimer disease. Cell Adhesion & Migration. 2009;3(1):88-93.

[22] Cheignon C, Tomas M, Bonnefont-Rousselot D, Faller P, Hureau C, Collin F. Oxidative stress and the amyloid beta peptide in Alzheimer's disease. Redox Biology. 2018;14: 450-464.

[23] Wang L, Yin Y, Liu X, Shen P, Zheng Y, Lan X et al. Current understanding of metal ions in the pathogenesis of Alzheimer's disease. Translational Neurodegeneration. 2020; 9(1).

[24] Jomova K, Valko M. Advances in metal-induced oxidative stress and human disease. Toxicology. 2011;283 (2-3):65-87.

[25] Arrigoni F, Prosdocimi T, Mollica L, De Gioia L, Zampella G, Bertini L. Copper reduction and dioxygen activation in Cu–amyloid beta peptide complexes: insight from molecular modelling. Metallomics. 2018;10(11): 1618-1630.

[26] Tõugu V, Tiiman A, Palumaa P. Interactions of Zn(ii) and Cu(ii) ions with Alzheimer's amyloid-beta peptide. Metal ion binding, contribution to fibrillization and toxicity. Metallomics. 2011;3(3):250.

[27] Budimir A. Metal ions, Alzheimer's disease and chelation therapy. Acta Pharmaceutica. 2011;61(1):1-14.

[28] Telling N, Everett J, Collingwood J, Dobson J, van der Laan G, Gallagher J et al. Iron Biochemistry is Correlated with Amyloid Plaque Morphology in an Established Mouse Model of Alzheimer's Disease. Cell Chemical Biology. 2017;24 (10):1205-1215.e3.

[29] Everett J, Brooks J, Lermyte F, O'Connor P, Sadler P, Dobson J et al. Iron stored in ferritin is chemically reduced in the presence of aggregating Aβ(1-42). Scientific Reports. 2020;10(1).

[30] Cheignon C, Tomas M, Bonnefont-Rousselot D, Faller P, Hureau C, Collin F. Oxidative stress and the amyloid beta peptide in Alzheimer's disease. Redox Biology. 2018;14: 450-464.

[31] Miller Y, Ma B, Nussinov R. Zinc ions promote Alzheimer A aggregation

via population shift of polymorphic states. Proceedings of the National Academy of Sciences. 2010;107(21): 9490–5.

[32] Lee M-C, Yu W-C, Shih Y-H, Chen C-Y, Guo Z-H, Huang S-J, et al. Zinc ion rapidly induces toxic, off-pathway amyloid-β oligomers distinct from amyloid-β derived diffusible ligands in Alzheimer's disease. Scientific Reports. 2018;8(1).

[33] Ventriglia M, Brewer GJ, Simonelli I, Mariani S, Siotto M, Bucossi S, et al. Zinc in Alzheimer's Disease: A Meta-Analysis of Serum, Plasma, and Cerebrospinal Fluid Studies. Journal of Alzheimer's Disease. 2015;46(1):75–87.

[34] Flora S, Pachauri V. Chelation in Metal Intoxication. International Journal of Environmental Research and Public Health. 2010;7(7):2745-2788.

[35] Squitti R, Zito G. Anti-Copper Therapies in Alzheimers Disease: New Concepts. Recent Patents on CNS Drug Discovery. 2009;4(3):209-219.

[36] Huang W, Wei W, Shen Z. Drug-like chelating agents: a potential lead for Alzheimer's disease. RSC Adv. 2014;4 (94):52088-52099.

[37] Cherny R, Barnham K, Lynch T, Volitakis I, Li Q, McLean C et al. Chelation and Intercalation: Complementary Properties in a Compound for the Treatment of Alzheimer's Disease. Journal of Structural Biology. 2000;130(2-3):209-216.

[38] Crapper McLachlan D, Smith W, Kruck T. Desferrioxamine and Alzheimer's Disease. Therapeutic Drug Monitoring. 1993;15(6):602-607.

[39] Liu G, Garrett M, Men P, Zhu X, Perry G, Smith M. Nanoparticle and other metal chelation therapeutics in Alzheimer disease. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease. 2005;1741(3):246-252.

[40] Sultan, Sadia et al. "Effect of iron chelator desferrioxamine on serum zinc levels in patients with beta thalassemia major." *The Malaysian journal of pathology* vol. 37,1 (2015): 35-8.

[41] Lambert, Catherine et al. "Bis (thiosemicarbazone) copper complexes: mechanism of intracellular accumulation." *Journal of biological inorganic chemistry : JBIC : a publication of the Society of Biological Inorganic Chemistry* vol. 18,1 (2013): 59-69. doi: 10.1007/s00775-012-0949-1

[42] Mckenzie-Nickson S, I. Bush A, J. Barnham K. Bis(thiosemicarbazone) Metal Complexes as Therapeutics for Neurodegenerative Diseases. Current Topics in Medicinal Chemistry. 2016;16 (27):3058-3068.

[43] Esmieu C, Guettas D, Conte-Daban A, Sabater L, Faller P, Hureau C. Copper-Targeting Approaches in Alzheimer's Disease: How To Improve the Fallouts Obtained from in Vitro Studies. Inorganic Chemistry. 2019;58 (20):13509-13527.

[44] Zhu X, Victor T, Ambi A, Sullivan J, Hatfield J, Xu F et al. Copper accumulation and the effect of chelation treatment on cerebral amyloid angiopathy compared to parenchymal amyloid plaques. Metallomics. 2020;12 (4):539-546.

[45] Hegde, Muralidhar L., P. Bharathi, Anitha Suram, Chitra Venugopal, Ramya Jagannathan, Pankaj Poddar, Pullabhatla Srinivas, et al. "Challenges Associated with Metal Chelation Therapy in Alzheimer's Disease." *Journal of*

Alzheimer's Disease 17, no. 3 (2009): 457–68.

[46] Kavirajan, Harish, and Lon S Schneider. "Efficacy and Adverse Effects of Cholinesterase Inhibitors and Memantine in Vascular Dementia: a Meta-Analysis of Randomised Controlled Trials." *The Lancet Neurology* 6, no. 9 (2007): 782–92.

[47] Zatta, P., M. Raso, P. Zambenedetti, W. Wittkowski, L. Messori, F. Piccioli, P. L. Mauri, and M. Beltramini. "Copper and Zinc Dismetabolism in the Mouse Brain upon Chronic Cuprizone Treatment." *Cellular and Molecular Life Sciences* 62, no. 13 (2005): 1502–13.

Chapter 11

Perturbation of Cellular Redox Status: Role of Nrf2, a Master Regulator of Cellular Redox

Lokesh Gambhir, Garima Tyagi, Richa Bhardwaj, Neha Kapoor and Gaurav Sharma

Abstract

Regulation of cellular redox homeostasis determines the fate of the cell. Perturbation in redox status is known to elicit multiple cellular pathways. Role of oxidative stress modulation in channelizing the cell towards apoptosis or rescuing the cell by activating pro-survival pathways, depends on the levels of generated oxidative stress. High levels of generated oxidative stress induce cell death pathways whereas mild and low levels are known to elicit the cell survival pathways. Generation of ROS for a short duration of time inducing Redox ticking also triggers the pro-survival pathways inside the cell. Nrf2 is the redox sensitive prosurvival transcription factor which acts as master regulator of redox equilibrium. Nrf2 and its dependent genes including HO-1, GCLC, NQO1 etc. are involved in maintaining the cellular redox homeostasis. Role of Nrf2 as dual edges sword has been highlighted in past decade. The cross talk between the Nrf2 and NF-κB is at the focal point of building the redox response network. The present chapter is aimed at providing the insight on the role of Nrf2 and NF-κB as redox sensitive transcription factors in regulating cellular redox status. Further, the chapter brings in light the therapeutic potential of targeting Nrf2 under multiple clinical settings.

Keywords: redox homeostasis, Nrf2, NF-κB, therapeutic potential, cancer, neurodegeneration

1. Introduction

An equilibrium between the oxidants, reactive oxygen species and antioxidants attained by cell is defined as redox status of the cell, In case of any kinds of diseases or pathological conditions which disrupt this equilibrium thus creating an oxidized state, termed as oxidative stress [1]. This redox system essentially works in maintaining cellular homeostasis and cell survival. Reactive oxygen species (ROS) consist of reactive species like superoxide (O_2−) and hydroxyl radical (HO), along with nonradical species such as hydrogen peroxide (H_2O_2). Reactive nitrogen species (RNS) contains nitrogen containing reactive species including nitric oxide (NO) and peroxynitrite ($ONOO^-$). Agents that contribute to formation of these ROS/RNS may be exogenous sources like Chemicals

(e.g., PCB), irradiation (i.e., UV irradiation, x-ray, gamma-ray) or atmospheric pollutants or they may be endogenous the mitochondria, where O_2^- is generated by electron leakage from complex I and III of the electron-transport chain, membrane-associated NAD(P)H oxidase, cytochrome c oxidase, and xanthine oxidase. In case of any oxidative stress experienced by the cell various enzymatic and non- enzymatic antioxidant systems present in the cell are ready to combat. A major class of enzymatic antioxidant systems include multiple isoforms of SOD, (SOD1, SOD2, SOD3) found in the extracellular matrix, cytoplasm, mitochondrial intermembrane space, nucleus, and lysosomes. Another enzymatic system which is responsible for conversion of reduced glutathione (GSH) to oxidized glutathione (GSSG), catalyzed by glutathione peroxidase (GPX) [2]. Non enzymatic systems include chemical antioxidants like glutathione (GSH), α-tocopherol (vitamin E), and ascorbic acid (vitamin C). GSH act as a cosubstrate in the reduction of H_2O_2 by GPx. It might also react with oxygen-free radical directly, similarly, vitamins E and C also reduce oxygen-free radicals. They act by trapping hydroxyl radicals and other reactive radicals and thus break radical chain reactions and form new less reactive radicals [3]. Reactive oxygen species (ROS) are reported to be involved in different cellular processes ranging from apoptosis and necrosis to cell proliferation and carcinogenesis. Reports confirm the ECS (endocannabinoid system), may play an important role in the regulation of cellular redox homeostasis [4]. Endocannabinoids such as AEA are also known to mediate some of their cellular responses by targeting the non-selective cation channel TRPV1, whose activation has been linked to increased ROS production, AEA has also been reported to target the PPAR family of nuclear receptors, whose activation is known to induce the expression of antioxidant enzymes, including catalase and glutathione peroxidase 3 [5]. Thioredoxins (Trx), function as hydrogen donors to thioredoxin-dependent peroxide reductases. These have a Cys-Gly-Pro-Cys active site, which is indispensable for redox regulatory functions of thioredoxins. Two isoforms of Trx have been observed, these are Trx1 (expressed in the cytoplasm and the nucleus) and Trx2 (expressed in the mitochondria), which are very crucial for cell survival. This implicates its protective role against reactive oxygen species [6].

Cellular Redox Homeostasis is determined by the ability of a cell to maintain the balance between the magnitude of generated oxidative stress and the rate of its detoxification [7] Maintaining the redox balance is important for proper function and responses of cells. Any disturbance in the redox homeostasis induces oxidative stress mediated signaling cascade that could lead to cell death or induce adaptive survival responses. Outcome of perturbation in the redox balance depends on the magnitude of oxidative stress induced inside the cell [8]. The intracellular "redox homeostasis" or "redox buffering" capacity is maintained primarily by glutathione (GSH oxidized / reduced) and thioredoxin (TRX oxidized/reduced) redox couples. GSH/GSSG ratio represents the major cellular redox buffer and it is therefore used as an indicator of the redox environment of the cell. GSH, Trx and glutaredoxin rectifies the thiol modifications due to oxidative stress. GSH reductase and Trx reductase reduces the GSSG and oxidized Trx at the expense of NADPH. The reducing nature of GSH and Trx is pivotal for the clearance of peroxides by peroxidase and peroxiredoxin [9]. Basal levels of reactive oxygen species (ROS) are endogenously produced in the mitochondria due to partial reduction of oxygen, inflammatory reactions and enzyme linked reactions viz. NAPH oxidase, Xanthine oxidase, cytochrome c oxidase [10]. Induction of mild oxidative stress activates redox sensitive pro-survival pathways like Nrf2 that protects against the oxidative damage. High oxidative stress leads to induction of apoptosis. Owing to their high reactivity, high levels of generated ROS react with the molecule including proteins, carbohydrates, lipids and DNA in the vicinity non-specifically (**Figure 1**).

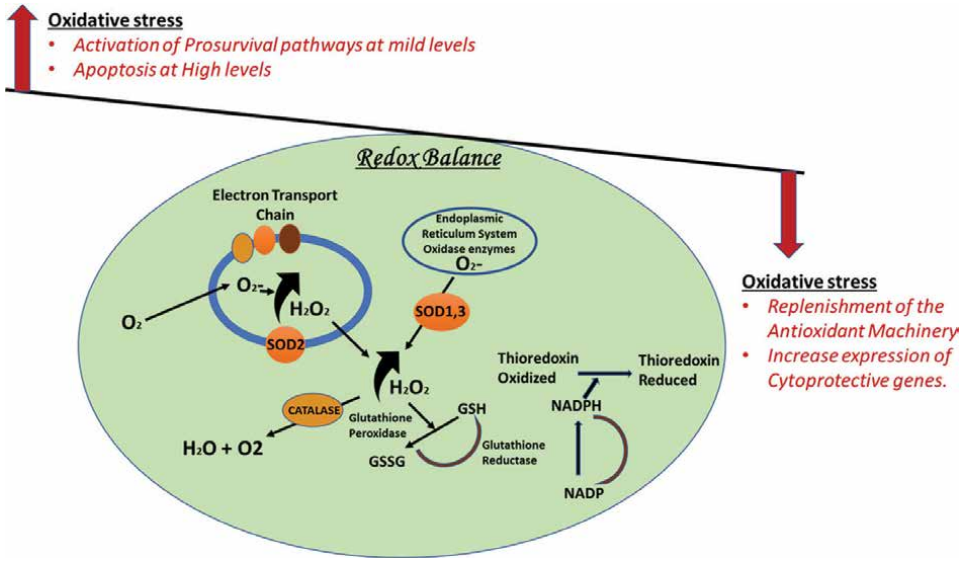


Figure 1.
Cellular redox homeostasis: Electron transport chain in mitochondria, endoplasmic reticulum and oxidases enzymes like NADPH oxidases and xanthine oxidase induces the generation of superoxide and peroxide radicals. The increase in hydrogen peroxide elevates the redox balance towards oxidative stress. Spatial-temporal regulation of the generated oxidative stress determines the cellular fate in term of apoptosis or cytoprotection. Activation & expression of transcription factors leading to the replenishment of antioxidant and cytoprotective machinery of the cell leads to restoration of redox balance.

This further results in impaired functioning of key cellular components directing the cell towards apoptosis [11]. However, generation of low levels of ROS acts as secondary messenger and activates numerous redox-sensitive pro-survival signaling pathways [12]. Spatio-temporal generation and regulation of ROS underlines their potential to contribute as secondary messenger from cell surface to the nucleus [13]. Scavenging of endogenous ROS can impair normal cellular response like production of cytokines and growth factors by T cells. Redox state of many proteins plays an important role during immune responses [14]. Critical cysteine residues present on proteins act as redox sensors and are prone to oxidation into sulfenic acids or disulphide formation or glutathionylation resulting in the modulation host immune responses [15]. The effect of oxidative stress on functions of these proteins depends on the concentration, duration and location of ROS generated inside the cell. A number of transcription factors and families have been identified which work in events of redox homeostasis or signaling, namely AhR, AP1, β-catenin, Egr-1, FOXO, HIF-1α, p53, NF-κB, Nrf2, Sp1, TTF. The present chapter is aimed at narrating the role of Nrf2 and NF-κB in redox environment and their redox regulation. Nrf2 being the master regulator is further detailed as putative target with therapeutic potential under multiple clinical settings.

2. NF-κB: redox sensitive transcription factor

NF-κB plays an important role in regulating the immune and inflammatory response. NF-κB is a ubiquitous transcription factor [16]. p52/p100, NF-κB p50/p105, C-REL, RELA/p65 and RelB constitute the Nuclear factor-κB (NF-κB) family

of transcription factors. These factors mediate the transcription of target genes by binding to a specific DNA element, κB enhancer, as various hetero- or homo-dimers [17]. They also regulate the various biological responses such as immune responses, cell differentiation, cell proliferation, survival, stress response and inflammation. But the most studied and well-known function of NF-κB is in the inflammation, regulating the pro inflammatory cytokines, activation, differentiation and effector functions of T cells [18]. NF-κB has the ability to detect the stimuli such as infectious agents, UV radiation, ROS, Tissue injury, lipopolysaccharide (LPS), and free radicals which activate NF-κB [19]. The basic mechanism involves the tissue injury which activates NF-κB, dissociates IκB as a result of which the NF-κB enters the cell nucleus and activate the DNA to enhance the inflammatory cytokines [20]. Regulation of NF-κB activity is achieved through various post-translational modifications of the core components of the NF-κB signaling pathways. There are two pathways by which NF-κB is regulated; the canonical and the alternative pathway [21]. The canonical pathway is responsible for the installation of pro inflammatory cytokines, chemokines and other inflammatory mediators which directly engage into inflammation and act indirectly. Activation of the non-canonical NF-κB pathway involves different signaling molecules and leads to the predominant activation of the p52/RelB dimer. An "alternative" NF-κB pathway is activated by TNF-family cytokines—lymphotoxin b (TNFSF3) CD40 ligand (CD40L and TNFSF5), B cell activating factor (BAFF and TNFSF13B), and receptor activator of NF-κB ligand (RANKL and TNFSF11).

2.1 The canonical pathway

The canonical pathway is provoked by the pro-inflammatory cytokines, ligands of varied immune receptors and involves the rapid and transient activation of IκB kinase [22]. NF-κB activity at sites of inflammation is associated with activation of the canonical pathway and RelA- or cRel-containing complexes [23]. In the pathway, NF-κB/Rel proteins are tethered which are inhibited by IκB proteins. Pro inflammatory cytokines, lipopolysaccrahaide, growth factors and antigen receptors activate the IKK complex (IKKβ, IKKα and NEMO) [24]. The complex then phosphorylates IκB proteins which lead to ubiquitination and proteasomal degradation, freeing NF-κB/Rel complex. Active NF-κB/Rel complex is further activated by post transcriptional modifications and translocate to the nucleus, where either alone or in combination with other transcription factors including AP-1, Ets and STAT and induce target gene expression [25].

2.2 The alternative pathway

NF-κB2 p100/RelB complexes are inactive in cytoplasm. Signaling in LTβR, CD40, BR3 activate kinase NIK [26] which in turn activate IKKα complex that phosphorylate C terminal residue in NF-κB2 p100 which leads to ubiquitination and proteasomal processing to NF-κB2 p52 and translocate to nucleus to target gene expression [27]. The pathway regulates important aspects of immune functions, including lymphoid organ development, the cross-priming function of dendritic cells, B cell survival and germination center reactions, generation and maintenance of effector and memory t cells, antiviral innate immunity [28]. The pathway is responsible for inflammatory disease, kidney inflammation, metabolic inflammation and central nervous system inflammation [29]. Recent evidence suggests that NF-κB also has a role in regulating the activation of inflammasomes. Dysregulated NF-κB activation is a hallmark of chronic inflammatory diseases. Therefore, a better understanding of the mechanism that

underlies NF-κB activation and pro-inflammatory function is of great significance for therapeutic strategies in the treatment of inflammatory diseases.

3. Redox modulation of NF-κB

Imbalance in redox state is redox modulation. The redox state of cells controls the activation and inhibition of NF-κB, as in the state of oxidative stress that can both activate and inhibit NF-κB by targeting the upstream kinases [30]. Activation of NF-κB by regular signaling is well known, however NF-κB activation also depends on redox state of cells in three possible ways: (i) many NF-κB -activating substances cause the production of reactive oxygen species (ROS) superoxide, H_2O_2, lipoxygenase products or act as oxidants on their own, (ii) NF-κB activation can be caused by superoxide H_2O_2 or organic hydroperoxide in some cell lines when no physiological stimulation is present, and (iii) A wide range of NF-κB inhibitors inhibit NF-κB – activation and antioxidants that are chemically unrelated. These observations have led to a consensus that NF-κB activation is related to some oxidative reaction. Molecules like thioredoxin, escalates the activity of NF-κB to bind DNA under oxidative stress [31]. A component of dynein motor complex LC-8 also participates in redox regulation of NF-κB. It activates NF-κB on exposure of TNFα and results in ROS production which oxidizes LC-8 and its dissociation from IκBα thus leading to NF-κB activation. Reportedly, NF-κB activation has anti- oxidant and pro-oxidant roles, the former involves the suppression of ROS accumulation, autophagy promotion, Inhibition of JNK activation and increased anti-oxidant targets whereas the pro-oxidant role includes the induction of pro-oxidation genes. One of the most important molecules in regulating redox modulation is hydrogen peroxide, it has been a question of debate, if H_2O_2 is involved in redox activation of NF-κB. As indicated in literature, TNFα induced activation of NF-κB mediated by H_2O_2. TNFα is a strong activator of NF-κB, that induces superoxide formation in mitochondria. As in Wurzberg cells where H_2O_2 directly activates NF-κB. The findings were found to be inefficient when lymphoblastoid cell lines, Jurkat cells showed no results of NF-κB activation by H_2O_2 [31, 32]. Various exogenous and endogenous sources can enhance the redox reaction. Redox reactions play a huge role in inflammation specifically in lung inflammation where oxidative injury is most common due to its structure and function. ROS production is an immune response against inhaled pathogens and pollutants like cigarette smoke, automobile exhaust. Excess production of endogenous ROS leads to chronic inflammatory lung disease such as chronic obstructive pulmonary disease, asthama and pulmonary fibrosis. Oxidative stress produced by cigarette smoke activates NF-κB by activating IKK complex which interferes with the chromatin modifications that escalate the transcription of pro-inflammatory genes [33].

4. Nrf2: the master regulator

The nuclear factor erythroid 2 (NFE2)-related factor 2 (Nrf2) is a member of the cap 'n' collar (CNC) subfamily of basic region leucine zipper (bZip) transcription factors including nuclear factor erythroid-derived 2 (NFE2) and NRF1, NRF2, and NRF3. There are seven conserved NRF2-ECH homology (Neh) domains within NRF2 gene, with different functions to control NRF2 transcriptional activity. The bZip in the Neh1 domain acts to activate gene transcription by forming dimer with small

musculoaponeurotic fibrosarcoma proteins (sMAF). Neh2 domain mediates NRF2 ubiquitination and degradation as it contains ETGE and DLG motifs which act together with Kelch domain of Kelch-like-ECH-associated protein 1 (KEAP1) [34]. The Neh3-5 domains find their role as transcriptional activation domains, Neh6 domain works to mediates Nrf2 degradation in cells experiencing oxidative stress. Neh7 domain mediates interaction with retinoic X receptor alpha (RXRα), which represses Nrf2 activity. It is involved in the control of development of labial and mandibular segment of Drosophila by basic leucine zipper DNA binding domain (bZip) homeotic gene [35].

Removal of Nrf2 alters the defense machinery of the cell against oxidative stress. Knocking out Nrf2 has no effect on the mortality of the mice. Basal level expression of Nrf2 in the cytoplasm ensures the production of cytoprotective proteins to exert normal physiological redox homeostasis [36]. Modulation in redox status is known to activate prosurvival redox sensitive Nrf2 pathway. Under normal condition Nrf2 is sequestered in cytoplasm by the inhibitor KEAP-1. Abrogation of KEAP-1 binding leads to translocation of Nrf2 to the nucleus mediated by nuclear localization signal. In nucleus Nrf2 forms a heterodimer with the co-transcription factor MAF. The heterodimers bind to the corresponding antioxidant response element and induces the expression of downstream cytoprotective and antioxidant enzymes [37]. The Nrf2 system is considered to be a major cellular defense mechanism against cellular oxidative stress. Nrf2 plays an important role in cellular defense and in improving the removal of ROS by activating downstream genes that encode phase II detoxifying enzymes and antioxidant enzymes, such as GCLM, NQO1, HMOX1, GPX, and glutathione S-transferases (GST) [38]. Nrf2 controls the expression of key components of the glutathione (GSH) and thioredoxin (TXN) antioxidant system, as well as enzymes involved in NADPH regeneration, ROS and xenobiotic detoxification, heme metabolism, thus playing a fundamental role in maintaining the redox homeostasis of the cell. Excessive ROS production causes oxidative stress to increase mitochondrial DNA damage, further promotes the activation of oncogenes or the inactivation of anti-oncogenes, which facilitates its tumorigenic signaling pathways and tumor progression. Nrf2/ARE pathway protects cells against oxidative stress via regulating the expression of Sestrin 2 gene as evident by monitoring the expression of downstream antioxidants. Sestrin 2 has strong antioxidant capacity and can provide cell with cytoprotective against various harmful stimuli. Sestrin blocks mTOR expression and mitigates the accumulation of ROS [39].

5. Redox regulation of Nrf2

Redox regulation underlines the cellular homeostasis. Regulation of redox sensitive transcription factors play a pivotal role in determining the cellular fate. Nrf2 being a master regulator is at the focal point of maintaining and regulating the cellular redox equilibrium. Perturbation in redox equilibrium is known to modulate the Nrf2 activation and hence effect the cellular fate [19]. Spatio-temporal generation of oxidative stress determines the graded activation of redox sensitive mediators. Mild oxidative stress is known to activate Nrf2 pathway and increases the cytoprotective proteins. Redox based activation of Nrf2 is attributed to the presence of more than 20 critical cysteine residues present in the KEAP-1 protein (**Figure 2**) [40]. 273 and cys288 have been shown as critical for abrogating KEAP-1 mediated inhibition of Nrf2. Mutation in these residues render activation of Nrf2 by inhibiting the Cul3-E3-KEAP-1 mediated degradation of Nrf2. The mutation did not had any effect on the

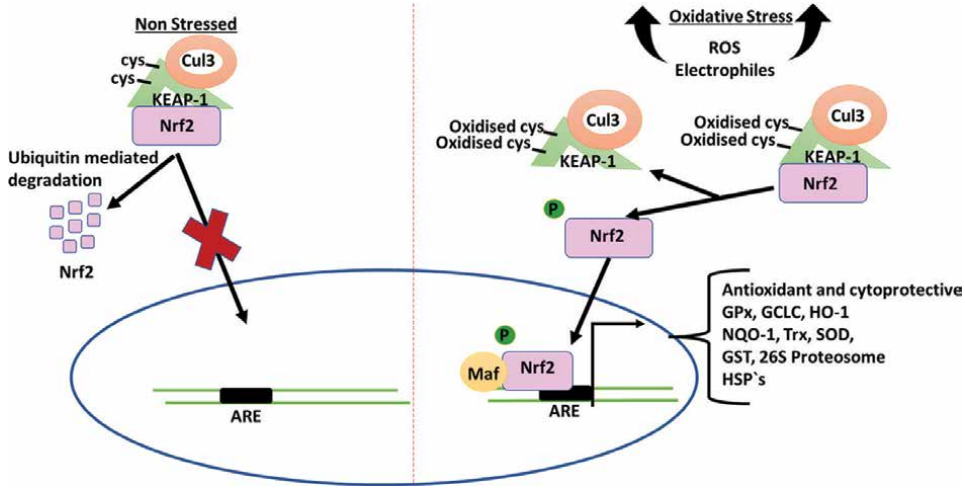


Figure 2.
Redox regulation of Nrf2 pathway. Under non-stressed condition, Nrf2 is sequestered by its inhibitor KEAP-1 in the cytoplasm by inducing ubiquitin mediated Nrf2 degradation. Increase in ROS and electrophiles induces oxidative stress which results in oxidation of critical cysteine residues in the redox sensitive transcription factors. Cysteine residues of KEAP-1 are oxidized to release Nrf2 which translocates to the nucleus to express the downstream genes of antioxidant and cytoprotective potential. Thus, redox perturbation and tickling induces the Nrf2 pathway that act as master regulator for maintaining cellular redox equilibrium in turn governing the cellular fate.

detachment of the KEAP-1:Nrf2 complex, thus allowing the nuclear translocation of free Nrf2. Further, cys151 was also implicated in redox modulator-based activation of Nrf2 pathway. Perturbation in cellular redox status by prooxidants have been shown to induce cellular oxidative stress and increase the glutathionylation of proteins. 1,4 Naphthoquinone treatment induced glutathionylation of KEAP-1 for inducing Nrf2 pathway owing to its prooxidant nature. Apart from the cytoprotective nature of Nrf2, its role as a redox sensitive anti-inflammatory transcription factor has been well documented. Multiple reports have highlighted redox modulation based modulating role of Nrf2 in ameliorating immune-pathologies [41]. Nrf2 knockout mice exhibited increased bronchial inflammation, prolonged inflammation, high susceptibility for autoimmune syndrome, elevated lymphocyte proliferation and impaired redox homeostasis. Nrf2 dependent proteins including HO-1 inhibits the cytokine secretion, leukocyte migration, adhesion and suppressed LPS induced production of tumor necrosis factor-a (TNF-a) and nitric oxide (NO) in murine macrophages. Ablation in HO-1 protein increases the susceptibility towards autoimmune diseases [42].

6. Therapeutic potential of Nrf2

Excessive reactive oxygen species are threat to cells redox homeostasis which are major cause of oxidative stress leading to maladies like cellular dysfunction in aging, cardiovascular disease, renal dysfunction, diabetes, cancer is some to name a few. Antioxidant therapies play an important role in combating the progress of these diseases but the results are not satisfactory therefore there is an urgent need for a solution which activates endogenous antioxidant defenses. The redox-sensitive transcription factor NF-E2 related factor 2 (Nrf2) plays a significant role in synchronizing cellular antioxidant defenses and maintaining redox homeostasis [43]. Nrf2 is the

"master regulator" of the antioxidant response, it modulates the expression of many genes, that include antioxidant enzymes, immune and inflammatory responses, tissue remodeling and fibrosis, carcinogenesis and metastasis. NRF2 activation by Keap1 binding is one of the major pathways that senses the oxidative stress, particularly there are Four reactive cysteine residues identified in Keap1 are most likely nominees for being the direct sensors of oxidative stress, various alternative mechanisms for Nrf2 activation were discovered, which are dependent upon kinase pathways, these include mitogen-activated protein kinases (MAPK), phosphatidylinositol-3 kinase and atypical protein kinase(s) C. Cell culture experiments report many compounds which show the ability to activate Nrf2. Large number of Nrf2 activators are principally naturally-occurring and plant-derived such as sulforaphane and curcumin and found in foods, but synthetic compounds have also shown to act as activators for instance bardoxolone methyl [44].

The Therapeutic potential of Nrf2 and its activators have been studied in various diseases. Some of them are as follows:

6.1 Diabetic nephropathy

It is the leading cause of chronic kidney disease, some mechanisms contribute to the onset and pathogenesis of diabetic nephropathy, including genetic and hemodynamic factors, oxidative stress, and cytokine signaling. Diabetes triggers oxidative stress through different ways, such as advanced glycation end-product accumulation and activation of polyol pathway, protein kinase C pathway, and renin angiotensin-aldosterone system. Loss or decrease in expression of SOD or glutathione in renal diseases are overcome by restoring and check the progression of disease. Nrf2 regulates expression of genes through ARE (antioxidant response elements) in their promoters to neutralize free radicals and accelerate removal of environmental toxins. Protective role of Nrf2 against renal damage has been demonstrated on streptozotocin induced diabetic rats, wherein it was shown that it slows the progression of diabetic nephropathy, Nrf2-mediated protection works through the negative regulation of TGF-b1 and p21/WAF1Cip1 (p21) [45]. Reports suggest the Nrf2-dependent anti-oxidative and anti-inflammatory effects of digitoflavone in streptozotocin-induced diabetic nephropathy, *in vitro* using SV40-transformed mouse mesangial cells (SV40-Mes13), results showed that Digitoflavone activated Nrf2, reduced oxidative damage, inflammation, TGF-β1 expression, extracellular matrix protein expression and mesangial cell hyperplasia [46]. Enhancement of NRF2 activity in the renal tubules considerably improves damage related to kidney injury and prevents its progression to chronic kidney disease (CKD) by reducing oxidative stress. KEAP1-NRF2 system along with Nrf2 activators have also been proposed to be a target for renal defense, wherein KEAP 1 inhibitors like CDDO-methyl ester, a synthetic triterpenoid are the main targets for the study as they work by inhibiting KEAP1-Nrf2 bonding which lets stabilized and free Nrf2 to translocate to nucleus and activate downstream genes [47]. Another strategy employed is suppressing the degradation of Nrf2 via inhibiting proteasome activity by inhibitors like minocycline, Increasing of Nrf2 concentrations within the cells have been found to be effective against the renal damage, reports suggest that Zinc helps elevating the Nrf2 protein level within the nucleus and upregulated the expression of Nrf2 downstream enzymes by encouraging inhibition of Nrf2 nuclear promoter Fyn mediated by Akt/GSK-3β, Resveratrol and its analogue (polydatin) have reported to activated Nrf2/ARE pathway through upregulating Sirt1 (NAD-dependent histone deacetylase in the nucleus) in glomerular mesangial cell [48].

6.2 Cancer

Systematic studies of carcinogenesis specify an important role of endogenous oxidative damage to DNA, and an imbalance of cellular redox homeostasis that is balanced by elaborate defense and repair processes [49]. Pancreatic cancer is the most fatal diseases, it has very high rate of metastasis, Keap1-Nrf2 pathway is an emerging target for PC prevention and therapy. Certain modulators like UHRF1 (ubiquitin-like containing PHD and RING finger domains 1) is overexpressed in pancreatic cancer and are correlated to tumor growth. UHRF1 suppresses Keap1 expression by promoter methylation, this leads to Nrf2 activation. MBD 1 and p62 have been reported to inhibit ROS and promote tumor growth and drug resistance by inducing Nrf2 accumulation, nuclear translocation and activation. Nrf2 activation by a has also been found to inhibit PC cell growth and induce apoptosis by upregulating HO-1 [50]. The compound D3T (3*H*-1,2-dithiole-3-thione) has been shown to increase the nuclear accumulation of Nrf2, Honaucin A, natural marine-based compound, obtained from cyanobacteria, forms a covalent bond with the sulfhydryl groups on KEAP1, resulting in the activation of Nrf2, phenol, polyphenol, or triterpenoid majorly form class of compounds that activate Nrf2. Sulforaphane is highly electrophilic molecule, it non-covalently binds to sulfhydryl groups of KEAP1 resulting in Nrf2 activation. Sulforaphane can also activate antioxidant response elements (AREs) associated with Nrf2. Micro RNA's (miRNAs) miR-141, miR-432-3p, miR-200a, have also shown to modulate the activities of KEAP1 and NRF2 in ovarian carcinoma cell lines, breast cancer, esophageal squamous cell carcinoma (ESCC), endometrial cancer tissues, miR-7, directly targeted KEAP1 mRNA in neuroblastoma SH-SY5Y cells, where it activated NRF2-dependent transcription of the antioxidant genes HMOX1 and GCLM [49]. Aberrant Nrf2 activation with in cancer cells may be due to somatic mutations within the Nrf2, KEAP1, or CUL3 genes, or the increase of KEAP1 interacting proteins, such as p62/Sqstm1 and p21, or it may be due to cysteine modification by oncometabolites such as fumarate all of the above activities may confer resistance to cancer cells and hence form the major targets for the cancer therapies. Nrf2 inducers are reported to hasten the detoxification of carcinogens (often electrophiles) from the cell and hence protect from chemical carcinogenesis Nrf2 inhibitors like brusatol work as protein synthesis inhibitors, ML385, a thiazole-indoline compound binds to Neh1. Therefore, Nrf2 inducers and Nrf2 inhibitors may function as anticancer drugs, with different effects on different targets, Nrf2 inducers work in order to protect normal cells from carcinogens, whereas Nrf2 inhibitors suppress the proliferation of cancer cells that have acquired aberrant Nrf2 activation or Nrf2 addiction [51]. Keap1-deficient mice showed upregulation of detoxifying enzymes, including GST and NQO1, and higher Nrf2 activation [52]. Various epigenetic regulations like hypermethylation, histone modifications control the expression of Nrf2 and hence may form targets to different therapeutic strategies [53].

6.3 Pulmonary fibrosis or lung injury and inflammation

Pulmonary fibrosis is a progressive and irreversible disease; it is characterized by an increase in differentiation and of fibroblasts to myofibroblasts and excessive accumulation of extracellular matrix in lung tissue. A study reports antifibrotic function of sulforaphane (SFN), an NRF2 activator, was largely dependent on a long noncoding RNA [54]. Therapeutic potential of thymoquinone (TQ) in bleomycin-induced lung fibrosis (BMILF) were also investigated and it was seen that it decreases

expressions of Nrf2, Ho-1 and TGF-β. Nrf2/Ho-1 signaling pathway is a principal target for TQ protective effect against BMILF in rats [55]. The protective role of Nrf2 is mediated by PPARγ in hypoxia-induced Acute Lung Injury (ALI). Reports reveal that overexpression of Brg1 increases Nrf2 activity and reduces ROS and inflammatory factors in lung tissues. In lipopolysaccharide (LPS)-induced lung inflammation the defensive role of the PI3K/Akt-dependent activation of the Nrf2-HO-1 pathway was revealed in mice treated with desoxyrhapontigenin. Nrf2 knockout resulted in a worsening of asthma symptoms. Protective role of Nrf2 in emphysema induced mice can be correlated by its activation in alveolar macrophages. The role of Nrf2 dysfunction in COPD may be the result of loss of DJ-1. DJ-1 overexpression activates Nrf2 and inhibits apoptosis of alveolar type II cells that are undergoing Cigarette smoking-induced oxidative stress and inflammatory response. DJ-1 induces the activation of Nrf2 and increases the expression of downstream antioxidant machinery to reduce the oxidative stress. The underlines anti-inflammatory effects are attributed to the expression of HO-1. These findings highlight the role of DJ-1 as putative target for cigarette smoking induced lung diseases [56].

6.4 Neurodegeneration

Neurodegenerative conditions may be results of various primary causes which include including expression of certain gene alleles, toxicant administration, aging, protein aggregation, proteasomal or autophagic dysfunction, inflammation, neuronal apoptosis, oxidative stress, mitochondrial dysfunction, and interactions between neurons and glia. The earliest degenerative condition to be associated with oxidative stress was aging. Some diseases characterized as neurodegenerative are Parkinson's disease, amyotrophic lateral sclerosis (ALS), and Alzheimer's disease, Huntington's disease, Friedrich's ataxia, multiple sclerosis, and stroke. Nrf2 activation provides neuroprotection against oxidative stressors and mitochondrial toxins, including hydrogen peroxide, tert-butyl hydroperoxide, 6-hydroxydopamine, 3-nitropropionic acid (3-NP), 1-methyl-4-phenylpyridinium (MPP), and rotenone [57]. In various studies conducted water derivative of artemisinin namely artesunate and lipid soluble derivative artemisinin, artemether both show to enhance the activation of Nrf2 via increasing its nuclear translocation and binding to downstream antioxidant response elements, as well as through suppressing ROS-dependent p38 MARK and NF-κB pathways [58]. Report suggests that SFN, an isothiocyanate compound that occurs naturally and can be derived from cruciferous vegetables such as broccoli is capable of activating Nrf2, Results show that SFN is able to cross the blood brain barrier, activate Nrf2-dependent gene expression in the basal ganglia, eventually protecting nigral dopaminergic neurons from cell death induced by MPTP. Wide variety of bioactive compounds like resveratrol, curcumin, naphthazarin, genistein, and carnosic acid and berberin have been reported as Nrf2 activators that show positive effects in neurodegenerative disorders by protecting dorsal root ganglion (DRG) neurons from glucose-induced injury also by antioxidant activity in primary spinal cord astrocytes exposed to H_2O_2 [59].

7. Conclusion

Cellular redox equilibrium is pivotal for normal cellular functioning and responses. Impairment in regulation and maintenance of redox homeostasis underlines the pathogenesis of multiple associated diseases. Thus, identifying the key players in redox

regulation is of prime interest. Nrf2 and NF-KB are the two most pivotal and embroiled redox sensitive transcription factors that underlines the maintenance of redox balance. Thus, the two pathways are at the epicenter of investigation with clinical significance. The Nrf2 is the master regulator of cellular redox status. The Nrf2 and its dependent genes are responsible for cytoprotection, immunoregulation, maintaining cellular antioxidant levels, reducing drug toxicities etc. Presence of multiple critical cysteine residues in KEAP-1, inhibitor of Nrf2, renders redox sensitivity in activation of Nrf2 pathway. Aberrant expression and regulation of Nrf2 pathway has been implicated in various pathologies including cancer, diabetes, neurodegeneration etc. Multiple researchers have demonstrated the targeting of Nrf2 as key strategy to curb inflammation and associated disorders. Apart from Nrf2, another redox sensitive transcription factor is NF-κB. Aberrant activation of NF-κB pathway has been implicated in inflammation, immunity, differentiation, cell growth, tumorigenesis and apoptosis. NF-κB also contains cysteine residues which act as sensors for redox modulation. Recent advances have highlighted the cross talk between Nrf2 and NF-κB as putative target for strategic drug development. Further, in depth clinically relevant exploration of the cross talk is warranted. The triangulate interplay of cellular redox, Nrf2 and NF-κB have immense potential to generate the therapeutic benefits via serving a putative target for discovering and developing novel drugs.

Conflict of interest

There is no actual or potential conflict of interest.

Author details

Lokesh Gambhir[1*], Garima Tyagi[2], Richa Bhardwaj[3], Neha Kapoor[1] and Gaurav Sharma[1]

1 School of Applied Sciences, Suresh Gyan Vihar University, Jaipur, Rajasthan, India

2 Department of Biotechnology, School of Basic and Applied Sciences, Shri Guru Ram Rai University, Dehradun, Uttarakhand, India

3 Department of Botany, IIS University, Jaipur, Rajasthan, India

*Address all correspondence to: gambhir.lokesh@gmail.com

References

[1] Tauler RP. Redox status. In: Mooren FC, editor. Encyclopedia of Exercise Medicine in Health and Disease. Berlin, Heidelberg: Springer; 2012. DOI: 10.1007/978-3-540-29807-6_167

[2] Trachootham D, Lu W, Ogasawara MA, Valle NR, Huang P. Redox regulation of cell survival. Antioxidants & Redox Signaling. 2008;**10**(8):1343-1374

[3] Xie ZZ, Liu Y, Bian JS. Hydrogen sulfide and cellular redox homeostasis. Oxidative Medicine and Cellular Longevity. 2016;**2016**:6043038

[4] Lipina C, Hundal HS. Modulation of cellular redox homeostasis by the endocannabinoid system. Open Biology. 2016;**6**(4):150276

[5] Mydin RB, Okekpa SI. Reactive oxygen species, cellular redox homeostasis and cancer. In: Homeostasis-An Integrated Vision. Rijeka: IntechOpen; 2018

[6] Panieri E, Santoro MM. ROS homeostasis and metabolism: A dangerous liason in cancer cells. Cell Death & Disease. 2016;**7**(6):e2253

[7] Kong H, Chandel NS. Regulation of redox balance in cancer and T cells. Journal of Biological Chemistry. 2018; **293**(20):7499-7507

[8] Meyer AJ. The integration of glutathione homeostasis and redox signaling. Journal of Plant Physiology. 2008;**165**(13):1390-1403

[9] Zorov DB, Juhaszova M, Sollott SJ. Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. Physiological Reviews. 2014;**94**(3):909-950

[10] Di Meo S, Reed TT, Venditti P, Victor VM. Role of ROS and RNS sources in physiological and pathological conditions. Oxidative Medicine and Cellular Longevity. 2016;**2016**:1245049

[11] Schieber M, Chandel NS. ROS function in redox signaling and oxidative stress. Current Biology. 2014;**24**(10): R453-R462

[12] Sauer H, Wartenberg M, Hescheler J. Reactive oxygen species as intracellular messengers during cell growth and differentiation. Cellular Physiology and Biochemistry. 2001;**11**(4):173-186

[13] Mittal M, Siddiqui MR, Tran K, Reddy SP, Malik AB. Reactive oxygen species in inflammation and tissue injury. Antioxidants & Redox Signaling. 2014;**20**(7):1126-1167

[14] Paulsen CE, Carroll KS. Cysteine-mediated redox signaling: Chemistry, biology, and tools for discovery. Chemical Reviews. 2013;**113**(7):4633-4679

[15] Ma Q. Role of Nrf2 in oxidative stress and toxicity. Annual Review of Pharmacology and Toxicology. 2013; **53**:401-426

[16] Liu T, Zhang L, Joo D, Sun SC. NF-κB signaling in inflammation. Signal Transduction and Targeted Therapy. 2017;**2**:17023. DOI: 10.1038/sigtrans.2017.23

[17] Oeckinghaus A, Ghosh S. The NF-kappaB family of transcription factors and its regulation. Cold Spring Harbor Perspectives in Biology. 2009;**1**(4):a000034. DOI: 10.1101/cshperspect.a000034

[18] Hoesel B, Schmid JA. The complexity of NF-κB signaling in inflammation and cancer. Molecular Cancer. 2013;**12**(1):86

[19] Bhattacharyya A, Chattopadhyay R, Mitra S, Crowe SE. Oxidative stress: An essential factor in the pathogenesis of gastrointestinal mucosal diseases. Physiological Reviews. 2014;**94**(2):329-354. DOI: 10.1152/physrev.00040.2012

[20] Tak PP, Firestein GS. NF-kappaB: A key role in inflammatory diseases. The Journal of Clinical Investigation. 2001;**107**(1):7-11. DOI: 10.1172/JCI11830

[21] Lawrence T. The nuclear factor NF-kappaB pathway in inflammation. Cold Spring Harbor Perspectives in Biology. 2009;**1**(6):a001651. DOI: 10.1101/cshperspect.a001651

[22] Shih VF, Tsui R, Caldwell A, Hoffmann A. A single NF-κB system for both canonical and non-canonical signaling. Cell Research. 2011;**21**(1):86-102. DOI: 10.1038/cr.2010.161

[23] Adli M, Merkhofer E, Cogswell P, Baldwin AS. IKKα and IKKβ each function to regulate NF-κB activation in the TNF-induced/canonical pathway. PloS One. 2010;**5**(2):e9428

[24] Israël A. The IKK complex, a central regulator of NF-kappaB activation. Cold Spring Harbor Perspectives in Biology. 2010;**2**(3):a000158. DOI: 10.1101/cshperspect.a000158

[25] Häcker H, Karin M. Regulation and function of IKK and IKK-related kinases. Science's STKE: Signal Transduction Knowledge Environment. 2006;**357**:re13. DOI: 10.1126/stke.3572006re13

[26] Sun SC. Non-canonical NF-κB signaling pathway. Cell Research. 2011;**21**(1):71-85. DOI: 10.1038/cr.2010.177

[27] Sun SC. The non-canonical NF-κB pathway in immunity and inflammation. Nature Reviews Immunology. 2017;**17**(9):545-558. DOI: 10.1038/nri.2017.52

[28] Cildir G, Low KC, Tergaonkar V. Noncanonical NF-κB signaling in health and disease. Trends in Molecular Medicine. 2016;**22**(5):414-429

[29] Noort AR, van Zoest KP, Weijers EM, Koolwijk P, Maracle CX, et al. NF-κB-inducing kinase is a key regulator of inflammation-induced and tumour-associated angiogenesis. The Journal of Pathology. 2014;**234**(3):375-385

[30] Flohé L, Brigelius-Flohé R, Saliou C, Traber MG, Packer L. Redox regulation of NF-kappa B activation. Free Radical Biology and Medicine. 1997;**22**(6):1115-1126

[31] Lingappan K. NF-κB in oxidative stress. Current Opinion in Toxicology. 2018;**7**:81-86

[32] Gloire G, Piette J. Redox regulation of nuclear post-translational modifications during NF-κB activation. Antioxidants & Redox Signaling. 2009;**11**(9):2209-2222

[33] Moi P, Chan K, Asunis I, Cao A, Kan YW. Isolation of NF-E2-related factor 2 (Nrf2), a NF-E2-like basic leucine zipper transcriptional activator that binds to the tandem NF-E2/AP1 repeat of the beta-globin locus control region. Proceedings of the National Academy of Sciences. 1994;**91**(21):9926-9930

[34] He F, Ru X, Wen T. NRF2, a transcription factor for stress response and beyond. International Journal of Molecular Sciences. 2020;**21**:4777. DOI: 10.3390/ijms21134777

[35] de la Vega MR, Chapman E, Zhang DD. NRF2 and the hallmarks of cancer. Cancer Cell. 2018;**34**(1):21-43

[36] Velichkova M, Hasson T. Keap1 regulates the oxidation-sensitive shuttling of Nrf2 into and out of the nucleus via a Crm1-dependent nuclear export mechanism. Molecular and Cellular Biology. 2005;**25**(11):4501-4513

[37] Xiong L, Xie J, Song C, Liu J, Zheng J, Liu C, et al. The activation of Nrf2 and its downstream regulated genes mediates the antioxidative activities of xueshuan Xinmaining tablet in human umbilical vein endothelial cells. Evidence-Based Complementary and Alternative Medicine. 2015;**2015**: 187265

[38] Xue D, Zhou X, Qiu J. Emerging role of NRF2 in ROS-mediated tumor chemoresistance. Biomedicine & Pharmacotherapy. 2020;**131**:110676

[39] Lin TY, Cantley LC, DeNicola GM. NRF2 rewires cellular metabolism to support the antioxidant response. A Master Regulator of Oxidative Stress-The Transcription Factor Nrf2. IntechOpen. 2016:107-108. DOI: 10.5772/65141

[40] Gambhir L, Sharma V, Kandwal P, Saxena S. Perturbation in cellular redox homeostasis: Decisive regulator of T cell mediated immune responses. International Immunopharmacology. 2019;**67**:449-457

[41] Gambhir L, Checker R, Thoh M, Patwardhan RS, Sharma D, Kumar M, et al. 1,4-naphthoquinone, a pro-oxidant, suppresses immune responses via KEAP-1 glutathionylation. Biochemical Pharmacology. 2014;**88**:95-105

[42] Chapple SJ, Siow RC, Mann GE. Crosstalk between Nrf2 and the proteasome: Therapeutic potential of Nrf2 inducers in vascular disease and aging. The International Journal of Biochemistry & Cell Biology. 2012;**44**(8):1315-1320

[43] Hybertson BM, Gao B, Bose SK, McCord JM. Oxidative stress in health and disease: The therapeutic potential of Nrf2 activation. Molecular Aspects of Medicine. 2011;**32**(4-6):234-246

[44] Zheng H, Whitman SA, Wu W, Wondrak GT, Wong PK, Fang D, et al. Therapeutic potential of Nrf2 activators in streptozotocin-induced diabetic nephropathy. Diabetes. 2011; **60**(11):3055-3066

[45] Nezu M, Suzuki N, Yamamoto M. Targeting the KEAP1-NRF2 system to prevent kidney disease progression. American Journal of Nephrology. 2017;**45**(6):473-483

[46] Lu Y, Zhang Y, Lou Y, Cui W, Miao L. Sulforaphane suppresses obesity-related glomerulopathy-induced damage by enhancing autophagy via Nrf2. Life Sciences. 2020;**258**:118153

[47] Ames BN, Gold LS, Willett WC. The causes and prevention of cancer. Proceedings of the National Academy of Sciences. 1995;**92**(12):5258-5265

[48] Panieri E, Buha A, Telkoparan-Akillilar P, Cevik D, Kouretas D, Veskoukis A, et al. Potential applications of NRF2 modulators in cancer therapy. Antioxidants. 2020;**9**(3):193

[49] Taguchi K, Yamamoto M. The KEAP1–NRF2 system in cancer. Frontiers in Oncology. 2017;**7**:85

[50] Qin JJ, Cheng XD, Zhang J, Zhang WD. Dual roles and therapeutic potential of Keap1-Nrf2 pathway in pancreatic cancer: A systematic review. Cell Communication and Signaling. 2019;**17**(1):121. DOI: 10.1186/s12964-019-0435-2

[51] Telkoparan-Akillilar P, Panieri E, Cevik D, Suzen S, Saso L. Therapeutic

targeting of the NRF2 signaling pathway in cancer. Molecules. 2021;**26**(5):1417

[52] Basak P, Sadhukhan P, Sarkar P, Sil PC. Perspectives of the Nrf-2 signaling pathway in cancer progression and therapy. Toxicology Reports. 2017;**4**:306-318

[53] Liu P, Luo G, Dodson M, Schmidlin CJ, Wei Y, Kerimoglu B, et al. The NRF2-LOC344887 signaling axis suppresses pulmonary fibrosis. Redox Biology. 2021;**38**:101766

[54] Ahmad A, Alkharfy KM, Jan BL, Ahad A, Ansari MA, Al-Jenoobi FI, et al. Thymoquinone treatment modulates the Nrf2/HO-1 signaling pathway and abrogates the inflammatory response in an animal model of lung fibrosis. Experimental Lung Research. 2020;**46**(3-4):53-63

[55] Carlson J, Price L, Deng H. Nrf2 and the Nrf2-interacting network in respiratory inflammation and diseases. In: Nrf2 and its Modulation in Inflammation. Champions: Springer; 2020. pp. 51-76

[56] Calkins MJ, Johnson DA, Townsend JA, Vargas MR, Dowell JA, Williamson TP, et al. The Nrf2/ARE pathway as a potential therapeutic target in neurodegenerative disease. Antioxidants & Redox Signaling. 2009;**11**(3):497-508

[57] Lu BW, Baum L, So KF, Chiu K, Xie LK. More than anti-malarial agents: Therapeutic potential of artemisinins in neurodegeneration. Neural Regeneration Research. 2019; **14**(9):1494

[58] Milani P, Ambrosi G, Gammoh O, Blandini F, Cereda C. SOD1 and DJ-1 converge at Nrf2 pathway: A clue for antioxidant therapeutic potential in neurodegeneration. Oxidative Medicine and Cellular Longevity. 2013;**2013**:836760. DOI: 10.1155/2013/836760

[59] Oeckinghaus A, Ghosh S. The NF-κB family of transcription factors and its regulation. Cold Spring Harbor Perspectives in Biology. 2009;**1**(4): a000034

Chapter 12

ROS Regulation Mechanism for Mitigation of Abiotic Stress in Plants

Asha Kumari, Mahendar Singh Bhinda, Sachin Sharma, Manoj Kumar Chitara, Ashim Debnath, Chandan Maharana, Manoj Parihar and Binny Sharma

Abstract

Plants respond to various stresses during their lifecycle among which abiotic stress is the most severe one comprising heat, cold, drought, salinity, flooding, etc. which take a heavy toll on crop yield worldwide in every corresponding year. ROS has a dual role in abiotic stress mechanisms where, at high levels, they are toxic to cells while at the same time, the same molecule can function as a signal transducer that activates a local as well as a systemic plant defense response against stress. The most common ROS species are Hydrogen peroxide (H2O2), Superoxide anions (O2-), Hydroxyl radicals (OH-), and Singlet oxygen (1O2) which are results of physiological metabolism often controlled by enzymatic and non-enzymatic antioxidant defense systems. ROS generally accumulate in plants during abiotic and biotic stress conditions resulting in oxidative damage which ultimately leads to programmed cell death. Many ROS scavenging pathways have been well studied against stress responses. Through careful manipulation of ROS levels in plants, we can enhance stress tolerance in plants under unfavorable environmental conditions. This chapter presents an overview of ROS regulation in plants and the essential enzymes involved in the abiotic stress tolerance mechanisms which are thoroughly discussed below.

Keywords: Plants, ROS, Abiotic stress, Signal transducer, antioxidants

1. Introduction

Drought, temperature, salinity, flooding and heavy metal toxicity are the examples of abiotic stressors. These multiple abiotic stressors sometimes occur at the same time [1, 2] and cause significant reduction in crop production. To satisfy the demands of food security for sustainable development in the era of a rising population and climate change, scientists predict a vital need for a "second green revolution" to produce higher yield and yield stability under non-optimal and adverse growing conditions through a combination of approaches based on recent advances in functional genomics [3, 4]. Plants have evolved a range of biological and biochemical responses to

coping up with adverse climatic conditions, including the activation of many stress-responsive genes and the synthesis of different structural proteins via complex signaling pathways, to confer resistance to abiotic stress conditions [5]. Reactive oxygen species (ROS) are byproducts of plant metabolic processes and are produced in a range of cellular compartments including chloroplasts [6], mitochondria [7], and peroxisomes [7, 8]. ROS not only cause irreversible DNA damage and cell death, but they also serve as important signaling molecules, regulating growth in plants under stress conditions. This suggests that ROS plays a dual role in vivo depending on their level of reactivity, production site, and ability to penetrate the cell membrane [9]. Reactive oxygen species (ROS), which include hydrogen peroxide (H2O2), superoxide radical (O2•-), hydroxyl radical (OH•), and singlet oxygen (1O2), are harmful byproducts of basic metabolic processes in living organisms [9, 10]. In plants, oxygen (O2), the source of all ROS, is stable and not very reactive [11]. Many excellent reviews have focused on ROS metabolism [9, 12], ROS sensory and signaling networks [9, 13, 14], and the involvement of ROS in developmental and stress response processes [12, 13]. The majority of these reviews, however, provided an overall retrospective for the model plant *Arabidopsis* [15]. They discussed enzymatic and non-enzymatic antioxidants and their roles in abiotic stress responses. However, the anti-oxidant system's regulation mechanisms, or even the key components involved in ROS regulation and abiotic stress resistance, has yet to be compiled in crop plants. In this chapter, we provide insight into current knowledge on the regulation of ROS homeostasis in crop plants. The genes that have been recognized in ROS homeostasis regulation affecting abiotic stress tolerance in crop plants were summarized in particular.

2. ROS role in plant growth and development

Despite the continuous efforts and gains made in agriculture development during recent decades, many stress factors continue to harm the crop growth and productivity. Most of the crop plants thrive in suboptimal environmental surroundings. Stressful conditions are the main factor preventing them from exhibiting their maximum genetic competence in terms of growth and reproduction, and as a result, plant productivity suffers as an outcome of these aberrant circumstances [15–17]. These pressures resulted in significant productivity and economic declines around the world. These stresses might be either biotic or abiotic. Pathogens (viruses, bacteria, and fungi), insects, herbivores, and rodents are all examples of biotic stresses. On the other hands, drought (water scarcity situation), salinity (high concentration of salt), cold (chilling and frost), heat (high temperature), flooding (water excess), radiation (high-intensity ultraviolet and visible light), contaminants, and toxins (heavy metals, pesticides, and aerosols), and soil nutrient deprivation are all examples of abiotic stresses [16, 18]. Any of these conditions either separately or in combinations may have varying degrees of influence on plant growth and development (**Table 1**).

Environmental factors influence plant growth and development through morphological, physiological, biochemical, and molecular alterations. The plant organelle metabolic paths are vulnerable to variation in environmental factors [13]. Tolerance can be attained by plant breeding or cultural activities that mitigate damages and require knowledge of the plant's stress response and how it affects individual plants and plant processes [42]. Various mechanisms linked with abiotic stress instruct plant cells to develop oxygen radicals and their derivatives referred to as reactive oxygen species (ROS). Furthermore, the development of reactive oxygen species (ROS) is a

Stress type	Plant Sp.	Stress condition	Loss result	Reference
Salinity	Soybean (*Glycine max* L.)	Application of (0, 33, 66 and 99 mM NaCl)	Significantly ↓ shoot length by 24%, 32% and 47% , and shoot and dry weight by 46%, 61% and 80% and 44%, 65% and 83% at 33, 66 and 99 mM NaCl respectively and root fresh, dry weight by 23%, 20% and 53%, 53% at 66 and 99 mM NaCl respectively	[19]
	Miscanthus × *giganteus*	NaCl concentrations (0, 2.86, 5.44, 7.96, 10.65, 14.68, 17.5, 19.97 and 22.4 dS m−1	Significantly ↓ biomass yield by 50% at 10.65 dS m−1 NaCl, root dry weight reduced by 61% at 22.4 dS m−1 NaCl	[20]
	Maize	0, 60, 120, 180 and 240 mM NaCl	Significantly ↓ in germination percentage (77.4%), germination rate (32.4%), length of radicle (79.5%) and plumule (78%), seedling length (78.1%) and seed vigor (95%) are obtained in highest level of salinity (240 mM)	[21]
	Tomato	1% and 3% NaCl	Significantly reduction in germination percentage 77.60% at 3% NaCl,	[22]
	Egg plant cultivars - Lagra Negra (LN), China-A2 (CH) and Black Beauty (BB)	NaCl (0, 50, 100, 150 and 200 mM)	Significantly ↓ survival of cultivar at 100 (50, 40, 30%), 150 (15, 0, 0) and 200 (0, 0, 0% or no survival of the plants)	[23]
	Lentil (*Lens culinaris* Medik.)	100 mM NaCl for 4 days	Significantly ↓ the growth (33%) and seedling fresh weight (44%)	[24]
Water logging	Maize var. DH605 and ZD958	Waterlogging for 3 and 6 days at third extended leaf stage (V-3, V-6), six extended leaf stage (V-3, V-6) and 10th day after flowering stage (10VT-3, 10VT-6), no waterlogging (CK)	Grain yields of DH605 in treatments V3-3, V3-6, V6-3, V6-6, 10VT-3 and 10VT-6 are 18.6 %, 30.3 %, 12.6 %, 18.8 %, 5.7 % and 13.9 % lower than in the control (CK), respectively. ZD958 yields in treatments V3-3, V3-6, V6-3, V6-6, 10VT3 and 10VT-6 are lower than CK yields by 15.6 %, 30.4 %, 13.0 %, 21.4 %, 5.9 % and 12.8 %, respectively	[25]
	Wheat	Waterlogging for 14 days at 22 days after sowing	Shoot dry weight ↓ by 37% and grain yield by 32% compared with the non-waterlogged plants	[26]
	Mung bean (*Vigna radiata*) var. MH–1K–24	Waterlogging at vegetative stage (30 days after sowing) for 3, 6 and 9 days	Photosynthetic loss at 3, 6 and 9 days are 43, 51, and 63 %, respectively, while grain yield	[27]

Stress type	Plant Sp.	Stress condition	Loss result	Reference
			loss was 20, 34 and 52 % respectively.	
	Chinese kale (*Brassica oleracea* var. *alboglabra*) and Caisin (*Brassica rapa* subsp. *parachinensis*)	Waterlogging or water deficit for 19 days in case of Chinese kale and 14 days in case of Caisin	Significantly ↓ plant fresh (90%) and dry weight (80%), leaf area (86%) and leaf number (38%) in Chinese kale; no impact on leaf number in Caisin but ↓ plant fresh and dry weights and leaf area by 60–70%	[28]
Drought/ Water deficit	Wheat	Plants are kept at normal day/ night (21/ 15°C) temperature and moisture content was maintained at 30% field capacity	Grain yield reduced by 53.05% compared to control	[29]
	Eggplant cultivars - Lagra Negra (LN), China-A2 (CH) and Black Beauty (BB)	Drought stimulated by PEG (0, 3, 8 and 10%)	↓ survival of cultivar at 3 % (90, 75, 70%), 8% (60, 45, 40%) and 10% (10, 0, 0% survival)	[23]
	Black gram (*Vigna mungo* L.) cultivar T9, KU-301 and green gram (*Vigna radiata* L.) cultivar Pratap, SG21-5 SG 21-5	A temporary rain shed are constructed in the field with PVC (polyvinyl chloride) film (of about 0.15 mm thickness and 85% of transmittance) to avoid rainfall	↓ Seed yield (T9-31.28%, KU 301- 48.52%, Pratap-37.12%, SG 21-5- 56.98%)	[30]
	Tomato genotypes viz., LE 1, LE 27, LE 57, LE 114, LE 118, LE 125, CO 3, PKM 1, TH CO 2 and TNAU TH CO 3	25 days old seedlings were transplanted and drought was imposed at first day after transplanting onwards based on IW/CPE, 0.5 IW/CPE for drought stress and 1.0 IW/CPE for control are maintained by irrigation the field at regular interval based cumulative pan evaporation.	Overall yield loss of tomato fruits up to 52 per cent under field condition, highest yield loss of 83.18 and 81.51 per cent are shown by LE 125 and LE 1 respectively.	[31]
Toxic/ heavy metals	Wheat	$CdCl_2 .H_2O$ (98%) @ 0, 5, 20, 50 and 80 mg L^{-1}	Significantly ↓ in root length (70.4%), shoot length (81.2%), percent germination (68%) and germination index (76.8%) at 80 mg L-1 Cd compared to control	[32]
	Maize	$CuSO_4 \cdot 5H_2O$ @ 0, 1, 10, 100, 1000, 5000, 10000 µmol/L in Hoagland culture medium	Significantly ↓ root activity in 1 µmol/L (18.3%), 10 µmol/ L (62.7%), and then decrease slowly-slowely	[33]

Stress type	Plant Sp.	Stress condition	Loss result	Reference
	Maize (*Zea mays* L.) cultivars Run Nong 35 and Wan Dan 13	Cd (0, 75, 150, 225, 300, 375 μM)	Grain yield is reduced in the range of 4–11 % under different Cd toxicity levels	[34]
	Tomato (cultivar PKM –1)	$ZnSO_4.7H$ O (0, 50, 100, 150, 200 and 250 mg kg^{-1} soil)	Significantly ↓ in root length 21.86, 25.40 and 33.3 and shoot length 7.46, 26.73, 31.72%, leaf area 29.53, 45.79, 48.02, root dry weight 27.46, 37.20, 54.37 and shoot dry weight 16.78, 32.91, 41.86% @ 150, 200, 250 mg/kg^{-1} respectively	[35]
	Pepper (*Capsicum annuum* L.)	0.1mM $PbCl_2$ and 0.1 mM $CdCl_2$	Significantly ↓ in total dry mass, root dry mass and shoot dry mass under both heavy metal and in combination compered to control	[36]
	Bean (*Phaseolus vulgaris*)	Pot soil contaminated with As (III) 20, 50 mg/kg and As (V) 20, 50 mg /kg	Significantly ↓ in shoot dry weight (40.85%) in As (V), shoot biomass in As (III) 49.3% & As (V) 63.88%, Dry matter yield of roots in 20 mg/kg As (V) 34.42, 46.18, 59.87 and 50 mg/kg As (V) 43.82, 56.09, 71.67%	[37]
Heat stress/ High temperature	Maize	In pot trial 32/22 °C (max/min temperature, control), 36/26 °C, and 40/30 °C for 14 consecutive days bracketing flowering	At 40/30 °C grain yield, seed-set, and grain number significantly ↓ by 73.6, 76.4, and 77.6%	[38]
	Wheat	In field condition during hot area in Iran	Significantly ↓ in grain yield (46.63%), 1000-kernel weight (20.61%) and grain filling duration (20.42%)	[39]
	Soybean	Double crop growing season in field	Significantly ↓ seed yield (29.5%) compare to previous year	[40]
	Field pea (*Pisum sativum* L.);	Field pea crop exposed three distinct windows normal, moderately and late sowing to heat stress environment	Significantly ↓ in seed set (7–14%) and 100-seed weight (6–16%)	[41]

Table 1.
Effect of various abiotic stresses on plant growth, development and production.

crucial mechanism in higher plants, since it is used to relay cellular signaling information in reaction to fluctuating environmental conditions.

As the crop yield is depending on the plant's capability to respond to various forms of environmental stresses, most of which causes oxidative stress and increases concentration of reactive oxygen species (ROS). Increased ROS accumulation is closely

related to increased environmental stress. A variety of biotic and abiotic factors can disrupt the balance between ROS production and the scavenging process, and responsible for raising their levels in intra-cellular [10]. All of these are accountable to cause serious oxidative injury to the plants, limiting their growth parameters and revenue ultimately (**Figure 1**).

Reactive oxygen species (ROS) also play a role in a variety of processes, including cell growth, production, and comeback to biotic and abiotic environmental inducements, as well as programmed cell death and signal transduction. Stressors, hormones, growth, and a variety of additional metabolic pathways can all arouse ROS formation, which can then trigger other pathways or serve as direct defense compounds in the plant body [43]. But, when ROS synthesis exceeds cellular scavenging potential, it disturbs the cellular redox homeostasis and produce ROS [44, 45]. To counter these stresses, plants have antioxidant pathways that scavenge excess ROS and avoid cell damage. Thus, plant synthesis and quenching are out of equilibrium, resulting in yield losses due to oxidative disruption. Though, it is difficult to identify this drop to oxidative damage due to the many processes involved in ROS synthesis; however, stresses and oxidative damage are interconnected and are liable for yield reduction [46].

Therefore, understanding the oxidative appliances in plants might be an aid in the growth of plants that are best suited to their surroundings. Plants stimulate antioxidant defense mechanisms in response to stress, which helps in the continuation of cell constituent's structural integrity and, potentially reduces oxidative injury. Plant defense is aided by several antioxidant enzymes. As a result, maintaining a high antioxidant ability to abolish toxic levels of ROS has been concurrent to improved crop plant capacity towards stress tolerance.

Manipulating ROS scavenging enzyme organizations is a likely method for producing transgenic plants that are more tolerant to a variety of stress situations; however, more research is needed for this since many enzymes and isoforms are

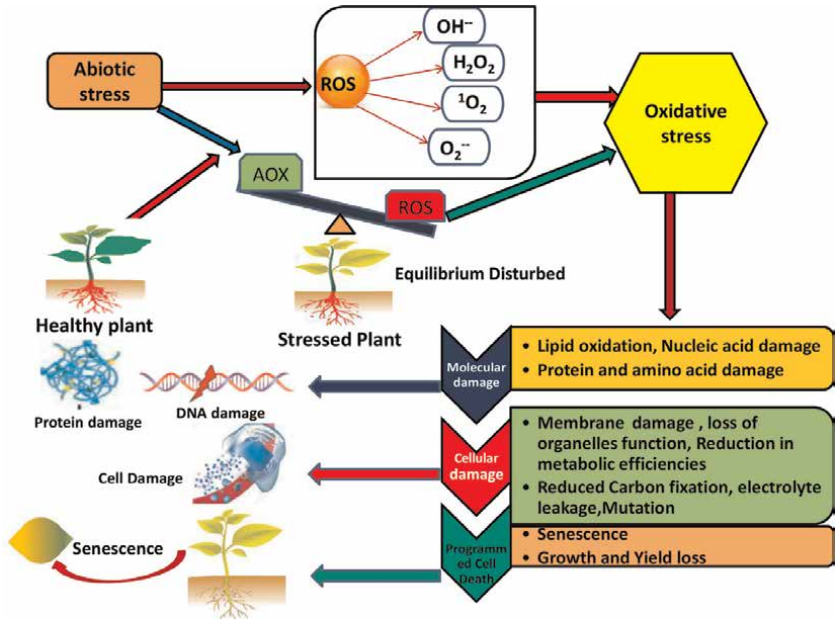


Figure 1.
Effect of oxidative stress on plant growth. ROS (Reactive oxygen species),AOX (Antioxidants).

involved, and ROS is only one of the promising issues of plant tolerance to environmental and biotic stresses [47].

2.1 Plant antioxidant defense system overview

To minimize possible harm to cellular components, as well as to sustain growth, metabolism, development, and total yield, the balance between ROS generation and removal at the intracellular level must be closely controlled and/or competently processed. Antioxidants scavenge ROS and/or regulate ROS development, either directly or indirectly [48]. This antioxidant defence system comprises low-molecular-weight non-enzymatic antioxidants and some antioxidant enzymes [49]. Non-enzymatic components include tocopherol, carotenoids, phenolic compounds, flavonoids, alkaloids, and non-protein amino acids, besides cellular redox buffers like ascorbate (AsA) and glutathione (GSH) [50–52].

Numerous antioxidant enzymes, like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), peroxidase (POX), polyphenol oxidase (PPO), peroxiredoxins (PRXs), Thioredoxins (TRXs), and ascorbate-glutathione (AsAGSH) cycle enzymes, such as ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), and glutathione reductase (GR) are the parts of the enzymatic components of the antioxidant defense organization [53–56]. The above non-enzymatic antioxidants function in combination with antioxidant enzymes to keep the balance between ROS synthesis and detoxification [52, 54].

3. ROS involvement in plant stress responses

The disruption of the equilibrium between the formation of reactive oxygen species (ROS) and antioxidant defense systems, resulting in an unsustainable accumulation of ROS and oxidative stress induction in the plant body, is one of the most imperative effects of environmental stresses. Both enzymatic and non-enzymatic antioxidant defense systems maintain the balance between reclamation and ROS generation when exposed to extreme environmental conditions [57]. Based on their concentration in plants, reactive oxygen species (ROS) can play both harmful and advantageous functions. ROS are unwelcome and dangerous byproducts of natural cellular metabolism at elevated amounts. But, serve as a second messenger in intracellular signaling flows that facilitate a variety of retorts in plant cells at low concentrations. It causes oxidative destruction to lipids, proteins, and DNA, resulting in changes in intrinsic membrane features such as fluidity, ion transfer, enzyme activity loss, protein crosslinking, protein synthesis inhibition, and DNA damage, all of which contribute to cell death. Thus, ROS disrupt biomolecules and cause genetically programmed cell death events at high concentrations. Higher plants have an extensive and very robust plant ROS setup, which is made up of antioxidant enzymes and antioxidant particles, that keeps ROS levels under control to avoid oxidative harm. Environmental factors like heat [58], cold [59], drought [60], Al toxicity [61], organic pollutants (OPs) [62], and pathogens [63, 64] have all been revealed to bring ROS production in plant cells.

On the other hand, changes in ROS levels over time and space are inferred as signals for a variety of biological events, including growth, development, resistance to abiotic stress factors, appropriate response towards pathogen, and cell death. The

molecular communication connected with ROS arbitrated signal transduction, which leads to gene expression management, is one of the essential early stress responses in the plant's acclamatory output. By altering the cell's redox equilibrium, ROS might function as a "second messenger," modifying the actions of particular proteins or gene expression. At any level of plant growth, the network of redox signals composes metabolism to regulate energy generation and consumption, interfering with primary signaling agents (hormones) to respond to evolving environmental nods. The consequence or fine-tuning of biological reactions to changed ROS levels is determined by interactions with other signaling molecules. Despite the recent identification of several constituents of the ROS signaling system, understanding how ROS-derived signals are incorporated to ultimately control biological processes like plant growth, development, stress adaptability as well as programmed cell death remains a challenge. To offset the negative impacts of oxidative stress, plants engage their antioxidant defense mechanism. Antioxidant defense ability, instead, vary according to plant species and genotype, stress kind, and period of exposure.

3.1 ROS involvement in water stress

Water stress is a common environmental restriction that plants frequently faced during their lives, restricting survival, reproduction, and ultimately productivity. Drought stress causes stomata closing, decrease CO2 entrance, and compromised photosynthetic rate, as well as discrepancy in the light acquire and usage and changed photochemistry in chloroplasts, triggering ROS excessive formation [49, 65]. The production of reactive oxygen species (ROS), which is assumed to lead to cellular injury, is one of the main and serious alterations due to drought stress. Though, a signaling function for ROS in activating the ROS scavenging mechanism, which might award defence or resistance to stress, has recently been discovered. This scavenging system is made up of antioxidant enzymes including SOD, catalase, and peroxidases, as well as antioxidant substances such as ascorbate and reduced glutathione; the oxidative load is largely governed by the balance between ROS formation and scavenging. Drought stress undoubtedly causes ROS production as a primary plant reaction, which might be regulated by hormones such as ABA and ethylene, which may also perform a downstream function. Unless ROS scavenging by antioxidant systems is disrupted, a high amount of ROS might exacerbate stress made harm to most cellular components [66].

3.2 Plant antioxidant defense against drought

According to Nahar et al. [67], a drought exposed *V. radiate* seedlings had lower AsA/DHA and GSH/GSSG ratios, but higher APX, GR, GPX, and GST activities, compared to control, which added to drought, persuaded oxidative loss tolerance. Rady et al. [68] reported raised H2O2 (26.2 percent) and O2 (51 percent) production, as well as elevated SOD, CAT, and APX activities by 110 percent, 66 percent, and 77 percent, respectively and also considerably amplified AsA, GSH, and -tocopherol level, in *S. Lycopersicum* cv. *Login* 935 treated to drought stress (60 percent FC for 20 days). Improved tolerance for drought stress via the antioxidant framework regulation has also been demonstrated in several chemical priming techniques. Antoniou et al. [69], who found that pre-treatment of *M. sativa* plants with melatonin led to enhanced CAT activity and lower H2O2 amount relative to untreated plants.

3.3 ROS involvement in salinity stress

Salinity carries oxidative stress by striking various impediments like ion toxicity, osmotic stress, nutritional deficiency, and toxicity, all of which leading to ROS overproduction and oxidative stress [70].

Rehman et al. [71] identified a 2.5- and 3-fold rise in H2O2 generation, as well as a 2- and a 3-fold upsurge in thiobarbituric acid reactive substances (TBARS) concentration, under 100 and 200 mM sodium chloride (NaCl) salinity conditions, respectively, compared to control which exhibits salt-induced oxidative stress.

3.4 Plant antioxidant defense against salinity

Many plant studies have revealed that regulating the antioxidant mechanism reduces the impact of salt stress in various plant species. Researchers have shown that antioxidant enzyme activity varies according to salt level, exposure length, and plant developmental stages [72, 73]. Vighi et al. [74] found a difference in response between salt-tolerant (BRS Bojuru) and salt-sensitive (BRS Pampa) rice cultivars and established that the OsAPX3, OsGR2, OsGR3, and OsSOD3-Cu/Zn genes were the main differentiator markers among these two genotypes. Alzahrani et al. [75] revealed elevated SOD, CAT, GR, and AsA stages in faba bean genotypes, when H2O2 levels rose beyond 90% under salinity stress, indicating the control of antioxidant response during salt stress and its alleviation.

Alsahli et al. [76] observed that a 2-fold increase in SOD, CAT, and APX activity and lowered 3-fold H2O2 in comparison to untreated control plants when salicylic acid (SA) was applied under salt-stressed in wheat.

Similarly, the antioxidant responses under salt stress conditions were controlled in sour orange through exogenous application of polyamines as reported by Tanou et al. [77], whereas in sorghum with simultaneous treatment of jasmonic acid (JA) and humic acid boosted APX activity, resultant in salt tolerance revealed by Ali et al. [78].

3.5 Plant antioxidant defense against high temperature

Plants' antioxidant defense mechanisms are triggered in response to high temperature (HT) stress [79, 80] although antioxidant capability varies amongst species as well as resistant and susceptible genotypes [81]. Reduced SOD and CAT activity, as well as repressed OsSOD, OsCAT, and OsAPX2 expression resulted in a 1.27-fold increased H2O2 accumulation in germinating rice seeds in high-temperature stress, according to Liu et al. [82]. Sarkar et al. [83] discovered increased CAT and POX activities in wheat genotypes during high-temperature stress (30°C).

3.6 Plant antioxidant defense against low temperature

Low-temperature stress causes plants to activate their antioxidant defense appliance to counteract negative consequences. Cucumber (*C. sativus* cv. *Xinyan* 4) seedlings were open to low-temperature stress (15/8°C day/night) for the period of 8 days and reported that 3- and 2-fold increased Cu-ZnSOD and Fe-SOD activities, respectively, in response to increased H2O2 and O2*-production [84]. Besides, after low-temperature stress, *Cynodondactylon, Capsella bursa pastoris*, and *Citrus reticulata* showed considerably enhanced CAT activity [85–87]. Higher APX activity was seen in *Jatropha macrocarpa* in reaction to increase H2O2, which enhanced low-temperature

stress tolerance, but lower APX activity (>6-fold) in *J. curcas* was connected to enhanced sensitivity under low-temperature circumstances [88]. Cheng et al. [89] studied *Citrulluslanatus* by exposing low-temperature stress (10/5 0C, 7 days) and found that the antioxidant defence system was activated, with GSH/GSSG and AsA/DHA ratios increasing considerably just a day after exposure in comparison to the control trial. Wang et al. [90] reported increased AsA and GSH levels in transgenic apple seedlings under low temperature stress in response to elevated H2O2 concentration (8°C, 12 hours). Han et al. [91] subjected 14-days old rice seedlings to low temperature (12°C, 6 days) stress and found increased H2O2 content and O2*- accumulation, as well as enlarged SOD and CAT activity and also increased GSH/GSSG ratio.

3.7 Plant antioxidant defense against flooding

Numerous crop species have displayed their capability to continue under the flooded or waterlogged situation for brief or even extended durations through triggering antioxidant defense mechanisms. An experiment was conducted by Li et al. [92], using18 maize genotypes which were subjected to waterlogged conditions and revealed that after 2 days of stress, 12 genotypes had 19–57 percent greater SOD activity, 13 genotypes had 19.16–106.96 percent greater POD activity, and only 9 genotypes had 26–57 percent greater CAT activity. In sesame seedlings under waterlogged conditions, lower AsA content, while higher GSH and GSSG content, as well as H2O2 content, was detected in a time-dependent way [93]. During extended (8 days) WL stress, although, AsA-GSH cycle enzymes were not controlled similarly, with considerably increased APX and MDHAR activity and considerably decreased DHAR and GR activity.

Furthermore, Park and Lee [94] found that when the Antarctic plants. *antarctica* was exposed to waterlogged (for 7 days), it accumulated about 52 percent more H2O2 and had 91 percent more CAT activity than controls.

3.8 Plant antioxidant defense against toxic metals

Metal toxicity tolerance is favorably coupled with improved antioxidant activity towards ROS detoxification and metal chelation [95, 96]. GST, one of the most important antioxidants, aids GSH in reducing metal toxicity by conjugating with them [97]. GSH also acts as a cytosolic predecessor of phytochelatins (PC), which are metal binders and catalyse the shuttle of metal ions and other xenobiotic that expedites the compound passage into the cell vacuole [81, 98]. The undertaking of cytosolic metals/metalloids ions into the vacuole in passive form lessens cellular toxicity [95]. Furthermore, both GST and GSH play a role in the accumulation of certain flavonoids (anthocyanin), which are metal binders and might follow a similar path to the vacuole [99, 100].

4. ROS generation and removal in the plants

Reactive oxygen species (ROS) are a broad term that includes the radical and non-radical form of species, formed due to incomplete oxygen metabolism. Radical species include superoxide radical (O2•_), hydroxyl radical (•OH), alkoxyl (RO•) and peroxyl (ROO•) while non- radical species contains hydrogen peroxide (H2O2), singlet

oxygen (1O2), ozone (O3), and hypochlorous acid (HClO). Oxygen is a fundamental element found in the Earth's crust that evolved billions of years ago. Oxygen molecules (O2) are not only crucial for metabolism and respiration but also support life forms on the Earth. O2 are mainly evolved through the photosynthetic activities of cyanobacteria in ancient times. ROS are partially reduced or activated derivatives of oxygen molecules that are highly reactive and toxic and can cause potential damage to the plants which includes cellular destruction, damage to plant metabolism and growth along with damage to DNA, RNA, proteins, and lipids. Plants perform various metabolic processes viz., namely photosynthesis, respiration which leads to the production of reactive oxygen species in various cell organelles like mitochondria [7], peroxisomes [8], chloroplasts [6], etc. They are an unavoidable phenomenon that leads to the production of oxidative stress in plants. ROS can also be produced during abiotic and biotic stress responses in plants. Besides this, the presence of free metals (Fe, Cu, Mn) derived from the metallo-protein complex phenomenon also contributes to ROS production. The production of ROS is stimulated by many factors namely physiological responses in the plant cell organelles, hormonal signaling, pathogen attack, gravitropism which produces free radicals inside plants [101]. The stoichiometry of ROS reveals that oxygen contains two unpaired electrons in their outermost shell having similar spin quantum numbers. However, oxygen molecules can accept a single electron at a time in its outermost orbit due to spin restriction, resulting in the formation of ROS which is highly reactive and active in subsequent reactions [102]. ROS are atoms or groups of atoms that possess at least one unpaired electron. Oxygen is an indispensable part of aerobic reactions in the plant system and molecular reduction of O2 leads to the formation of reactive oxygen species various cell organelles which are highly reactive as of molecular oxygen. Photosynthesis is a crucial metabolic process performed by the plants in the chloroplast of plant cells due to the localization of photosynthetic apparatus in the chloroplast. Although the photosynthetic process is highly influenced by the generation of ROS (O2.-&1O2), the formation of superoxide radicals is associated with PSI. The photolysis of water molecules is a crucial phenomenon in the PSII system of photosynthesis which produces O2 thus favoring superoxide radical formation reaction in PSI of photosynthesis. Also, auto-oxidation of Iron-sulfur protein results in O2.- production in the subsequent process due to abundant Fred and low NADP. Furthermore, reduced Fd reacts with superoxide radicals to form H2O2 in the illuminated chloroplast [103]. However, the regulation of ROS production during photosynthetic processes has been enunciated in several studies [53, 58, 104]. Singlet oxygen is also produced in PSII during photosynthetic processes. However, the root and stems of rice plants mainly produced O2-Which might relate to their subsequent environment for adaptation [105]. Additionally, superoxide radicals can be generated during PSII by auto-oxidation of PSII electron acceptors and PQ [106]. The ROS formation in illuminated chloroplast occurs mainly due to stress conditions followed by the closing of stomata. The partial reduction of O2 molecule in the respiratory chain occurs in chloroplast which consists of NADPH dehydrogenase and terminal oxidase is termed as chlororespiration. This phenomenon is also a major source of ROS production in the chloroplast. The peroxisomes also mediate the formation of O2.-through ETC using NADH as an electron donor. Peroxisomes are single membrane-bound organelle that performs certain major functions in the plants like fatty acid β- oxidation, regulation of glyoxylate cycle, photorespiration, metabolism of ROS & ureides, etc. [107–109]. Additionally, peroxisomes also regulate the generation of ROS via various metabolic functions. For instance, H2O2 production in the peroxisomes facilitated the regulation of the photosynthetic carbon oxidation cycle in C3

plants. During the carbon oxidation cycle, oxygenation of RuBP (mediated by RuBisCO) regenerates NADP+ and harbors a major sink of electrons which in turn prevents photoinactivation of PSII in the case when CO2 concentration is lacking. Due to which RuBisCO stimulates oxygenation in place of carboxylation as temperature elevates. The glycolate thus generated by oxygenation of RuBisCO suffers oxidation upon translocation to peroxisomes from chloroplast produces H2O2 as a by-product in the cells [102, 110]. Photorespiration is a metabolic process that occurs in chloroplast, mitochondria & peroxisomes. It includes phosphoglycolate metabolism which involves light-dependent O2 uptake & CO2 release with peroxisomal glycolate oxidase generating (H2O2) in the cells. The mitochondrial metabolism generated a considerable amount of ROS like H2O2, hydroxyl radicals, superoxide, etc. In the plants, mitochondria regulate aerobic respiration includes ETC (electron transport chain), which formulates ROS production in the mitochondrial membrane. However, the mitochondria bestow the limited ROS production in plants possibly due to the presence of alternative oxidase (AOX) that catalyzes the tetravalent reduction of O2 by ubiquinone [10]. The series of metabolic reactions in mitochondria leads to the formation of ROS inside the organelle. The flavoprotein region of NADH dehydrogenase encourages the production of O2- anions during mitochondrial electron transport (MET). One of the effective inhibitors of MET antimycin A enhances the ROS production by blocking electron flow after ubiquinone and the reduced ubiquinone undergoes auto-oxidation by contributing electron to O2, forming O2- [111]. Additionally, researchers studied that ubiquinone also contributes to H2O2 production in MET [112, 113]. Several mitochondrial enzymes like aconitase and 1-galactono-γ lactone dehydrogenase (GAL) also contribute to ROS generation. Furthermore, the O2- also gets converted into stable form H2O2 by the mitochondrial form of SOD (Mn-SOD). H2O2 is further transformed to (OH.) through Fenton reaction which is removed by ascorbate- glutathione cycle enzyme in plant system. Such OH molecules are liable to mutations in ETC of the mitochondrial genome. ROS generation in mitochondria also possesses negative effects on proteins by oxidation, cleavage, degradation of backbones [106]. Mitochondrial dysfunction due to excessive ROS production under unfavorable circumstances induces PCD (programmed cell death) and necrosis in the plants. The respiratory burst oxidase homologue (RBOH) synonymous with membrane-bound NADPH oxidase (NOX) in the plants also contributes to O2.- formation through electron through from intracellular NADPH across the plasma membrane to O2 in apoplast [103]. NADPH oxidase has a well-established role in stress responses in plants. Neill et al, 2002 studied that RBOH- dependent O2.— generation enunciates lipid peroxidation, PCD. NADPH oxidase induces membrane damage, favors oxidative burst, and reduces plant metabolic and growth-related activities under certain undesirable conditions. The plant cell wall is a site of redox reactions which enables the H2O2- dependent reactions and consists of malate dehydrogenase and NADH oxidase. NADPH-dependent microsomal electron transport is also a potential source of superoxides in the plant system. O2– formation in microsomes is mainly progressed by auto-oxidation of cytochrome P-450 reductase and/or auto-oxidation of oxycytochrome –P-450 complex [114]. Thus, the plant system involves efficient methods of ROS production in different responses. Different cellular compartments enunciate constant ROS production as a byproduct of redox and aerobic reactions. It is interesting to know that under favourable conditions plant maintains redox homeostasis and counteract with ROS production strategies within its system. The system of higher plants is complex and posses various regulatory processes. It is important to understand that controlled ROS generation and oxidative

burst can be a fundamental part of the plant signaling and defense mechanism. Future acknowledgments and researches should be directed towards the detailed study of ROS production in various aspects in plants with its signaling implications with references to various molecules present in the plant system. Plants possess a peculiar ROS scavenging system to maintain ROS homeostasis and redox signaling in their system during oxidative stress. However, disrupted protective mechanisms in response to oxidative burst may affect ROS-mediated redox homeostasis and cause cell death in the plants [11]. It is well-acquainted fact that plant produces a considerable amount of ROS during various metabolic processes in several cell organelles like chloroplast, mitochondria, plasma membrane and many more. They are an inexorable part of plant metabolism which play important role in redox signaling under environmental stresses [115]. To accomplish ROS removal from plant system plants consists of several ROS scavenging system which can be categorized into enzymatic and non-n enzymatic defense mechanism. Plants contain various antioxidant enzymes to mediate ROS scavenging mechanism which includes superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione peroxidase (GPX), catalase (CAT), monodehydroascorbate reductase (MDHAR or MDAR), dehydroascorbate reductase (DHAR or DAR) and glutathione reductase (GR) (**Figure 2**). These antioxidant enzymes ensure plant survival by minimizing the deleterious effect of ROS and prevent its overaccumulation. SOD (1.15.1.1) is a ubiquitous enzyme that plays a

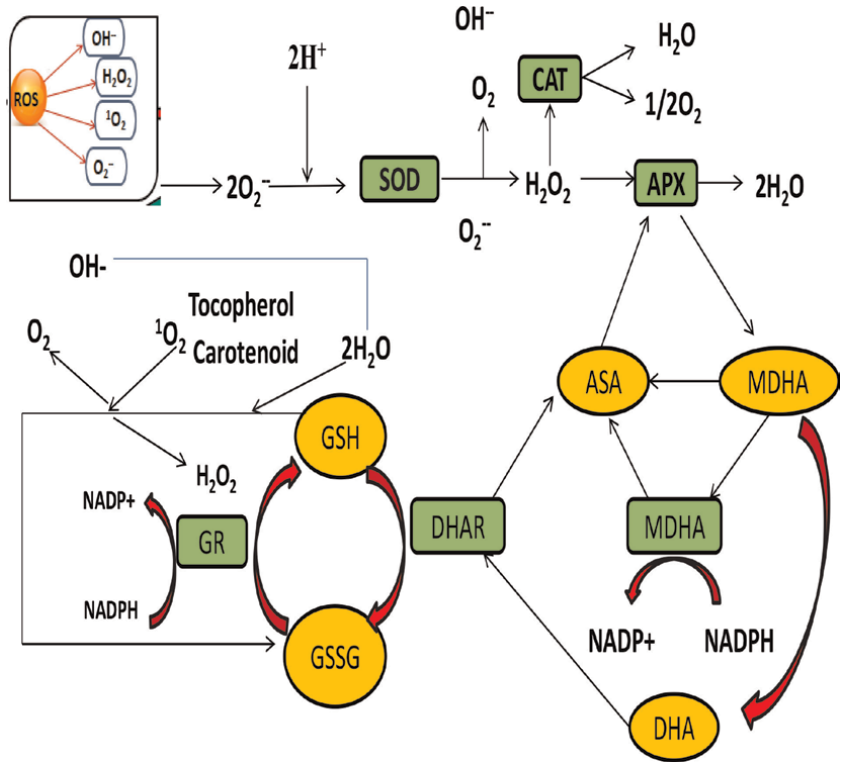


Figure 2.
Enzymatic and Non enzymatic Antioxidant mechanism to defend oxidative stressEnzymatic and non-enzymatic antioxidants in algae.ASC(Ascorbate),APX,(Ascorbate peroxidase),CAT Catalase, DHA Dehydroascorbate, GSH (Glutathione), GR Glutathione reductase , GSSG (Glutathione disulfide),MDHA (Monodehydroascorbate),SOD (Superoxide dismutase),DHA (Dehydroascorbate).

significant role in plant protection against oxidative stress. It catalyzes the dismutation of O2—to O2 and H2O. SOD has several isoforms and can be categorized as FeSOD, MnSOD, NiSOD, Cu/Zn SOD based on metal cofactors associated with the enzymes. The Arabidopsis genome contains three FeSOD (FSD1, FSD2, FSD3), one MnSOD (MSD1), and three Cu/Zn SOD (CSD1, CSD2, CSD3) type of genes [116, 117]. Similarly, the tomato genome consists of four Cu/Zn SOD, three FeSOD, and one MnSOD [118]. SOD gene family also have been discovered in many plant species like Musa acuminate, Sorghum bicolor, Populus trichocarpa, potato, pea, wheat, etc. However, transgenic approaches have also been described to study SOD responses in plants [10]. SOD isozymes have also been compartmentalized into mitochondria [119], peroxisomes [9], cytosol [116, 120], thylakoids [116, 121]. In plants, SOD is found in roots, leaves, fruits, and seeds where it functions significantly in the environment and oxidative stresses [122], photooxidative stress [123, 124], lateral root growth [119], germination [120], chloroplast development and flowering [121, 125]. Catalases (CAT) (E.C. 1.11.1.6) are a versatile antioxidant that helps in ROS scavenging mechanism in plants. They are ion containing homotetrameric proteins that catalyze the decomposition of H2O2 to H2O and O2 during the photorespiration process along with detoxification of H2O2[126]. Catalases are involved in antioxidant defense mechanism have been enunciated in many studies [127–132]. They also mediate in various physiological processes [45, 133–135]. The oxidative stress in plant cells can be maintained by enzymes of ascorbate- glutathione cycle. Ascorbate peroxidase (APX) (E.C.1.11.1.11) is another class of antioxidant enzyme that plays a vital role in scavenging H2O2 in chloroplast and cytosol in the plants. They are categorized into various forms based on their localization which is mainly chloroplast stromal soluble form (sAPX), chloroplast thylakoid bound form (tAPX), cytosolic form (cAPX) and glyoxisome membrane form (gmAPX) [136, 137]. They are heme-containing peroxidases possessing nine putative APX genes identified in Arabidopsis in cytosolic, chloroplast and peroxisomal regions of plant cells [138, 139] and sAPX in mitochondria [140]. They detoxify H2O2 through electron transfer from ascorbate to form monodehydroascorbate (MDHA). APX possesses several metabolic functions in H2O2 scavenging, plant responses to environmental stress, photoprotection, and plant development [115]. Another enzyme of ascorbate- glutathione cycle MDHAR (E.C.1.6.5.4) catalyzes the reverse reduction of MDHA to ascorbate in the presence of NAD(P)H [141] Foyer & Noctor, 2011). MDHAR is mainly localized in the cytosol, peroxisomes, mitochondria, and chloroplast. MDHAR is involved in stress tolerance, plant physiological processes, senescence, interaction, with endophytes has been explored in various studies [115, 142, 143]. DHAR is another enzyme that brings about the regeneration of ascorbate from DHA. DHARs (E.C.1.8.5.1) are monomeric enzymes that are identified in the Arabidopsis genome as DHAR1 and DHAR2 (cytosol), DHAR3 (chloroplast) [144]. Similar to APX, DHAR is also involved in the regulation of defense against environmental stress in various species [145, 146]. Glutathione reductase (GR) (E.C.1.6.4.2) is another potent enzyme mainly localized in chloroplast, mitochondria and cytosol. It catalyzes the reduction of glutathione and contains FAD-binding domain & NADPH- binding domain which carries out an enzymatic activity. Isozymes of GR have been widely studied in Arabidopsis which plays a vital role in various plant physiological responses [115].

The non-enzymatic antioxidant defense mechanism includes several low molecular mass ROS scavenging molecules like glutathione, ascorbic acid (AsA), flavonoids, carotenoids, tocopherols, alkaloids which aid in the removal of H2O2, singlet oxygen, and other ROS molecules. The antioxidant defense mechanism of AsA during

oxidative burst has been well acquainted through several studies. AsA (commonly known as Vit C) is water-soluble, localized in many plant cell organelles stimulates the quenching superoxide hydroxyl radicals and singlet oxygen produced during oxidative stress. Despite these, it also reduces H2O2 to H2O via ascorbate peroxidase reaction [147]. AsA regulates antioxidant defense mechanisms in response to various environmental stresses [148]. The non-enzymatic antioxidant system functions along with an enzymatic system to counteract the negative effect of ROS in plants. Reduced glutathione (GSH) is another class of low M.W thiol tripeptide antioxidant molecule commonly dominated in the cytosol, ER, mitochondria, chloroplast, vacuoles, peroxisomes & apoplast. GSH mediates multiple functions in plants. It plays a vital role in plant physiological functions like cell differentiation, growth, senescence, and many more. Precisely, it is also known for its antioxidant defense system in oxidative stress. It scavenges H2O2, 1O2, OH., O2.–, and reduces them to produce GSSG as a by-product. GSSG can also be generated through GSH. GSH plays a crucial part in regenerating AsA via the ascorbate- glutathione cycle. GSSG gets converted to GSH through denovo synthesis or by GR. Similar to glutathione another potential antioxidant molecule also includes proline, amino acids, alkaloids, polyamines, terpenes, amines, phenolics like compounds that scavenge ROS in plants. Carotenoids, on the other hand, are a group of lipophilic antioxidants that are present in a wide variety of organisms including plants. They have a well-established role in photosynthesis and protect photosynthetic machinery in response to ROS production. They scavenge 1O2, thus preventing generation by reacting with 3Chl* and excited Chl (Chl*), regulates the xanthophylls cycle. Similar to carotenoids, α- tocopherol is also a protector of the cell membrane in response to ROS production. α- tocopherol quench excess energy, safeguard lipids, and scavenges ROS formed during photosynthesis. It usually reacts with lipid radicals RO., ROO. And RO* at membrane- water interface and gets reduced to TOH. Which is then interacts with GSH & AA [149]. Flavonoids like flavonols, flavones, isoflavones, and anthocyanins are diverse in the plant kingdom and also plays a crucial role in the various physiological process mainly pigmentation in flowers, fruits, and seeds. They mitigate the negative effects of ROS produced in plants during photosynthesis. Additionally, they also scavenges1O2 along with repairing chloroplast membrane [150].

5. ROS regulation with genes and tolerance to abiotic stress tolerance in crops

Plants are evolved with multiple signaling pathways to control various sets of genes for generating different classes of protein to cope up with abiotic stress. These highly regulated genes play a very important role in ROS activation and regulation. Functional genomics helps us to identify more than 1000 stress-responsive genes in plants [151]. These genes have been characterized into different classes such as protein kinases and phosphatases, transcription factors, enzymes, molecular chaperones, and other functional proteins. The different genes involved in the regulation of ROS homeostasis and response to abiotic stresses have been categorized in plants (**Figure 3**; **Table 2**).

5.1 Protein kinases and phosphatases

Mitogen-activated protein kinases (MAPK) are the important gene groups in ROS signaling and regulation. Many studies have been conducted in plants on MAPK

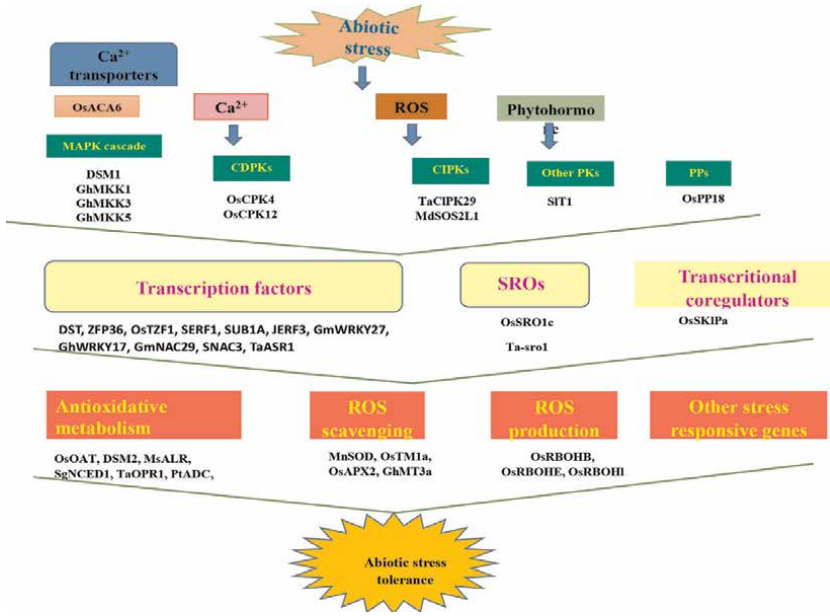


Figure 3.
Representative diagram showing several major genes/factors involved in ROS regulation and provides abiotic stress tolerance response in plants. Abbreviations: calcium-dependent protein kinase (CDPK); calcineurin B-like protein-interacting protein kinase (CIPK); mitogen-activated protein kinase (MAPK); protein kinase (PK); similar to RCD one (SRO).

cascades [174]. In cotton, two MAPK kinases (MAPKKs) have been characterized (GhMKK1 and GhMKK5) which are responsible for the homeostasis of ROS and abiotic stress tolerance [152]. Due to overexpression of GhMKK1 in tobacco, tolerance to salt and drought stresses have been observed by exhibiting ROS scavenging along with activities of antioxidant enzymes [175]. In transgenic tobacco plants, when BnMKK1 gene has been introduced, it triggers ABA signaling and leads to drought sensitivity and water loss [158] whereas GhMKK5 gene reduces salt and other abiotic stresses [152]. Plants showing overexpression of GhMKK5 leads to the up-regulation of ROS-related genes resulted in hypersensitive reaction with an accumulation of H2O2 [176]. In another study, a gene called GhMKK3 helps in regulating drought tolerance. Overexpression of this gene in tobacco induces stomatal closure due to activation of ABA-responsive gene along with a reduction in stomatal numbers [153, 177]. In some cases, two or more genes (GhMKK3 and HgPIP1) work together in connection with HgMPK7 gene for the production of drought and ABA-activated MAPK modules [178]. A drought-hypersensitive mutant1 (DSM1) of MAPK gene has been identified in rice which shows the sensitive response to oxidative stress [154]. In rice plants, two calcium-dependent protein kinase (CDPK) genes, OsCPK12 and OsCPK4 enhance tolerance to salt and drought stress respectively [155]. CBL-interacting protein kinase (CIPK) gene TaCIPK29 in wheat also is salt tolerance with ROS regulation mechanism (**Table 3**).

In transgenic tobacco, activities of ROS- scavenging enzymes have been increased along with the expression of transporter genes which leads to abiotic stress (salt stress) tolerance [156]. Another CIPK gene (MdSOS2L1) showed abiotic stress (salt tolerance) tolerance in crops like tomato and apple. Increased antioxidant metabolites (malate, procyanidin) and ROS scavenging enzymes are the mechanisms found after

Functional Class	Functional Protein	Genes involved	Plant concern	Abiotic Stress Resistance	ROS regulation	Reference
Protein Kinase and phosphatase						
MAPKs	MAPKK	*GhMKK1*	*G.hirsutum*	Drought and salt stress	ROS scavenging	[152, 153, 175]
	MAPKKK	*DSM1*	*O. sativa*	Drought stress	ROS scavenging	[154]
CDPK	Calcium-dependent protein kinase	*OsCPK12*	*O. sativa*	Salt stress	ROS production and scavenging	[155]
	Calcium-dependent protein kinase	*OsCPK4*	*O. sativa*	Drought and salt stress	ROS scavenging.	
CIPK	CBL-interacting protein kinase	*TaCIPK29*	*T. aestivum*	Salt stress	ROS scavenging	[156]
	CBL-interacting protein kinase	*MdSOS2L1*	*Malus x domestica*	Salt stress	ROS scavenging; antioxidative metabolism	[157]
Protein phosphatase	Protein phosphatase 2C	*OsPP18*	*O. sativa*	Drought and oxidative stress	ROS scavenging	[158]
Transcription factors						
AP2/ERF	ERF	*SERF1*	*O. sativa*	Salt stress	ROS signaling	[159]
	ERF	*SUB1A*	*O. sativa*	Drought, submerge and oxidative stress	ROS scavenging	[160]
	ERF	*JERF3*	*S.lycopersicum*	Drought, salt and freezing stress	ROS scavenging	[161]
Zinc finger	C2H2 zinc finger	*DST*	*O. sativa*	Drought and salt stress	ROS scavenging	[162]
	C2H2 zinc finger	*ZFP36*	*O. sativa*	Drought and oxidative stress	ABA-induced antioxidant defense	[163]
	CCCH zinc finger	*OsTZF1*	*O. sativa*	Drought, salt and oxidative stress	ROS scavenging	[164]
NAC	NAC	*GmNAC29*	*G. max*	Drought and salt stress	ROS production	[165]
	NAC	*SNAC3*	*O. sativa*	Drought, heat and oxidative stress	ROS scavenging	[166]
WRKY	WRKY	*GmWRKY27*	*G. max*	ROS production	Drought and salt stress	[165]

Functional Class	Functional Protein	Genes involved	Plant concern	Abiotic Stress Resistance	ROS regulation	Reference
	WRKY	*GhWRKY17*	*G. hirsutum*	ROS scavenging	Drought and salt stress	[167]
Other nuclear proteins						
SRO protein	SRO	*OsSRO1c*	*O. sativa*	Drought and oxidative stress	ROS scavenging	[168]
	SRO	*Ta-sro1*	*T. aestivum*	Osmotic, salt and oxidative stress	ROS production and scavenging	[33]
ABA metabolism	Carotene hydroxylase	DSM2	*O. sativa*	Drought and oxidative stress	antioxidative metabolism	[169]
	9-cis-epoxycarotenoid	*SgNCED1*	*S. guianensis*	Drought and salt stress	ABA-induced antioxidant defense	[170]
ROS scavenging	MnSOD	*MnSOD*	*N. plumbaginifolia*	Drought stress	ROS scavenging	[171]
	APX	*OsAPX2*	*O. sativa*	Drought, salt and cold stresses	ROS scavenging	[172]
Calcium transporters	type IIB Ca2+ATPase	*OsACA6*	*O. sativa*	Drought and salt stress	ROS scavenging	[173]

Table 2.
Major representative genes of crops involves in abiotic stress tolerance through ROS regulation.

Stress condition	Experimental condition	Plant sp.	Management strategy	Result	Reference
For salt stress plants are applied 12 dS m^{-1} NaCl after 2 weeks of germination	Pot	Maize	Se application (20 mg/L)	Improved shoot fresh and dry weight compared to control	[179]
For salt stress each pot pre-treated with 150 mM of NaCl for four weeks before AM fungus treatment	Pot	Rice	AM fungus *Claroideoglomus etunicatum* (isolate EEZ 163) @ 700 infective propagules per pot at sowing time just below rice seedlings	Significantly ↑ shoot dry weight (156%), root length (63%) under 150 mM NaCl concentration compared to un-inoculated control plants	[180]
For salt stress seedling are dip into Hoagland's medium containing 200 mM salt solution	Pot	Wheat	Seedling allowing in Hoagland's medium containing 200 mM salt solution and 5 ml PGP bacterial solution (*Bacillus* sp. (EN1), *Zhihengliuella* sp. (EN3), *S. succinus* (EN4), *Bacillus gibsonii* (EN6), *Oceanobacillus* sp. (EN8), *Halomonas* sp. (IA), and *Thalassobacillus* sp. (ID), *Halobacillus* sp) @ 1×10^9 cfu mL^{-1}	Significantly ↑growth rates of the plants are 67.5%, 64.4%, 62.2%, 76.3%, 70.6%, 73.5% and 78.1% for EN1, EN3, EN4, EN6, EN8, IA and ID as well as ↑ total fresh weight and length of root and shoot of wheat seedlings compared to control	[181]
Irrigated by saline water with 5 salinity levels: S1: 2 dS m^{-1}, S2: 4 dS m^{-1}, S3: 6 dS m^{-1}, S4: 8 dS m^{-1}, and S5: 10 dS m^{-1}	Pot	Tomato	Nano-fertilizer consisting of 79.19% $CaCO_3$ and 4.62% $MgCO_3$% areapplied through foliar spraying in three concentrations: N1: 0.5 g L^{-1}, N2: 0.75 g L^{-1}, and N3: 1 g L^{-1}.	Significantly ↑ average number of clusters and flowering, fruit set (especially in N1/S1: 65%), yield of fruit compare to control (N0/S1: 26% fruit set)	[182]
Marigold seedlings were exposed to four levels of drought stresses (100% (D_0), 75% (D_1), 50% (D_2), and 25% (D_3) according to water	Pot	Marigold	The inoculum (5 g of soil containing spores of AM fungi *Glomus constrictum* Trappe) are placed 3 cm below the surface of the soil (before sowing) to	Significantly ↑ plant height (7.7, 4.9, 5.5 and 16.2%), dry weight of shoot (19.14, 25.6, 5.88 and 31%) and flower (42.6, 9.6 and 21.8%,	[183]

Stress condition	Experimental condition	Plant sp.	Management strategy	Result	Reference
holding capacity of the soil			produce mycorrhizal pants	respectively) compared to non-AM inoculated plant	
For water stress plants are supplied 50%, 75%, 100% water after germination	Pot	Sorghum	Surface sterilized seeds are dipped in inoculums (*Streptomyces laurentii* EU-LWT3-69 and *Penicillium* sp. strain EU-DSF-10) for 2 h and sown in pot	Significantly ↑ plant root and shoot length, dry weight of biomass compared to un-inoculated plants	[184]
Exposure of inoculated plant to a 8-week drought-stress	Pot	Timothy (Phleum pratense L.)	At 3 weeks post seeding, each seedling was inoculated by pipetting 0.5 mL of phosphate buffer containing 10^6 CFU of *B. subtilis* strain B26	Significantly ↑ in shoot and root biomass by 26.6 and 63.8% compared to un-inoculated plants	[185]
For salt stress, 150 mM NaCl applied after 5 weeks of transplantation or at 5 to 6 leaf stage, and For drought stress, plant supplied of half of the water required for normal irrigation	Pot	Cucumber (*Cucumis sativus*) (cv. Cador, cv. Venus)	Foliar spray with Si (2.25 mM) as K_2SiO_3 to stressed plant at 10 days interval	Significantly ↑ in the R/S ratio by > 20 % in Cador and about 15 % in Venus cultivars	[186]
For flooding stress, 8 weeks older plants are flooded with deionised water for 15 days	Pot	*Ocimum sanctum*	Seedling treated with ACC deaminase-containing rhizobacteria bacterial inoculum of Fd2 (*Ach romobacte rxylosoxidans*), Bac5 (*Serratia ureilytica*), Oci9 (*Herbaspirillum seropedicae*) and Oci13 (*Ochrobac trum rhizosphaerae*) @ 10^8 CFU/ml before imposing stress	Significantly ↑ in fresh weight Fd2 (46.5%) followed by Oci13 (45.1%), Bac5 (26.5%) and Oci9 (16.6%), root weight in Fd2 (37%), shoot length 76.3, 41.1, 31.3 and 19.7%, number of leaves is 41.9, 37.7, 16 and 11%, number of nodes in Fd2 (72%) Bac5 (66%), Oci9 (33%) and Oci13 (27%) respectively.	[187]
For flooding stress, 5 month older	Pot	Muscadine Grape	control (aerated plants), aerated +	Si significantly ↑ the dry weight of	[188]

Stress condition	Experimental condition	Plant sp.	Management strategy	Result	Reference
plants are induced flooding/hypoxia stress by limiting the oxygen supply to the nutrient solution in hydroponic units		(*Muscadinia rotundifolia* Michx.)	Si (250 ppm), aerated + SiNP (250 ppm), hypoxia stress, hypoxia stress + Si (250 ppm), and hypoxia stress + SiNPs (250 ppm)	root, shoot, and total weight 20, 30 and 15%, Si + hypoxia stress ↑ 125, 120 and 125%, SiNPs + control ↑ 30, 46 and 20% and overall improvement is 155% compared to untreated control	
Heat stress treatment imposed on plants after 4 weeks of emergence and raised temperature by 2°C each day to avoid osmotic shock until the desired temperature level (45±2°C) are achieved	Pot	Tomato	Plants are sprayed 45 days after sowing with sulphur (S) @ 2, 4, 6, and 8 ppm for 2 times at 15 and 22 days after heat induction	In thermo tolerant cultivar @ 6 ppm S significantly ↑ maximum shoot (38.3 cm) and root (12.3 cm) length, shoot fresh (46.65g) and dry (14.57g) weights, average root fresh (12.21g) and dry (6.44g) weight, and in thermo sensitive cultivar fresh weight of fruit (42.1g), shoot fresh (42.14g) and dry (13.16g) weight, root fresh (12.21g) and dry (5.3g) weight compared to @ 2, 4, 8 ppm and untreated control	[189]
Heat stress treatment in Pot: at squaring, flowering and ball formation Field: April (medium temperature), early May (high temperature) and mild-June (optimum temperature) at squaring, flowering and boll formation stage	Pot and field	Cotton	Foliar spray of K, Zn and B @ 1.5, 0.2 and 0.1 % one day before heat treatment	Pot: Significantly ↑ seed cotton yield (SCY) in K (21%) Zn (16%) and B (7%) and average ball weight compared to control Field: Significantly ↑ seed cotton yield (SCY) in April (15%) and May (17%) thermal regimes and average ball weight compared to control in both year	[190]
For heat stress treatment at V_3	Pot	Soybean	2 days after transplantation,	Significantly ↑ in shoot length	[191]

Stress condition	Experimental condition	Plant sp.	Management strategy	Result	Reference
stage plants are exposed for 5 and 10 days			50 ml of freshly diluted *Bacillus cereus* SA1 culture (10^9 CFU/mL) are inoculated to each pot and repeated further 2 times after 5 days	(15.08%), root length (14.63%), fresh and dry weight (27.28 and 12.39%, respectively) after 5 and similar pattern followed by 10 days	
1 week after bacterial drench treatment, each pot are watered with 20 ml tap water and then heat and drought stressed by withholding watering for 5-7 days at 35°C in a growth chamber	Pot	Chinese cabbage	Seedlings (four-leaf stage) were drenched with bacterial suspensions (*Bacillus aryabhattai* H26-2 and H30-3) @ 10^7 cells/ml, 1 ml/g of potting mixture	Significantly ↑ in fresh weight (2.4% in H30-3) and number of lateral root (10.95 and 1.5% in H30-3 and H26-2, respectively) compare to control	[192]
For drought stress pots are irrigated with 80, 60 and 40% of water holding capacity for 60 days	Pot	Common bean (*Phaseolus vulgaris* L)	Before sowing seeds are treated with H_2O_2 for 4 hour	Significantly ↑ in root (8.15, 2.72%) and shoot (21.09, 10.52%) length, root fresh (25, 10%) and dry (31.25, 15.38%) weight, shoot fresh (21.12, 6.6%) and dry (21.68, 5.79%) weight in 60 and 40% WHC, respectively.	[193]
Drought stress induced in the pots artificially by irrigating the pot with a PEG-6000 (g/L of water) nutrient solution	Pot	Black gram and garden pea	Seed treatment with bacterial inoculum in individual with RJ12, RJ15 and RJ46 and in consortium with RJ12 + RJ15, RJ12 + RJ46, RJ15 + RJ46 (1×10^8 CFU^{-1})	Significantly ↑ in germination percentage (3-13, 7.7-17%), root length (21.15-55, 48.27-64.7%) and shoot length (26.66-35.67, 13.33-22.15%) varies in both crop plant respectively	[194]
For cold stress treatment pot are kept at 8±2°C for 60 days	Pot	Wheat	Seed treatment with charcoal based inoculum of the bacterial (*Pseudomonad*) culture 10^8 cfu g^{-1}	Significantly ↑ in shoot (4.7-26.1%), root length (27.9–70.5%), root (1.04–2.04-fold), and shoot biomass (1.25–1.66-fold) as compare to control	[195]

Stress condition	Experimental condition	Plant sp.	Management strategy	Result	Reference
For cold-stress 40 day old seedlings are transferred at 15/10°C (day/night) for two weeks	Pot	Cucumber	Each pot inoculated with 10 g of the AM fungi *R. irregularis*	Significantly ↑ shoot (7%) and root (25%) dry matter per gram compared to control	[196]
Heavy metal stress of Cadmium ($CdSO_4 \cdot 8H_2O$) treatments (0, 100 and 200 mg/l)	Pot	Mustard	SA (1.0 mM) are sprayed in the evening (10 ml/plant) to plants mixed with tween-20 every alternate day from the 1st day of treatment up to day 45	Significantly ↑ shoot (7%) and root (5%) fresh weight and Shoot and root dry weight (5%) @ 200 mg/l and 1 mM SA concentration compared to control	[197]
For heavy metal stress of Cr, plants after 30 days of germination, both soil and sand pots are irrigated with Cr as K2Cr2O7 solution (500 ml pot^{-1}) of 0, 0.25, and 0.5 mM in 7-day interval	Pot	Wheat	Glycinebetaine in 0.1 % Tween-20 solution are applied at 0 and 100 mM on the leaves until runoff at both tillering and booting vegetative stages	Significantly ↑ plant height, root length, kernel length, and number of tillers per plant compared to control	[198]
For heavy metal stress of Hg, seedlings are treated with $HgCl_2$ at 60 and 100 μM concentration for 7 days	Pot	Rice	Application of different combination with $HgCl_2$ of SNPs @ 100, 200 μM or KNO_2 @ 200 μM or $K_4Fe(CN)_6$ @ 200 μM or TIBA 1 μM	Significantly ↑ shoot and root length compare in all treatments to Hg treated plant	[199]
For heavy metal stress of Al, plants are treated with Al (500 μM) + salicyclic acid at different concentration for 2 weeks in Hoagland solution	Pot	Tomato var. SIRI and GOWRI	Application of salicyclic acid (SA) @ 100, 250 and 500 μM concentration on seedling which is mix in Hoagland for 2 weeks	Significantly ↑ plant growth in SIRI (65-85%) and GOWRI (20-55%)	[200]

Table 3.
Various management options to control various abiotic stresses in plants

molecular characterization of MdSOS2L1 gene [157]. In many cellular functions, protein phosphatase plays a crucial role in signal transduction with a process called dephosphorylation. A protein phosphatase gene, OsPP18 (PP2C) has been identified

in rice crops, which shows drought resistance response via ABA-independent pathways and regulating ROS homeostasis [158]. PPC2 genes have been identified and characterized in the genome of *Medicagotruncatula* [201]. Genome studies in Brassica [202] maize, tomato, and Arabidopsis also indicated the presence of PP2C gene families, responsible for abiotic stress tolerance [203, 204]. Among the two subfamilies of PP2C, subfamily A is used in ABA-dependent stress responses and B subfamily is MAPK regulators [205].

5.2 Transcriptional factors

Transcriptional factors (TFs) play an important role as a regulatory protein that could change the expression of stress-responsive genes and enhance tolerance to abiotic stress in plants. There are many studies on transcription factors that show their role in abiotic stress management in plants [206–208]. In-plant abiotic stress responses, members of AP2/ERF, zinc finger, NAC, and WRKY families have been identified and characterized to play a major role in the regulation of ROS homeostasis [209–211]. AP2/ERF (APETALA2/ethylene response factor) group of transcription factors regulates various abiotic stress responses and are found in certain rice varieties. They can acclimatize in stress conditions and minimize the energies consumed via gibberellin and ethylene responsiveness [212]. Although in the early stage of abiotic stresses, ROS levels act as an adaptation signal but the key components of ROS signaling are still unknown. In rice, ERF transcription factor SERF1 plays a very important role in molecular signaling (H2O2 mediated response) during the resistance response against salinity tolerance [159]. Another factor SUB1A reduces gibberellin response and ethylene production in submerged rice genotypes and conserves carbohydrates for future use. After the flooding subsides, plants go through severe leaf desiccation [204] which leads to ROS accumulation in plant tissues [206]. SUB1A boosts submergence tolerance by activating ROS-scavenging genes and also induces ABA responsiveness while activating stress genes [160, 213]. The JERF3 gene was also found to be involved in abiotic stress tolerance in tomato (*S. Lycopersicum)* by modulating ROS regulation and also influence the expression of genes involved in oxidative, osmotic stress responses which ultimately reduces ROS accumulation [161].

Zinc finger domains(s) were reported to be one of the most important transcription factors used in ROS regulation for abiotic stress tolerance in Arabidopsis and other plant species [214]. Based on the location and number of protein residues, zinc finger proteins are classified into several groups such as C2H2, CCCH, C2C2, and C3HC4 [211]. Gene DST accumulates H2O2 in the guard cell of the rice plant and enhances abiotic stress (drought and salt) tolerance while increasing the closure of stomata [162]. In rice crops, two other zinc finger proteins (ZFP179 and ZFP36) also help in the regulation of ROS homeostasis and abiotic stress tolerance [163, 215]. Another protein OSTZF1 enhances the expression of ROS-scavenging enzymes and genes responsible for redox homeostasis which helps in modulating abiotic stress resistance [164].

TF families also include the NAC group, which is one of the largest TF families with approximately 300 members among rice and Arabidopsis [216, 217]. This group of TFs helps in abiotic stress tolerance through ROS regulation. In Soybean, GmNAC2 transcription factor involves in signaling pathways of ROS and modulate the expression of ROS-scavenging genes [218]. Another NAC TF gene-SNAC3 has been identified in rice crops which regulates positively during drought stress and high temperature enhances abiotic stress tolerance by controlling ROS-related enzymes [219].

Another TF family WRKY is widely involved in Arabidopsis and Rice which has more than 100 genes only in these two plants [208]. These WRKY genes regulate both biotic and abiotic stress responses [220]. The WRKY transcription family is consists of a highly conserved region WRKYGQK heptapeptide at the C terminus and at the N- terminus a zinc-finger motif is present. These WRKY domains bind to W-box in the promoter regions and regulate various physiological responses [201, 221]. In rice WRKY genes reduces the oxidative stress tolerance effects by enhancing ROS and ABA functions. In transgenic soybean, the GmWRKY27 gene enhances drought and salt tolerance response by reducing ROS levels [165]. Another WRKY gene-GhWRKY17 in cotton involves abiotic stress tolerance by regulating ROS level and also by modifying ABA signaling pathways [167].

5.3 SRO proteins

SRO proteins which are also known as SIMILAR TO RCD ONE, are characterized as plant-specific proteins. Their domain characterization shows that they contain a C-terminal RCD1-SRO-TAF4 (RST) domain, N-terminal WWE domain, and a poly (ADP-ribose) polymerase catalytic (PARP) domain. In rice, the OsSRO1c gene targets abiotic stress (drought) related transcription factor (SNAC1), accumulates H2O2 in plant cells which leads to a reduction in water loss by reducing stomatal aperture [168]. OsNAC5 and ONAC095 have also been found to enhance drought and oxidative stress tolerance in rice [7]. In wheat crops, overexpression of the Ta-sro1 gene helps in cellular homeostasis with the regulation of ROS (through ROS-mediated enzymes) and provides salinity tolerance [208].

5.4 ABA metabolism-related proteins

Abscisic acid (ABA) plays an important role as a phytohormone that induces abiotic stress tolerance response in plants. In rice during drought condition, mutant gene dsm2 have been identified which synthesize β-carotene hydroxylase which is a precursor of ABA. Overexpression of *DSM2* enhances stress-related ABA-responsive gene expression and increases xanthophylls which lead to resistance response in abiotic and oxidative stresses [169]. In ABA catabolism, another hydroxylase-encoding gene-OsABA8ox3 is involved and controls oxidative stress under abiotic stress conditions [209]. In transgenic tobacco plants, overexpression of *the SgNCED1* gene (9-*cis*-epoxycarotenoid dioxygenase gene from *Stylosanthesguianensis*) increases ABA content and provides tolerance response to salt and drought stresses. This tolerance response is associated with the inducing production of NO and H2O2 along with the activation of ROS-scavenging enzymes [170].

5.5 ROS-scavenging proteins/enzymes

The presence of ROS-scavenging enzymes such as SOD, APX, and CAT in every cellular compartment of crop plants helps in ROS detoxification and protects against several abiotic stresses [52]. In water scarcity condition, improved yield and survival rate is observed in transgenic alfalfa crop due to the presence of MnSOD gene [171]. In transgenic rice plants under cold stress conditions, APX gene- OsAPX1 shows an increased percentage of spikelet fertility whereas overexpression of OsAPX2gene increased drought stress tolerance as compared with wild-type plants [172].

5.6 Ca2+ transporters and binding proteins

For the growth, development, and stress tolerance in plants, Calcium (Ca2+) controls several signaling pathways. P-type Ca2+ATPases or antiporters maintain the basal cytosolic level by regulating the influx and efflux of Ca2+ across the membranes. In rice crops, OsACA6 gene has been isolated and characterized. In tobacco plants, overexpression of the OsACA6 gene reduces ROS accumulation and induces expression of stress-responsive genes which leads to drought and salinity tolerance [173]. In transgenic lines, this gene controls cellular ion homeostasis and ROS-scavenging pathways which give tolerance response to Cd2+ stress [222].

6. Conclusion and future prospects

In the field of plant stress and ROS production, key sources, mechanism and various antioxidant enzymes to counteract the ROS are well reported. However, ROS homeostasis, signal transduction and interaction among various cellular compartments towards signaling are largely unknown and need to be addressed. Many studied reported that many antioxidant remain involve in ROS regulation but their inter and intra compartmental coordination to adjust ROS during stress condition is poorly understood. Therefore, to develop a conceptual and comprehensive framework, a combination of transcriptome, proteome, and metabolome approaches is required to understand ROS development, signaling pathways and their management.

Plants need robust and comprehensive adaption mechanisms to combat under stress conditions. For better stress resistance and ROS homeostasis many specific genes responsible for stress resistance has been identified in rice and transgenic plants. However, most of the ROS associated genes are studied for the expression of antioxidant enzymes activity and large field scale testing of transgenic plants for stress tolerance is very limited. Thus, in order to improve the abiotic stress tolerance by homeostasis of ROS, functions of associated genes and mechanism to control the ROS signaling pathways require detailed investigation. In future, these ROS associated genes and QTLs can be used in breeding and genetic engineering programme for the development of abiotic stress resistance cultivar.

DOI: http://dx.doi.org/10.5772/intechopen.99845

Author details

Asha Kumari[1], Mahendar Singh Bhinda[1], Sachin Sharma[3], Manoj Kumar Chitara[4], Ashim Debnath[2], Chandan Maharana[1], Manoj Parihar[1]* and Binny Sharma[5]

1 ICAR—Vivekananda Parvatiya Krishi Anushandhan Sansthan, Almora, Uttarakhand, India

2 Department of Genetics and Plant Breeding, College of Agriculture, Acharya Narendra Deva University of Agriculture and Technology, Ayodhya, Uttar Pradesh, India

3 Department of Soil Science and Agricultural Chemistry, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

4 Department of Plant Pathology, College of Agriculture, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

5 Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

*Address all correspondence to: manojbhu7@gmail.com

References

[1] Mittler R, Blumwald, E. Genetic engineering for modern agriculture: challenges and perspectives. Annual Review of Plant Biology. 2010;61:443–462. doi: 10.1146/annurev-arplant-042809-112116

[2] Hu H,Xiong, L. Genetic engineering and breeding of drought-resistant crops. Annual Review of Plant Biology. 2014; 65:715–741. doi: 10.1146/annurev-arplant-050213-040000

[3] Zhang A, Jiang M, Zhang J, Ding H, Xu S, Hu X, et al. Nitric oxide induced by hydrogen peroxide mediates abscisic acid-induced activation of the mitogen-activated protein kinase cascade involved in antioxidant defense in maize leaves. New Phytologist. 2007;175:36–50. doi: 10.1111/j.1469-8137.2007.02071.x

[4] Eckardt NA, Cominelli E, Galbiati M, Tonelli C. The future of science: food and water for life. The Plant Cell. 2009;21:368–372. doi: 10.1105/tpc.109.066209

[5] Hirayama T, Shinozaki K. Research on plant abiotic stress responses in the post-genome era: past, present and future. The Plant Journal. 2010;61:1041–1052. doi: 10.1111/j.1365-313X.2010.04124.x

[6] Dietz KJ, Turkan I, Krieger-Liszkay A. Redox- and reactive oxygen species-dependent signaling into and out of the photosynthesizing chloroplast. Plant Physiology2016;171:1541–1550. doi: 10.1104/pp.16.00375.

[7] Huang S, Van Aken O, Schwarzländer M, Belt K, Millar AH. The roles of mitochondrial reactive oxygen species in cellular signaling and stress response in plants.Plant physiology. 2016;171(3):1551-1559.

[8] Sandalio LM, Romero-Puertas MC. Peroxisomes sense and respond to environmental cues by regulating ROS and RNS signaling networks. Annals of Botany. 2015;116:475–485. doi: 10.1093/aob/mcv074.

[9] Miller G, Suzuki N, Ciftci-Yilmaz S, Mittler R. Reactive oxygen species homeostasis and signalling during drought and salinity stresses. Plant Cell& Environment. 2010;33:453–467. doi: 10.1111/j.1365-3040.2009.02041.x

[10] Apel K, Hirt H.Reactive oxygen species: metabolism, oxidative stress, signal transduction. Annual Review of Plant Biology. 2004;55:373-399.

[11] Mittler R. ROS are good. Trends in Plant Science. 2017;22:11–19. doi: 10.1016/j.tplants.2016.08.002

[12] Noctor G, Mhamdi A, Foyer CH. The roles of reactive oxygen metabolism in drought: not so cut and dried. Plant Physiology. 2014;164:1636–1648. doi: 10.1104/pp.113.233478

[13] Suzuki N, Koussevitzky S, Mittler R, Miller G. ROS and redox signalling in the response of plants to abiotic stress. Plant, Cell &Environment. 2012;35(2): 259-270. DOI: 10.1111/j.1365-3040.201102336.x

[14] Baxter A, Mittler R, Suzuki N. ROS as key players in plant stress signaling. J of Experimental Botany. 2014;65:1229–1240. doi: 10.1093/jxb/ert375

[15] Gill SS, Tuteja N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry. 2010;48(12):909-930. DOI: 10.1016/j.plaphy.2010.08.016

[16] Mahajan S, Tuteja N. Cold, salinity and drought stresses: an overview. Archives of Biochemistry and Biophysics. 2005;444:139-158. DOI: 10.1016/j.abb.2005.10.018

[17] Atkinson NJ, Urwin PE. The interaction of plant biotic and abiotic stresses: from genes to the field. Journal of Experimental Botany. 2012;63(10): 3523-3544. DOI: 10.1093/jxb/ers100

[18] Cancado GMA. The importance of genetic diversity to manage abiotic stress. In: Shanker A, editor. Abiotic Stress in Plants - Mechanisms and Adaptations.InTechOpen;2011. pp. 351-366. DOI: 10.5772/22397

[19] Weisany W, Sohrabi Y, Heidari G, Siosemardeh A, Ghassemi-Golezani K. Changes in antioxidant enzymes activity and plant performance by salinity stress and zinc application in soybean (*'Glycine max*'L.). Plant Omics. 2012;5(2):60.

[20] Stavridou E, Hastings A, Webster RJ, Robson PR. The impact of soil salinity on the yield, composition and physiology of the bioenergy grass *Miscanthus×giganteus*. GCB Bioenergy. 2017;9(1):92-104.

[21] Khodarahmpour Z, Ifar M, Motamedi M. Effects of NaCl salinity on maize (*Zea mays* L.) at germination and early seedling stage. African Journal of Biotechnology. 2012;11(2):298-304.

[22] Singh J, Sastry ED, Singh V. Effect of salinity on tomato (*Lycopersicon esculentum* Mill.) during seed germination stage. Physiology and Molecular Biology of Plants. 2012;18(1):45-50.

[23] Zayova E, Philipov P, Nedev T, Stoeva D. Response of in vitro cultivated eggplant (*Solanum melongena* L.) to salt and drought stress. AgroLife Scientific Journal. 2017;6(1):276-282.

[24] Hossain MS, Alam MU, Rahman A, Hasanuzzaman M, Nahar K, Al Mahmud J, Fujita M. Use of iso-osmotic solution to understand salt stress responses in lentil (*Lens culinaris* Medik.). South African Journal of Botany. 2017;113:346-354.

[25] Ren B, Zhang J, Dong S, Liu P, Zhao B. Effects of duration of waterlogging at different growth stages on grain growth of summer maize (*Zea mays* L.) under field conditions. Journal of Agronomy and Crop Science. 2016; 202(6):564-575.

[26] Robertson D, Zhang H, Palta JA, Colmer T, Turner NC.Waterlogging affects the growth, development of tillers, yield of wheat through a severe, but transient, N deficiency. Crop and Pasture Science. 2009;60(6):578-586.

[27] Kumar P, Pal M, Joshi R, Sairam, RK. Yield, growth and physiological responses of mung bean [*Vigna radiata* (L.) Wilczek] genotypes to waterlogging at vegetative stage. Physiology and Molecular Biology of Plants. 2013;19(2): 209-220.

[28] Issarakraisila M, Ma Q, Turner DW. Photosynthetic and growth responses of juvenile Chinese kale (*Brassica oleracea* var. *alboglabra*) and Caisin (*Brassica rapa* subsp. *parachinensis*) to waterlogging and water deficit. Scientia horticulturae. 2007;111(2):107-113.

[29] Qaseem MF, Qureshi R,Shaheen H. Effects of pre-anthesis drought, heat and their combination on the growth, yield and physiology of diverse wheat (*Triticum aestivum* L.) genotypes varying in sensitivity to heat and drought stress. Scientific reports. 2019;9(1):1-12.

[30] Baroowa B, Gogoi N. Morpho-physiological and Yield responses of Black gram (*Vigna mungo* L.) and Green

gram (*Vigna radiata* L.) genotypes under Drought at different Growth stages. Research Journal of Recent Sciences. 2016;5(2):43-50.

[31] Sivakumar R, Nandhitha GK, Nithila S. Impact of drought on chlorophyll, soluble protein, abscisic acid, yield and quality characters of contrasting genotypes of tomato (*Solanum lycopersicum*). Current Journal of Applied Science and Technology.2017;21(5):1-10.

[32] Ahmad I, Akhtar MJ, Zahir ZA, Jamil A. Effect of cadmium on seed germination and seedling growth of four wheat (*Triticum aestivum* L.) cultivars. Pakistan Journal of Botany. 2012;44(5): 1569-1574.

[33] Liu JJ, Wei Z, Li JH.Effects of copper on leaf membrane structure and root activity of maize seedling. Botanical Studies. 2014;55(1):1-6.

[34] Anjum SA, Tanveer M, Hussain S, Bao M, Wang L, Khan I, et al. Cadmium toxicity in Maize (*Zea mays* L.): consequences on antioxidative systems, reactive oxygen species and cadmium accumulation. Environmental Science and Pollution Research. 2015;22(21): 17022-17030.

[35] Vijayarengan P,Mahalakshmi G. Zinc toxicity in tomato plants. World Applied Sciences Journal. 2013;24(5): 649-653.

[36] Kaya, C., Akram, N. A., Sürücü, A. and Ashraf, M. (2019). Alleviating effect of nitric oxide on oxidative stress and antioxidant defence system in pepper (Capsicum annuum L.) plants exposed to cadmium and lead toxicity applied separately or in combination. Scientia Horticulturae, 255, 52-60.

[37] Chandra S, Saha R, Pal P. Assessment of arsenic toxicity and tolerance characteristics of bean plants (*Phaseolus vulgaris*) exposed to different species of arsenic. Journal of Plant Nutrition. 2018; 41(3):340-347.

[38] Wang Y, Tao H, Tian B, Sheng D, Xu C, Zhou H, et al. Flowering dynamics, pollen, pistil contribution to grain yield in response to high temperature during maize flowering. Environmental and Experimental Botany. 2019;158:80-88.

[39] Modarresi M, Mohammadi V, Zali A, Mardi M. Response of wheat yield and yield related traits to high temperature. Cereal Research Communications. 2010; 38(1):23-31.

[40] Bihter ONAT, Bakal H, Gulluoglu L, Arioglu H.The effects of high temperature at the growing period on yield and yield components of soybean [*Glycine max* (L.)Merr] varieties. Turkish Journal of Field Crops. 2017;22 (2):178-186.

[41] Lamichaney A, Parihar AK, Hazra KK, Dixit GP, Katiyar PK, Singh, D,et al. Untangling the Influence of Heat Stress on Crop Phenology, Seed Set, Seed Weight, Germination in Field Pea (*Pisum sativum* L.). Frontiers in Plant Science. 2021;12:437.

[42] Peterson RKD, Higley LG. Biotic Stress and Yield Loss. 1st edn. Washington: CRC Press; 2000. pp. 261. ISBN 0-8493-1145-4.

[43] Bailey-Serres J, Mittler R. The roles of reactive oxygen species in plants cells. Plant Physiology. 2006;141(2):311. DOI: 10.1104/pp.104.900191

[44] Mullineaux PM and Baker NR. Oxidative stress: antagonistic signaling for acclimation or cell death? Plant Physiology. 2010;154:521-525.

[45] Sharma I, Ahmad P. "Catalase: a versatile antioxidant in plants," in Oxidative Damage to Plants, ed P. Ahmad (Srinagar: S.P. College). Academic Press; 2014. pp. 131–148.

[46] Wahid A, Farooq M, Siddique KHM. Implications of oxidative stress for plant growth and productivity. In: Pessarakli M, editor. Handbook of Plant and Crop Physiology. 3rdedn. LLC 6000 Broken Sound Parkway, Suite 300, Boca Raton, FL 33487 USA: Taylor & Francis Group;2014. pp. 549-556. DOI: ISBN13: 978-1-4665-5328-6

[47] Caverzan A, Casassola A, PatussiBrammer S. Reactive oxygen species and antioxidant enzymes involved in plant tolerance to stress. In: Arun K. Shanker and ChitraShanker, editors. Abiotic and biotic in stress plants - recent advances future perspectives. London: InTechOpen; 2016. pp. 463-480.

[48] Carocho M, Ferreira IC.A review on antioxidants, prooxidants and related controversy: Natural and synthetic compounds, screening and analysis methodologies and future perspectives. Food and Chemical Toxicology. 2013;51: 15–25.

[49] Hasanuzzaman M, Bhuyan M, Anee TI, Parvin K, Nahar K, Mahmud JA, Fujita M. Regulation of ascorbate-glutathione pathway in mitigating oxidative damage in plants under abiotic stress. Antioxidants. 2019; 8: 384.

[50] Gratão PL, Polle A, Lea PJ, Azevedo RA. Making the life of heavy metal-stressed plants a little easier. Functional Plant Biology. 2005;32:481-494.

[51] Scandalios JG. Oxidative stress: molecular perception and transduction of signals triggering antioxidant gene defenses. Brazalian Journal of Medical and Biological Research. 2005;38: 995-1014.

[52] Mittler R, Vanderauwera S, Gollery M, Van Breusegem F. Questions and future challenges. Trends in Plant Science. 2004;10(9):490-498.

[53] Asada K. Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiology. 2006;141:391–396.

[54] Mittler R. Oxidative stress, antioxidants and stress tolerance. Trends in Plant Science. 2002;7:405-410.

[55] Nath M, Bhatt D, Bhatt MD, Prasad R, Tuteja N. Microbe-mediated enhancement of nitrogen and phosphorus content for crop improvement. In Crop Improvement through Microbial Biotechnology; Prasad, R, Gill, S.S, Tuteja, N, Eds.; Academic Press: Cambridge, UK; 2018. pp. 291–301.

[56] Laxa M, Liebthal M, Telman W, Chibani K, Dietz KJ.The role of the plant antioxidant system in drought tolerance. Antioxidants. 2019;8:94.

[57] Hasanuzzaman M, Bhuyan MHMB, Zulfiqar F, Raza A, Mohsin SM, Mahmud JA, Fujita M, Fotopoulos V. Reactive Oxygen Species and Antioxidant Defense in Plants under Abiotic Stress: Revisiting the Crucial Role of a Universal Defense Regulator. Antioxidants. 2020a;9: 681. doi:10.3390/ antiox9080681

[58] Zhao Q, Zhou L, Liu J, Cao Z, Du X, Huang F, et al. Involvement of CAT in the detoxification of HT-induced ROS burst in rice anther and its relation to pollen fertility. Plant Cell Reports. 2018; 37:741–757. doi: 10.1007/s00299-018-2264-y

[59] Kawarazaki T, Kimura S, Iizuka A, Hanamata S, Nibori H, Michikawa M, et al. A low temperature-inducible protein AtSRC2 enhances the ROS producing activity of NADPH oxidase AtRbohF. Biochimica et Biophysica Acta. 2013;1833:2775-2780.doi: 10.1016/j.bbamcr.2013.06.024

[60] Lee S, Seo PJ, Lee HJ, Park CM.A NAC transcription factor NTL4 promotes reactive oxygen species production during drought-induced leaf senescence in Arabidopsis. The Plant Journal. 2012;70:831-844. doi: 10.1111/j.1365-313X. 2012.04932.x

[61] Wu Y, Yang Z, How J, Xu H, Chen L, Li K.Overexpression of a peroxidase gene (AtPrx64) of Arabidopsis thaliana in tobacco improves plant's tolerance to aluminum stress. Plant Molecular Biology. 2017;95:157-168. doi: 10.1007/s11103-017-0644-2

[62] Ahammed GJ, He BB, Qian XJ, Zhou YH, Shi K, Zhou J, et al.24-Epibrassinolide alleviates organic pollutants-retarded root elongation by promoting redox homeostasis and secondary metabolism in *Cucumi ssativus* L. Environmental Pollution. 2017;229: 922-931. doi: 10.1016/j.envpol.2017.07.076

[63] Kim DS, Hwang BK. An important role of the pepper phenylalanine ammonia-lyase gene (PAL1) in salicylic acid-dependent signalling of the defence response to microbial pathogens.Journal of Experimental Botany. 2014;65: 2295-2306. doi: 10.1093/jxb/eru109

[64] Yang C, Li W, Cao J, Meng F, Yu Y, Huang J, et al. Activation of ethylene signaling pathways enhances disease resistance by regulating ROS and phytoalexin production in rice. The Plant Journal. 2017;89:338-353. doi: 10.1111/tpj.13388

[65] Hasanuzzaman M, Nahar K, Khan MIR, Al Mahmud J, Alam MM, Fujita M. Regulation of reactive oxygen species metabolism and glyoxalase systems by exogenous osmolytes confers thermos-tolerance in *Brassica napus*. Gesunde Pflanzen. 2020b;72: 3-16.

[66] Kar RK.Plant responses to water stress, Role of reactive oxygen species. Plant Signaling & Behavior.2011;6(11): 1741-1745.

[67] Nahar K, Hasanuzzaman M, Alam MM, Rahman A, Mahmud JA, Suzuki T, Fujita M. Insights into spermine-induced combined high temperature and drought tolerance in mung bean: Osmoregulation and roles of antioxidant and glyoxalase system. Protoplasma. 2017;254:445–460.

[68] Rady MM, Belal HE, Gadallah FM, Semida WM.Selenium application in two methods promotes drought tolerance in *Solanum lycopersicum* plant by inducing the antioxidant defense system. Scientia Horticulturae. 2020;266:109-290.

[69] Antoniou C, Chatzimichail G, Xenofontos R, Pavlou JJ, Panagiotou E, Christou A, Fotopoulos V. Melatonin systemically ameliorates drought stress-induced damage in *Medicago sativa* plants by modulating nitro-oxidative homeostasis and proline metabolism. Journal of Pineal Research. 2017;62: e12401.

[70] Munns R, Tester M. Mechanisms of salinity tolerance.Annual Review of Plant Biology. 2008;59:651-681.

[71] Rehman S, Abbas G, Shahid M, Saqib M, Farooq ABU, Hussain M, Murtaza B, Amjad M, Naeem MA, Farooq A. Effect of salinity on cadmium tolerance, ionic homeostasis and oxidative stress responses in conocarpus exposed to cadmium stress: Implications

for phytoremediation. Ecotoxicology and Environment Safety. 2019;171:146–153.

[72] Cunha JR, Neto MCL, Carvalho FE, Martins MO, Jardim-Messeder D, Margis-Pinheiro M, Silveira JA. Salinity and osmotic stress trigger different antioxidant responses related to cytosolic ascorbate peroxidase knockdown in rice roots. Environmental and Experimental Botany. 2016;131: 58-67.

[73] Li Y, Cao XL, Zhu Y, Yang XM, Zhang KN, Xiao ZY, Wang H, Zhao JH, Zhang LL, Li GB. Osa-miR398b boosts H_2O_2 production and rice blast disease-resistance via multiple superoxide dismutases. New Phytologist. 2019;222: 1507-1522.

[74] Vighi I, Benitez L, Amaral M, Moraes G, Auler P, Rodrigues G, Deuner S, Maia L, Braga E.Functional characterization of the antioxidant enzymes in rice plants exposed to salinity stress. Biologia Plantarum. 2017; 61:540–550.

[75] Alzahrani SM, Alaraidh IA, Migdadi H, Alghamdi S, Khan MA, Ahmad P. Physiological, biochemical, antioxidant properties of two genotypes of *Vicia faba* grown under salinity stress. Pakistan Journal of Botany. 2019;51: 786-798.

[76] Alsahli A, Mohamed AK, Alaraidh I, Al-Ghamdi A, Al-Watban A, El-Zaidy M, Alzahrani, SM. Salicylic acid alleviates salinity stress through the modulation of biochemical attributes and some key antioxidants in wheat seedlings. Pakistan Journal of Botany. 2019;51:1551-1559.

[77] Tanou G, Ziogas V, Belghazi M, Christou A, Filippou P, Job D, Fotopoulos V, Molassiotis A. Polyamines reprogram oxidative and nitrosative status and the proteome of citrus plants exposed to salinity stress. Plant, Cell and Environment. 2014;37:864–885.

[78] Ali AYA, Ibrahim MEH, Zhou G, Nimir NEA, Jiao X, Zhu G, Elsiddig AMI, Suliman MSE, Elradi SBM, Yue W. Exogenous jasmonic acid and humic acid increased salinity tolerance of sorghum. Agronomy Journal.2020;112:871-884.

[79] Ding X, Jiang Y, He L, Zhou Q, Yu J, Hui D, Huang D. Exogenous glutathione improves high root-zone temperature tolerance by modulating photosynthesis, antioxidant and osmolytes systems in cucumber seedlings. Scientific Reports.2016;6:35424.

[80] Awasthi R, Gaur P, Turner NC, Vadez V, Siddique KH, Nayyar H. Effects of individual and combined heat and drought stress during seed filling on the oxidative metabolism and yield of chickpea (*Cicer arietinum*) genotypes differing in heat and drought tolerance. Crop and Pasture Science.2017;68: 823-841.

[81] Hasanuzzaman M, Hossain MA, Teixeira da Silva JA, Fujita M. Plant responses and tolerance to abiotic oxidative stress: Antioxidant defense is a key factor. In Crop Stress and its Management: Perspectives and Strategies; Bandi V, Shanker AK, Shanker C, Mandapaka M, Eds.; Springer: Berlin, Germany;2012. pp. 261-316.

[82] Liu J, Hasanuzzaman M, Wen H, Zhang J, Peng T, Sun H, Zhao Q. High temperature and drought stress cause abscisic acid and reactive oxygen species accumulation and suppress seed germination growth in rice. Protoplasma. 2019;256:1217-1227.

[83] Sarkar J, Chakraborty B, Chakraborty U. Temperature stress induced antioxidative and biochemical

changes in wheat (*Triticum aestivum* L.) cultivars. Journal of Plant Stress Physiology.2016:22-30.

[84] Zhao H, Ye L, Wang Y, Zhou X, Yang J, Wang J, Cao K,Zou Z. Melatonin increases the chilling tolerance of chloroplast in cucumber seedlings by regulating photosynthetic electron flux and the ascorbate-glutathione cycle. Frontiers in Plant Science. 2016;7: 1814.

[85] Shi H, Ye T, Zhong B, Liu X, Chan Z. Comparative proteomic and metabolomic analyses reveal mechanisms of improved cold stress tolerance in bermudagrass (*Cynodon dactylon* (L.) Pers.)by exogenous calcium. Journal of Integrative Plant Biology.2014;56:1064-1079.

[86] Wani MA, Jan N, Qazi HA, Andrabi KI, John R. Cold stress induces biochemical changes, fatty acid profile, antioxidant system and gene expression in Capsella bursa pastoris L. Acta Physiologie Plantarum. 2018;40:167.

[87] Mohammad rezakhani S, Hajilou J, Rezanejad F, Zaare-Nahandi F. Assessment of exogenous application of proline on antioxidant compounds in three citrus species under low temperature stress. Journal of Plant Interactions.2019;14:347-358.

[88] Spano C, Bottega S, Castiglione MR, Pedranzani HE. Antioxidant response to cold stress in two oil plants of the genus Jatropha.Plant, Soil and Environment.2017;63:271-276.

[89] Cheng F, Lu J, Gao M, Shi K, Kong Q, Huang Y, Bie Z. Redox signaling and CBF-responsive pathway are involved in salicylic acid-improved photosynthesis and growth under chilling stress in watermelon. Frontiers in Plant Science. 2016;7:1519.

[90] Wang QJ, Sun H, Dong QL, Sun TY, Jin ZX, Hao YJ, Yao YX.The enhancement of tolerance to salt and cold stresses by modifying the redox state and salicylic acid content via the cytosolic malate dehydrogenase gene in transgenic apple plants.Plant Biotechnology Journal.2016;14: 1986-1997.

[91] Han QH, Huang B, Ding CB, Zhang ZW, Chen YE, Hu C,et al. Effects of melatonin on anti-oxidative systems and photosystem II in cold-stressed rice seedlings. Frontiers in Plant Science.2017;8:785.

[92] Li W, Mo W, Ashraf U, Li G, Wen T, Abrar M,et al. Evaluation of physiological indices of waterlogging tolerance of different maize varieties in South China. Applied Ecology and Environmental Research. 2018;16: 2059-2072.

[93] Anee TI, Nahar K, Rahman A, Mahmud JA, Bhuiyan TF, Alam MU, et al. Oxidative damage and antioxidant defense in *Sesamum indicum* after different waterlogging durations. Plants. 2019;8:196.

[94] Park JS, Lee EJ. Waterlogging induced oxidative stress and the mortality of the Antarctic plant, *Deschampsia antarctica*. Journal of Ecology and Environment. 2019;43:29.

[95] Mahmud JA, Bhuyan MHMB, Anee TI, Nahar K, Fujita M, Hasanuzzaman M. Reactive oxygen species metabolism and antioxidant defense in plants under metal/ metalloid stress. In Plant Abiotic Stress Tolerance; Hasanuzzaman M, Hakeem K, Nahar K, Alharby H, Eds.; Springer: Cham, Switzerland;2019. pp. 221-257.

[96] Gratao PL, Alves LR, Lima LW. Heavy metal toxicity and plant

productivity: Role of metal scavengers. In Plant-Metal Interactions; Srivastava S, Srivastava AK, Suprasanna P, Eds.; Springer: Cham, Switzerland; 2019. pp. 49–60.

[97] Kumar S, Trivedi PK. Glutathione S-transferases: Role in combating abiotic stresses including arsenic detoxification in plants. Frontiers in Plant Science.2018;9:751.

[98] Chakravarthi S, Jessop CE, Bulleid NJ. The role of glutathione in disulphide bond formation and endoplasmic-reticulum-generated oxidative stress.EMBO Reports. 2006;7: 271–275.

[99] Petrussa E, Braidot E, Zancani M, Peresson C, Bertolini A, Patui S. et al. Plant flavonoids-biosynthesis, transport and involvement in stress responses. International Journal of Molecular Sciences.2013;14:14950-14973.

[100] Landi M, Tattini M, Gould KS. Multiple functional roles of anthocyanins in plant-environment interactions.Environmental and Experimental Botany.2015;119:4-17.

[101] Mori IC, Schroeder JI. Reactive oxygen species activation of plant Ca2þ channels.A signaling mechanism in polar growth, hormone transduction, stress signaling, hypothetically mechanotransduction.Plant Physiology. 2004;135(2): 702–708.

[102] Bhattacharjee S. ROS and Regulation of Photosynthesis. In: Reactive Oxygen Species in Plant Biology. Springer, New Delhi; 2019. pp. 107-125.https://doi.org/10.1007/978-81-322-3941-3_5.

[103] Corpas FJ, Gupta DK, Palma JM. Production sites of reactive oxygen species (ROS) in organelles from plant cells. InReactive oxygen species and oxidative damage in plants under stress. Springer, Cham; 2015. pp. 1-22. 10.1007/978-3-319-20421-5_1.

[104] Bhattacharjee S. ROS and oxidative stress: origin and implication. InReactive oxygen species in plant biology. Springer, New Delhi; 2019. pp. 1-31. https://doi.org/10.1007/978-81-322-3941-3_1.

[105] Yamauchi T, Yoshioka M, Fukazawa A, Mori H, Nishizawa NK, Tsutsumi N, et al. An NADPH Oxidase RBOH functions in rice roots during lysigenous aerenchyma formation under oxygen-deficient conditions. The Plant Cell. 2017;29:775–790. doi: 10.1105/tpc.16.00976.

[106] Gupta KJ, Igamberdiev AU. Compartmentalization of reactive oxygen species and nitric oxide production in plant cells: an overview. In: Gupta KJ, Igamberdiev AU (eds) Reactive oxygen and nitrogen species signaling and communications in plants. Springer International Publishing, Switzerland; 2015.pp. 1–14.

[107] del Rı´o LA, Corpas FJ, Sandalio LM, Palma JM, Gomez M, Barroso JB.Reactive oxygen species, antioxidant systems and nitric oxide in peroxisomes.Journal of Experimental Botany. 2002;53:1255–1272.

[108] Palma JM, Corpas FJ, del Rı´o LA. Proteome of plant peroxisomes: new perspectives on the role of these organelles in cell biology. Journal of Proteomics. 2019;9:2301–2312.

[109] Baker A, Paudyal R. The life of the peroxisome: from birth to death. Current Opinion in Plant Biology. 2014;22:39–47.

[110] Foyer CH, Noctor G. Redox sensing and signaling associated with reactive

oxygen in chloroplast, peroxysome and mitochondria. Physiologia Plantarum. 2003;119:355–364.

[111] Forman HJ, Boveris A. Free radicals in biology and medicine, vol 5. Academic Press, New York; 1982.pp. 65–90.

[112] Boveris A, Cadenas E. Mitochondrial production of superoxide anions and its relationship to the antimycin insensitive respiration. FEBS Letters. 1975;54(3):311–314.

[113] Maxwell DP, Nickels R, McIntosh L. Evidence of mitochondrial involvement in the transduction of signals required for the induction of genes associated with pathogen attack and senescence. The Plant Journal.2002; 29:269–79.

[114] Segal AW, Abo A.The biochemical basis of NADPH oxidase of phagocytes. Trends in Biochemical Sciences. 1993;18: 43–47

[115] Dvoˇrák P, Krasylenko Y, Zeiner A, Šamaj J, Takácˇ T. Signaling Toward Reactive Oxygen Species-Scavenging Enzymes in Plants. Frontiers in Plant Science. 2021;11:618-835. doi: 10.3389/ fpls.2020.618835.

[116] Kliebenstein DJ, Monde RA, Last RL. Superoxide dismutase in Arabidopsis: an eclectic enzyme family with disparate regulation and protein localization. Plant Physiology. 1998;118: 637–650. doi: 10.1104/pp.118.2.637.

[117] Pilon M, Ravet K, Tapken W. The biogenesis and physiological function of chloroplast superoxide dismutases. Biochimica et Biophysica Acta. 2011; 1807:989–998. doi:10.1016/j. bbabio.2010.11.002

[118] Feng K, Yu J, Cheng Y, Ruan M, Wang R, Ye Q, et al. The SOD Gene Family in Tomato: Identification, Phylogenetic Relationships, Expression Patterns. Frontiers in Plant Science. 2016; 7:1279. doi: 10.3389/fpls.2016.01279.

[119] Morgan MJ, Lehmann M, Schwarzländer M, Baxter CJ, Sienkiewicz-Porzucek A, Williams TC, et al. Decrease in manganese superoxide dismutase leads to reduced root growth and affects tricarboxylic acid cycle flux and mitochondrial redox homeostasis. Plant Physiology. 2008;147:101–114. doi: 10.1104/pp.107.113613.

[120] Dvoˇrák P, Krasylenko Y, Oveˇcka M, Basheer J, Zapletalová V, Šamaj J, et al. In-vivo light-sheet microscopy resolves localisation patterns of FSD1, a superoxide dismutase with function in root development and osmoprotection. Plant, Cell & Environment. 2020;44:68–87. doi: 10.1111/pce.13894

[121] Myouga F, Hosoda C, Umezawa T, Iizumi H, Kuromori T, Motohashi R, et al. A heterocomplex of iron superoxide dismutases defends chloroplast nucleoids against oxidative stress and is essential for chloroplast development in Arabidopsis. The Plant Cell. 2008;20:3148–3162. doi: 10.1105/ tpc.108.061341.

[122] Shafi A, Chauhan R, Gill T, Swarnkar MK, Sreenivasulu Y, Kumar S, et al. Expression of SOD and APX genes positively regulates secondary cell wall biosynthesis and promotes plant growth and yield in Arabidopsis under salt stress. Plant Molecular Biology. 2015;87:615–631. doi: 10.1007/s11103-015- 0301-6.

[123] Xing Y, Cao Q, Zhang Q, Qin L, Jia W, Zhang J. MKK5 regulateshigh light-induced gene expression of Cu/Zn superoxide dismutase 1 and 2 in Arabidopsis. Plant and Cell Physiology.2013;54:1217–1227. doi: 10.1093/pcp/pct072

[124] Gallie DR, Chen Z. Chloroplast-localized iron superoxide dismutases FSD2 and FSD3 are functionally distinct in Arabidopsis. PLOS One. 2019;14: e0220078. doi: 10.1371/journal.pone.0220078.

[125] Rizhsky L, Liang H, Mittler R.The water-water cycle is essential for chloroplast protection in the absence of stress. Journal of Biological Chemistry. 2003;278:38921–38925. doi: 10.1074/jbc.M304987200.

[126] Tuzet A, Rahantaniaina MS, Noctor G. Analyzing the function of catalase and the ascorbate-glutathione pathway in H2O2 processing: insights from an experimentally constrained kinetic model. Antioxidants and Redox Signaling. 2019;30:1238–1268. doi: 10.1089/ars.2018.7601.

[127] Ono M, Isono K, Sakata Y, Taji T. CATALASE2 plays a crucial role in long-term heat tolerance of *Arabidopsis thaliana*. Biochemical and Biophysical Resarch Communications.2020; 534:747–751.doi: 10.1016/j.bbrc.2020.11.006

[128] Bueso, E, Alejandro, S, Carbonell, P, Perez-Amador, M.A, Fayos, J, Bellés, J. M, et al. The lithium tolerance of the Arabidopsis cat2 mutant reveals a cross-talk between oxidative stress and ethylene. The Plant Journal. 2007;52: 1052–1065. doi: 10.1111/j.1365-313X.2007.03305.x. 40. 41.

[129] Zou JJ, Li XD, Ratnasekera D, Wang C, Liu WX, Song LF, et al. Arabidopsis Calcium-Dependent Protein Kinase8 and Catalase3 function in abscisic acid-mediated signaling and H2O2 homeostasis in stomatal guard cells under drought stress. The Plant Cell. 2015;27: 1445–1460. doi: 10.1105/tpc.15.00144.

[130] Nandi, Ankita, Liang-Jun Yan, Chandan Kumar Jana, and Nilanjana Das."Role of catalase in oxidative stress- and age-associated degenerative diseases." *Oxidative medicine and cellular longevity* 2019 .

[131] Hackenberg T, Juul T, Auzina A, Gwizdz S, Malolepszy A, Van Der Kelen K, et al. Catalase and No Catalase Activity1 promote autophagy-dependent cell death in Arabidopsis. The Plant Cell. 2013;25: 4616–4626. doi: 10.1105/tpc.113.117192

[132] Teh OK, Hofius D. Membrane trafficking and autophagy in pathogen-triggered cell death and immunity. Journal of Experimental Botany.2014;65: 1297–1312. doi: 10.1093/jxb/ert441.

[133] Yang Z, Mhamdi A, Noctor G. Analysis of catalase mutants underscores the essential role of CATALASE2 for plant growth and day length-dependent oxidative signalling. Plant Cell& Environment. 2019;42:688–700. doi: 10.1111/pce.13453.

[134] Zhang Y, Ji TT, Li TT, Tian YY, Wang LF, Liu WC. Jasmonic acid promotes leaf senescence through MYC2-mediated repression of Catalase2 expression in Arabidopsis. Plant Science. 2020;299:110-604. doi: 10.1016/j.plantsci.2020.110604.

[135] Palma, J. M, Mateos, R. M, López-Jaramillo, J, Rodríguez-Ruiz, M, González- Gordo, S, Lechuga-Sancho, A. M, et al. Plant catalases as NO and H_2S targets. Redox Biology.2020;34:101525. doi: 10.1016/j.redox.2020.101525

[136] Campa, A. Biological roles of plant peroxidases: Known and potential function. In: Peroxidases in Chemistry and Biology, eds. J. Everse KE, Everse MB, Grisham Boca Raton, FL: CRC Press; 1991. pp. 25–50.

[137] Bunkelmann JR, Trelease, RN. Ascorbate peroxidase: A prominent

membrane protein in oilseed glyoxysomes. Plant Physiology. 1996; 110: 589–598.

[138] Maruta T, Inoue T, Noshi M, Tamoi M, Yabuta Y, Yoshimura K, et al. Cytosolic ascorbate peroxidase 1 protects organelles against oxidative stress by wounding- and jasmonate-induced H_2O_2 in Arabidopsis plants. Biochimica et Biophysica Acta.2012;1820:1901–1907. doi: 10.1016/j.bbagen.2012.08.003.

[139] Maruta T, Sawa Y, Shigeoka S, Ishikawa T. Diversity and evolution of ascorbate peroxidase functions in chloroplasts: more than just a classical antioxidant enzyme? Plant and Cell Physiology.2016;57:1377–1386. doi: 10.1093/pcp/pcv203.

[140] Chew O, Whelan J, Millar AH. Molecular definition of the ascorbate-glutathione cycle in Arabidopsis mitochondria reveals dual targeting of antioxidant defenses in plants. Journal of Biological Chemistry.2003;278:46869–46877. doi: 10.1074/jbc.M307525200.

[141] Foyer CH. Noctor G. Ascorbate and glutathione: the heart of the redox hub. Plant Physiology. 2011;155:2–18. doi: 10.1104/pp.110.167569.

[142] Eltayeb AE, Kawano N, Badawi GH, Kaminaka H, Sanekata T, Shibahara T, et al. Overexpression of monodehydroascorbate reductase in transgenic tobacco confers enhanced tolerance to ozone, salt and polyethylene glycol stresses. Planta.2007;225:1255–1264. doi: 10.1007/s00425-006-0417-7

[143] Sultana S, Khew CY, Morshed MM, Namasivayam P, Napis S, Ho CL. Overexpression of monodehydroascorbate reductase from a mangrove plant (AeMDHAR) confers salt tolerance on rice. Journal of Plant Physiology. 2012;169:311–318. doi: 10.1016/j.jplph.2011.09.004.

[144] Rahantaniaina MS, Li S, Chatel-Innocenti G, Tuzet A, Issakidis-Bourguet E, Mhamdi A, et al. Cytosolic and chloroplastic DHARs cooperate in oxidative stress-driven activation of the salicylic acid pathway. Plant Physiology.2017;174:956–971. doi: 10.1104/pp.17.00317

[145] Yoshida S, Tamaoki M, Shikano T, Nakajima N, Ogawa D, IokiM, et al. Cytosolic dehydroascorbate reductase is important for ozone tolerance in *Arabidopsis thaliana*. Plant and Cell Physiology.2006;47:304–308. doi: 10.1093/pcp/pci246.

[146] Noshi M, Hatanaka R, Tanabe N, Terai Y, Maruta T, Shigeoka S. Redox regulation of ascorbate and glutathione by a chloroplastic dehydroascorbate reductase is required for high-light stress tolerance in Arabidopsis. Bioscience, Biotechnology, and Biochemistry.2016; 80:870–877. doi: 10.1080/09168451.2015.1135042.

[147] Foyer CHH, Lopez-Delgardo H, DatJF, Scott IM.Hydrogen peroxide and glutathioneassociated mechanisms of acclimatory stress tolerance and signaling. Physiologia Plantarum.1997; 100: 241–254.

[148] Akram NA, Shafiq F, Ashraf M. Ascorbic Acid-A Potential Oxidant Scavenger and Its Role in Plant Development and Abiotic Stress Tolerance. Frontiers in Plant Science.2017;8:613. doi: 10.3389/fpls.2017.00613.

[149] IgamberdievAU, Seregelyes C, Manac N,Hill RD.NADH-dependent metabolism of nitric oxide in alfalfa root cultures expressing barley hemoglobin. Planta.2004;219:95–102.doi:10.1007/s00425-003-1192-3.

[150] Agati G, AzzarelloE,PollastriS, TattiniM. Flavonoids as antioxidants in plants: location and functional significance. PlantScience.2012;196: 67–76.doi: 10.1016/j.plantsci.2012.07.014.

[151] You J,Chan Z. ROS regulation during abiotic stress responses in crop plants.Frontiers in Plant Science. 2015;6: 1092.

[152] Zhang H, Ni L, Liu Y, Wang Y, Zhang A, Tan M, et al. The C2H2-type Zinc Finger Protein ZFP182 is involved in Abscisic Acid-Induced Antioxidant Defense in Rice F. Journal of Integrative Plant Biology. 2012;54(7): 500-510.

[153] Li F, Li M, Wang P, Cox Jr KL, Duan L, Dever JK,et al. Regulation of cotton (*Gossypium hirsutum*) drought responses by mitogen-activated protein (MAP) kinase cascade-mediated phosphorylation of Gh WRKY 59. New Phytologist.2017;215(4):1462-1475.

[154] Ning J, Li X, Hicks LM, Xiong L. A Raf-like MAPKKK gene DSM1 mediates drought resistance through reactive oxygen species scavenging in rice. Plant Physiology.2010;152(2):876-890.

[155] Asano T, Hayashi N, Kobayashi M, Aoki N, Miyao A, Mitsuhara I, et al. A rice calcium-dependent protein kinase OsCPK12 oppositely modulates salt-stress tolerance and blast disease resistance. The Plant Journal. 2012;69: 26–36.

[156] Deng X, Hu W, Wei S, Zhou S, Zhang F, Han J, et al. TaCIPK29, a CBL-interacting protein kinase gene from wheat, confers salt stress tolerance in transgenic tobacco. PLOS One.2013;8: e69881.

[157] Hu DG, Ma QJ, Sun CH, Sun MH, You CX, Hao YJ. Overexpression of MdSOS2L1, a CIPK protein kinase, increases the antioxidant metabolites to enhance salt tolerance in apple and tomato. Physiologia Plantarum.2015;156 (2):201-214.

[158] You J, Zong W, Hu H, Li X, Xiao J, Xiong L. A Stress-responsive NaC1-regulated protein phosphatase gene rice protein phosphatase18 modulates drought and oxidative stress tolerance through abscisic acid-independent reactive oxygen species scavenging in rice. Plant Physiology.2014;166(4): 2100-2114.

[159] Schmidt R, Mieulet D, Hubberten HM, Obata T, Hoefgen R, Fernie AR, et al. Salt-Responsive ERF1 regulates reactive oxygen species–dependent signaling during the initial response to salt stress in rice. The Plant Cell.2013;25(6):2115-2131.

[160] Fukao T, Yeung E, Bailey-Serres J. The submergence tolerance regulator SUB1A mediates crosstalk between submergence and drought tolerance in rice. The Plant Cell. 2011;23(1):412-427.

[161] Wu L, Zhang Z, Zhang H, Wang XC, Huang R. Transcriptional modulation of ethylene response factor protein JERF3 in the oxidative stress response enhances tolerance of tobacco seedlings to salt, drought, freezing. Plant Physiology.2008;148(4):1953-1963.

[162] Huang XY, Chao DY, Gao JP, Zhu MZ, Shi M, Lin HX. A previously unknown zinc finger protein, DST, regulates drought and salt tolerance in rice via stomatal aperture control. Genes & Development.2009;23(15):1805-1817.

[163] Zhang H, Liu Y, Wen F, Yao D, Wang L, Guo J, et al. A novel rice C2H2-type zinc finger protein, ZFP36, is a key player involved in abscisic acid-induced antioxidant defence and oxidative stress

tolerance in rice. Journal of Experimental Botany.2014;65(20):5795-5809.

[164] Jan A, Maruyama K, Todaka D, Kidokoro S, Abo M, Yoshimura E, et al. OsTZF1, a CCCH-tandem zinc finger protein, confers delayed senescence and stress tolerance in rice by regulating stress-related genes. Plant Physiology.2013;161(3):1202-1216.

[165] Wang F, Chen HW, Li QT, Wei W, Li W, Zhang WK, et al. Gm WRKY 27 interacts with Gm MYB 174 to reduce expression of Gm NAC 29 for stress tolerance in soybean plants. The Plant Journal.2015;83(2):224-236.

[166] Fang Y, Liao K, Du H, Xu Y, Song H, Li X, et al.A stress-responsive NAC transcription factor SNAC3 confers heat and drought tolerance through modulation of reactive oxygen species in rice. Journal of Experimental Botany.2015;66(21):6803-6817.

[167] Yan Y, Jia H, Wang F, Wang C, Liu S, Guo X. Overexpression of GhWRKY27a reduces tolerance to drought stress and resistance to *Rhizoctonia solani* infection in transgenic *Nicotiana benthamiana*. Frontiers in Physiology. 2015;6:265.

[168] You J, Hu H, Xiong L.An ornithine δ-aminotransferase gene OsOAT confers drought and oxidative stress tolerance in rice. Plant Science. 2012;197:59-69.

[169] Du H, Wang N, Cui F, Li X, Xiao J, Xiong L. Characterization of the β-carotene hydroxylase gene DSM2 conferring drought and oxidative stress resistance by increasing xanthophylls and abscisic acid synthesis in rice.Plant Physiology.2010;154(3):1304-1318.

[170] Zhang Y, Tan J, Guo Z, Lu S, He S, Shu W, et al. Increased abscisic acid levels in transgenic tobacco over-expressing 9 cis-epoxycarotenoiddioxygenase influence H_2O_2 and NO production and antioxidant defences.Plant, Cell & Environment. 2009; 32(5), 509-519.

[171] McKersie BD, Bowley SR, Harjanto E, Leprince O. Water-deficit tolerance and field performance of transgenic alfalfa overexpressing superoxide dismutase. Plant Physiology.1996;111(4):1177-1181.

[172] Zhang Z, Zhang Q, Wu J, Zheng X, Zheng S, Sun X, et al. Gene knockout study reveals that cytosolic ascorbate peroxidase 2 (OsAPX2) plays a critical role in growth and reproduction in rice under drought, salt and cold stresses. PLOS One.2013;8(2): e57472.

[173] Huda KM, Banu MS, Garg B, Tula S, Tuteja R, Tuteja N. OsACA6, a P-type IIB Ca2+ ATPase promotes salinity and drought stress tolerance in tobacco by ROS scavenging and enhancing the expression of stress-responsive genes. The Plant Journal. 2013;76:997–1015

[174] Kalaivani N, Hasidah MS.The green MAPKs. Asian Journal of Plant Sciences. 2010;9(1):1-10.

[175] Lu W, Chu X, Li Y, Wang C, Guo X. Cotton GhMKK1 induces the tolerance of salt and drought stress, mediates defence responses to pathogen infection in transgenic *Nicotiana benthamiana*. PLoS One.2013;8(7):e68503.

[176] Zhang L, Li Y, Lu W, Meng F, Wu CA, Guo X. Cotton GhMKK5 affects disease resistance, induces HR-like cell death, reduces the tolerance to salt and drought stress in transgenic *Nicotiana benthamiana*. Journal of Experimental Botany. 2012a;63(10):3935-3951.

[177] Wang C, Lu W, He X, Wang F, Zhou Y, Guo X,Guo X. The cotton

mitogen-activated protein kinase kinase 3 functions in drought tolerance by regulating stomatal responses and root growth. Plant and Cell Physiology.2016; 57(8):1629-1642.

[178] Danquah A, de Zélicourt A, Boudsocq M, Neubauer J, Freidit Frey N, Leonhardt N, et al.Identification and characterization of an ABA-activated MAP kinase cascade in Arabidopsis thaliana. The Plant Journal.2015;82(2): 232-244.

[179] Ashraf MA, Akbar A, Parveen A, Rasheed R, Hussain I, Iqbal M. Phenological application of selenium differentially improves growth, oxidative defense and ion homeostasis in maize under salinity stress. Plant Physiology and Biochemistry. 2018;123: 268-280.

[180] Porcel R, Redondo-Gómez S, Mateos-Naranjo E, Aroca R, Garcia R, Ruiz-Lozano JM. Arbuscular mycorrhizal symbiosis ameliorates the optimum quantum yield of photosystem II and reduces non-photochemical quenching in rice plants subjected to salt stress. Journal of Plant Physiology. 2015;185: 75-83.

[181] Orhan F. Alleviation of salt stress by halotolerant and halophilic plant growth-promoting bacteria in wheat (*Triticum aestivum*). Brazilian Journal of Microbiology. 2016;47(3): 621-627.

[182] Sajyan TK, Naim L, Sebaaly Z, Rizkallah J, Shaban N, Sassine YN. Alleviating the adverse effects of salinity stress on tomato crop (*Solanum lycopersicum*) using nano-fertilizer as foliar application. In XXX International Horticultural Congress IHC2018: International Symposium on Water and Nutrient Relations and Management of 1253; 2018. pp. 33-40.

[183] Asrar AWA, Elhindi KM. Alleviation of drought stress of marigold (*Tagetes erecta*) plants by using arbuscular mycorrhizal fungi. Saudi Journal of Biological Sciences. 2011;18 (1):93-98.

[184] Kour D, Rana, KL, Kaur, T, Sheikh, I, Yadav, AN, Kumar, et al. Microbe-mediated alleviation of drought stress and acquisition of phosphorus in great millet (*Sorghum bicolour* L.) by drought-adaptive and phosphorus-solubilizing microbes. Biocatalysis and Agricultural Biotechnology. 2020;23:101501.

[185] Gagné-Bourque F, Bertrand A, Claessens A, Aliferis KA,Jabaji S. Alleviation of drought stress and metabolic changes in timothy (*Phleum pratense* L.) colonized with *Bacillus subtilis* B26. Frontiers in Plant Science. 2016;7:584.

[186] Ouzounidou G, Giannakoula A, Ilias I, Zamanidis P. Alleviation of drought and salinity stresses on growth, physiology, biochemistry and quality of two *Cucumis sativus* L. cultivars by Si application. Brazilian Journal of Botany. 2016;39(2):531-539.

[187] Barnawal D, Bharti N, Maji D, Chanotiya CS, Kalra A. 1-Aminocyclopropane-1-carboxylic acid (ACC) deaminase-containing rhizobacteria protect *Ocimum sanctum* plants during waterlogging stress via reduced ethylene generation. Plant Physiology and Biochemistry. 2012;58: 227-235.

[188] Iqbal Z, Sarkhosh A, Balal RM, Gómez C, Zubair M, Ilyas N, et al. Silicon alleviate hypoxia stress by improving enzymatic and non-enzymatic antioxidants and regulating nutrient uptake in muscadine grape (*Muscadinia rotundifolia* Michx.). Frontiers in Plant Science. 2020;11.

[189] Ali MM, Waleed Shafique M, Gull S, Afzal Naveed W, Javed T, Yousef AF et al. Alleviation of Heat Stress in Tomato by Exogenous Application of Sulfur. Horticulturae. 2021;7(2):21.

[190] Sarwar M, Saleem MF, Ullah N, Ali S, Rizwan M, Shahid MR, et al. Role of mineral nutrition in alleviation of heat stress in cotton plants grown in glasshouse and field conditions. Scientific Reports. 2019;9(1):1-17.

[191] Khan MA, Asaf S, Khan AL, Jan R, Kang SM, Kim KM, et al. Thermotolerance effect of plant growth-promoting Bacillus cereus SA1 on soybean during heat stress. BMC Microbiology. 2020;20(1):1-14.

[192] Da Jeong Shin SJY, Hong JK, Weon HY, Song J, Sang MK. Effect of *Bacillus aryabhattai* H26-2 and *B. siamensis* H30-3 on Growth Promotion and Alleviation of Heat and Drought Stresses in Chinese Cabbage. The Plant Pathology Journal. 2019;35(2):178.

[193] Abass, S. M. and Mohamed, H. I. (2011).Alleviation of adverse effects of drought stress on common bean (Phaseolus vulgaris L.) by exogenous application of hydrogen peroxide. Bangladesh Journal of Botany, 40(1), 75-83.

[194] Saikia J, Sarma RK, Dhandia R, Yadav A, Bharali R, Gupta VK,et al. Alleviation of drought stress in pulse crops with ACC deaminase producing rhizobacteria isolated from acidic soil of Northeast India. Scientific reports. 2018; 8(1):1-16.

[195] Mishra PK, Bisht SC, Ruwari P, Selvakumar G, Joshi GK, Bisht J.K, et al. Alleviation of cold stress in inoculated wheat (*Triticum aestivum* L.) seedlings with psychrotolerant Pseudomonads from NW Himalayas. Archives of microbiology. 2011;193(7):497-513.

[196] Ma J, Janouskova M, Ye L, BaiLQ, He CX.Role of arbuscular mycorrhiza in alleviating the effect of cold on the photosynthesis of cucumber seedlings. Photosynthetica. 2019;57(1):86-95.

[197] Ahmad P, Nabi G, Ashraf M. Cadmium-induced oxidative damage in mustard [*Brassica juncea* (L.)Czern.& Coss. plants can be alleviated by salicylic acid. South African Journal of Botany. 2011;77(1):36-44.

[198] Ali S, Chaudhary A, Rizwan M, Anwar HT, Adrees M, Farid M, et al. Alleviation of chromium toxicity by glycinebetaine is related to elevated antioxidant enzymes and suppressed chromium uptake and oxidative stress in wheat (*Triticum aestivum* L.). Environmental Science and Pollution Research. 2015;22(14):10669-10678.

[199] Chen Z, Zhang L, Zhu C. Exogenous nitric oxide mediates alleviation of mercury toxicity by promoting auxin transport in roots or preventing oxidative stress in leaves of rice seedlings. Acta Physiologiae Plantarum. 2105;37(9):1-9.

[200] Surapu V, Ediga A, Meriga B. Salicylic acid alleviates aluminum toxicity in tomato seedlings (*Lycopersicum esculentum* Mill.) through activation of antioxidant defense system and proline biosynthesis. Advances in Bioscience and Biotechnology. 2014;5: 777.

[201] Yang X, Li H, Yang Y, Wang Y, Mo Y, Zhang R, et al. Identification and expression analyses of WRKY genes reveal their involvement in growth and abiotic stress response in watermelon (*Citrullus lanatus*). PlOS One. 2018;13 (1):e0191308.

[202] Khan N, Fatima F, Haider MS, Shazadee H, Liu Z, Zheng, et al.Genome-wide identification and expression profiling of the polygalacturonase (PG) and pectin methylesterase (PME) genes in grapevine (*Vitis vinifera* L.). International Journal of Molecular Sciences. 2019;20(13):3180.

[203] Xue T, Wang D, Zhang S, Ehlting J, Ni F, Jakab S, et al. Genome-wide and expression analysis of protein phosphatase 2C in rice and Arabidopsis. BMC Genomics.2008;9(1):1-21.

[204] Singh A, Giri J, Kapoor S, Tyagi AK, Pandey GK. Protein phosphatase complement in rice: genome-wide identification and transcriptional analysis under abiotic stress conditions and reproductive development. BMC Genomics.2010;11(1):1-18.

[205] Fuchs S, Grill E, Meskiene I, Schweighofer A. Type 2C protein phosphatases in plants. The FEBS Journal.2013;280(2):681-693.

[206] Joshi R, Wani SH, Singh B, Bohra A, Dar ZA, Lone AA, et al. Transcription factors and plants response to drought stress: current understanding and future directions. Frontiers in Plant Science. 2016;7:1029.

[207] Kimotho RN, Baillo EH, and Zhang Z. Transcription factors involved in abiotic stress responses in Maize (*Zea mays* L.) and their roles in enhanced productivity in the post genomics era. Peer J. 2019;7:e7211.

[208] Nadarajah KK.ROS homeostasis in abiotic stress tolerance in plants. International Journal of Molecular Sciences.2020;21(15):5208.

[209] Yu S, Zhang L, Chen C, Li J, Ye S, Liu G, et al. Isolation and characterization of BnMKK1 responsive to multiple stresses and affecting plant architecture in tobacco. Acta Physiologiae Plantarum.2014a;36(6): 1313-1324.

[210] Ariel FD, Manavella PA, Dezar CA, Chan RL.The true story of the HD-Zip family.Trends in Plant Science.2007;12 (9):419-426.

[211] Fang Y, You J, Xie K, Xie W, Xiong L. Systematic sequence analysis and identification of tissue-specific or stress-responsive genes of NAC transcription factor family in rice. Molecular Genetics and Genomics.2008; 280(6):547-563.

[212] Ciftci-Yilmaz S, and Mittler R.The zinc finger network of plants.Cellular and Molecular Life Sciences.2008;65(7): 1150-1160.

[213] Fukao T, Xiong L. Genetic mechanisms conferring adaptation to submergence and drought in rice: simple or complex?.Current Opinion in Plant Biology.2013;16(2):196-204.

[214] Yeung E, van Veen H, Vashisht D, Paiva ALS, Hummel M, Rankenberg T, et al. A stress recovery signaling network for enhanced flooding tolerance in *Arabidopsis thaliana*.Proceedings of the National Academy of Sciences.2018;115 (26):E6085-E6094.

[215] Mittler R, Kim Y, Song L, Coutu J, Coutu A, Ciftci-Yilmaz S, et al. Gain- and loss-of-function mutations in Zat10 enhance the tolerance of plants to abiotic stress. FEBS Letters. 2006;580:6537–6542.

[216] Sun SJ, Guo SQ, Yang X, Bao YM, Tang HJ, Sun H, et al. Functional analysis of a novel Cys2/His2-type zinc finger protein involved in salt tolerance in rice. Journal of Experimental Botany.2010;61 (10):2807-2818.

[217] Riechmann JL,Ratcliffe OJ.A genomic perspective on plant transcription factors.Current Opinion in Plant Biology.2000;3(5):423-434.

[218] Nuruzzaman M, Manimekalai R, Sharoni AM, Satoh K, Kondoh H, Ooka H et al. Genome-wide analysis of NAC transcription factor family in rice. Gene.2010;465(1-2): 30-44.

[219] Jin H, Huang F, Cheng H, Song H, Yu D. Overexpression of the GmNAC2 gene, an NAC transcription factor, reduces abiotic stress tolerance in tobacco. Plant Molecular Biology Reporter.2013;31(2):435-442.

[220] Wu KL, Guo ZJ, Wang HH, Li J.The WRKY family of transcription factors in rice and Arabidopsis and their origins. DNA Research.2015;12(1):9-26.

[221] Ning P, Liu C, Kang J, LvJ. Genome-wide analysis of WRKY transcription factors in wheat (*Triticum aestivum* L.) and differential expression under water deficit condition. PeerJ.2017;5:e3232.

[222] Shukla D, Huda KMK, Banu MSA, Gill SS, Tuteja R, Tuteja N. OsACA6, a P-type 2B Ca^{2+} ATPase functions in cadmium stress tolerance in tobacco by reducing the oxidative stress load. Planta.2014;240(4):809-824.

Chapter 13

Reducing Compounds Roles in Oxidative Stress Relieving of Human Red Blood Cells

Giuseppe Gallo

Abstract

Oxidative stress is the consequence of an imbalance between pro-oxidant and antioxidant processes. Antioxidants that counteract reactive oxygen species do not all work the same way. Both resveratrol and the more powerful 4-hydroxytyrosol are excellent reducing agents. Polyphenol treatment (red wine polyphenols, resveratrol and catechin) is associated with a significant increase in anion permeability for chloride compared with control and 2.2′-azobis-2 amidinopropan dihydrochloride affected cells. Treatment with polyphenols was associated with a significant reduction in mean ± standard error of the mean membrane lipid peroxidation compared with control and 2.2′-azobis-2 amidinopropan dihydrochloride treatment. Hemolysis data are also obtained in the previously described conditions. 4-hydroxytyrosol is shown to significantly protect red blood cells from oxidative damage by 4-hydroxynonenal. But there are paradoxical effects like uric acid and creatinine. The obtained data evidence that both creatinine and uric acid levels have influence on the ratio of both malondialdehyde/protein and 4-hydroxynonenal/protein content on red blood cell ghosts, demonstrating their possible protective role against oxidative stress at low concentrations in blood and oxidizing power at higher concentrations. Finally, polyunsaturated fatty acids do not have all this reducing power.

Keywords: reactive oxygen species, resveratrol, 4-hydroxytyrosol, uric acid, creatinine, polyunsaturated fatty acids

1. Introduction

Chemical stress induced by the presence, in a living organism, of an excess of reactive chemical species, generally centered on oxygen (reactive oxygen species), secondary to an increased production of the same and / or to a reduced efficiency of the physiological systems of antioxidant defense. There are many antioxidants, among these there is resveratrol. The history of resveratrol (the *red wine* and pomegranate juice are rich in this substance, chemically it is a triidrostilbene "a derivative stilbenic", synthesized from the vine of red grapes.) 10 20 times more potent than Vitamin E and Glutathione is the most powerful of those produced by the body (prevents arteriosclerosis, cancer and recently even Alzheimer's and delays the appearance

of the characteristic signs of old age and can extend life by as much as 30%.). Another powerful antioxidant is hydroxytyrosol. The hydroxytyrosol (1- (2-hydroxy) ethyl-3,4-dihydroxybenzene) is classified as a phytochemical compound expressing strong antioxidant properties. The index ORAC (Oxygen Radical Absorbance Capacity, or the oxygen radical absorption capacity) for hydroxytyrosol totaled 40,000 μmolTE / g, about ten times greater than green tea at least twice compared to CoQ_{10}. In nature, hydroxytyrosol is found in high concentrations in olive vegetation water and also in lower concentrations in the olive leaf. Olives, leaves and olive pulp contain much higher amounts of hydroxytyrosol compared to olive oil. One study showed that a low dose of hydroxytyrosol in rats reduces the consequences of the side effects of oxidative stress induced by smoking [1]. Among the paradoxical antioxidants is creatinine. Creatinine (from the greek κρέας, Kreas, "flesh") is an intermediate of energy metabolism synthesized by the liver about (1 g / day) from arginine, S-adenosyl methionine and glycine and is used in mammalian muscles to regenerate ATP during the first seconds of muscle contraction. Recent reports showed that creatinine displays antioxidant activity which could explain its beneficial cellular effects. Some authors [2] have evaluated the ability of creatinine to protect human erythrocytes and lymphocytes against oxidative damage. Erythrocytes were challenged with model oxidants, 2, 2′-azobis (2-amidinopropane) dihydrochloride (AAPH) and hydrogen peroxide (H_2O_2) both in the presence and absence of creatinine. Incubation of erythrocytes with oxidants alone increased hemolysis, met-hemoglobin levels, lipid peroxidation [3] and protein carbonyl content. This was accompanied by decrease in glutathione cellular levels. Antioxidant enzymes and antioxidant power of the cell were compromised while the activity of membrane bound scavenger enzymes were lowered [2]. In a recent paper [4] authors demonstrated that hypertriglyceridemia affects Red Blood Cell (RBC) membrane oxidative status (increase of [malondialdehyde (MDA)] and [4-hydroxynonenal (HNE)] to membrane protein ratio) is increased by high levels of blood cholesterol, but selectively by hypertriglyceridemia [4, 5]. It is known that creatinine protects the erythrocytes by attenuating the AAPH and H_2O_2 induced alterations [5]. This protective effect was confirmed by electron microscopic analysis which showed that oxidant-induced cell damage was attenuated by creatinine. Thus, creatinine can function as a blood antioxidant, protecting cells from oxidative damage, genotoxicity and can potentially increase their lifespan. Another paradoxical antioxidant is uric acid. Uric acid is an organic molecule of natural origin. Urate is formed as a byproduct in living mammalian organisms higher in purine metabolism. It belongs to the group of oxypurines and is formed from a pyrimidine ring (α) condensed with an imidazole ring (β). Uric acid, despite being a major antioxidant in the human plasma, both correlates and predicts development of obesity, hypertension and cardiovascular disease, conditions associated with oxidative stress [6]. While one explanation for this paradox could be that a rise in uric acid concentration represents an attempted protective response by the host, we review the evidence that uric acid may function either as an antioxidant (primarily in plasma) or pro-oxidant (primarily within the cell). We suggest that it is the pro-oxidative effect of uric acid, that occurs in cardiovascular disease and may have a contributory role in the pathogenesis of this condition. Then finally there are the Polyunsaturated fatty acids. Polyunsaturated fatty acids (PUFAs) are fatty acids that contain more than one double bond in their backbone. This class includes many important compounds, such as essential fatty acids and those that give drying oils their characteristic property. Fatty acid composition and susceptibility to lipid peroxidation induced in vitro by (AAPH) were evaluated in human RBC membranes on days 30 and 180. n-3 PUFA treatment increased

eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) concentrations in RBC membranes in a time-dependent manner in all of the n-3 PUFA groups. These modifications occurred with concomitant dose- and time-dependent increases in the membrane unsaturation index. After 30 d of treatment with n-3 PUFAs, significantly increased in RBC membranes of the intermediate- and high-dose groups. Because of the higher concentration of this antioxidant in these groups, the susceptibility of RBC membranes to peroxidation was decreased. However, after 180 d of treatment, to baseline values and AAPH-induced lipid peroxidation increased in a dose-dependent manner. These results show that high doses of dietary n-3 PUFAs, as well as long-time treatments, affect human RBC susceptibility to lipid peroxidation by changes in fatty acid composition content [7].

2. Study on powerful antioxidants

2.1 Study on resveratrol

The reducing agent treatment efficacy was observed by evaluation of anion permeability for chloride, lipid peroxidation and hemolysis in RBCs. Anion permeability for chloride is an indicator of membrane protein damage and is evaluated in RBCs by the specific absorption of methemoglobin (C_M) at 590 and 635 nm after treatment of heparinized blood with $NaNO_2$. The measurement of the membrane lipid degradation is obtained by the determination of MDA. The lipid peroxidation susceptibility is observed after the oxidative stress induced by AAPH. The hemolysis assays are conducted on blood samples in phosphate buffer saline. To evaluate in human RBC the *in vitro* effects of AAPH and the antioxidant activity of polyphenols from red wine (resveratrol, catechin and naturally red wine), authors start the research evaluating RBC anion permeability for chloride. **Figure 1** shows the values of membrane anion permeability for chloride in both groups (controls and AAPH-treated RBCs). The statistical analysis of data shows that AAPH *in vitro* treatment lowers the parameter in comparison to all controls. Furthermore, the reducing agent treatment (T2-T4) also significantly recovers the activity up to values higher than AAPH treatment. The recovery of anion permeability for chloride is always lower than control data. Anion permeability for chloride values were slightly higher after red wine and resveratrol treatments (as reducing agents) in both experimental groups (controls and AAPH-treated RBCs), corresponding to a preserved architecture and anion permeability for chloride of the human erythrocyte membranes. To confirm the efficacy of oxidant and reducing treatments on human RBC *in vitro* authors evaluated lipid peroxidation, hemolysis and RBC morphology. In **Figure 2**, MDA levels are described in membranes from several varieties of treated and untreated RBC with AAPH, under the action of reducing agents or without treatment. On the contrary, as described in **Figure 2**, AAPH-induced increases of MDA levels are partially recovered by natural reducing agents at levels almost comparable to control the group. C2-C4 results always slow down MDA levels in RBC membranes, but catechin is less effective. Red wine, among them, is almost as powerful as resveratrol alone in both groups (controls and AAPH-treated RBCs). As shown in **Figure 3** time courses of RBC hemolyses are described at several incubation times (60, 120, 180 and 240 min) either under the action of natural oxidative pathways or by treatment with radical donor AAPH (60 mM). This experiment shows also the natural reducing agents partially recover the effect of both oxidative and reducing agent treatments. As described in all experiments, AAPH-

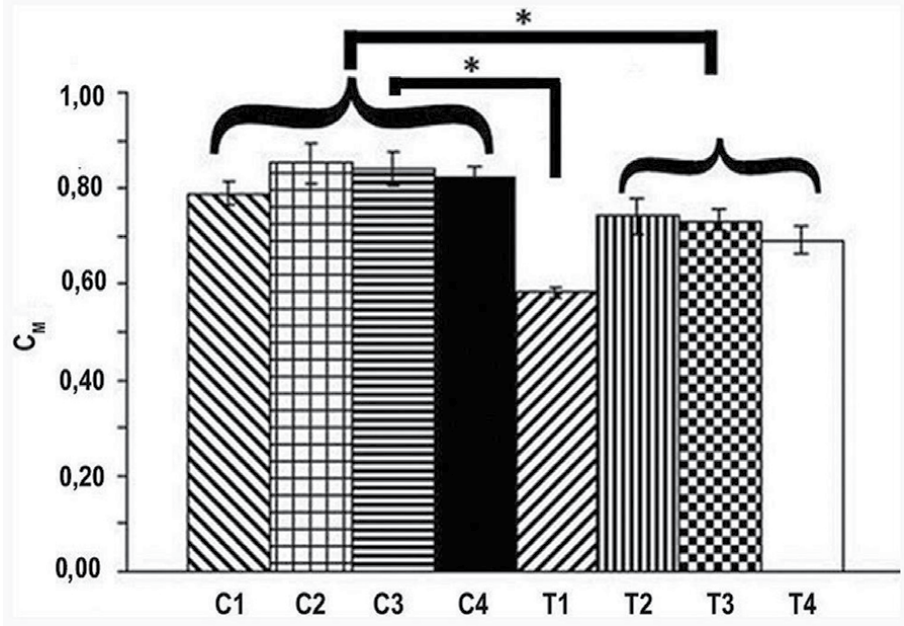


Figure 1.
*RBC membrane anion permeability for chloride. Data are mean ± standard error of the mean on 10 determinations. *P < 0.05 according to one-way analysis of variance and Bonferroni* post-hoc *test coupled data at each time were performed. C = control sample, T = treated sample [8] C1 = control; C2 = control + red wine 5, 2 mM (reducing power equivalents of gallic acid); C3 = control + resveratrol 5 μM; C4 = control + Catechin 50 μM; T1 = AAPH 60 mM; T2 = AAPH 60 mM + red wine 5,2 mM (reducing power equivalents of gallic acid); T3 = AAPH 60 mM + resveratrol 5 μM; T4 = AAPH 60 mM+ Catechin 50 μM.*

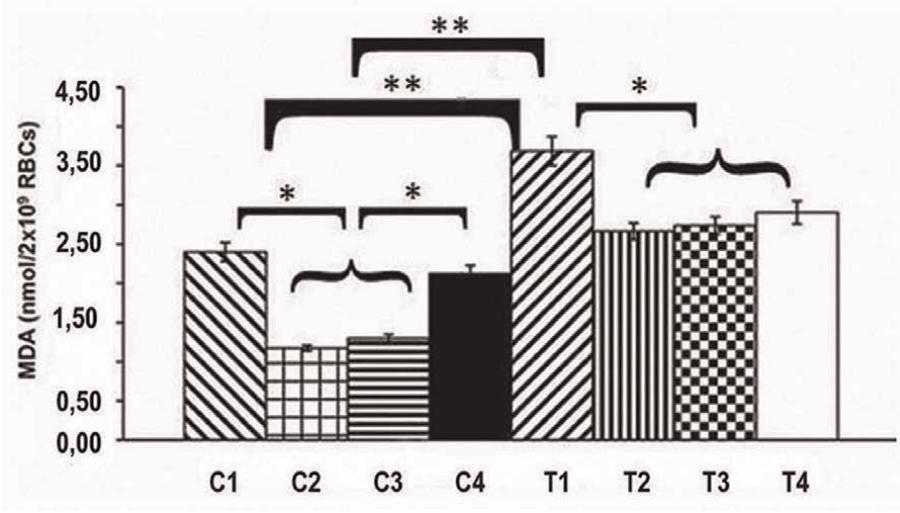


Figure 2.
*Effects of lipid peroxidation and induced by 2.2′-azobis-2 amidinopropan dihydrochloride (60 mM) (*t = 60 *min) on the malonyldialdehyde levels of human RBC membranes. Data are mean ± standard error of the mean on 10 determinations. Column labels are according to *P < 0.05 and **P < 0.01 according to one-way analysis of variance and Bonferroni* post-hoc *test for coupled data at each time were performed (**Figure 1**).*

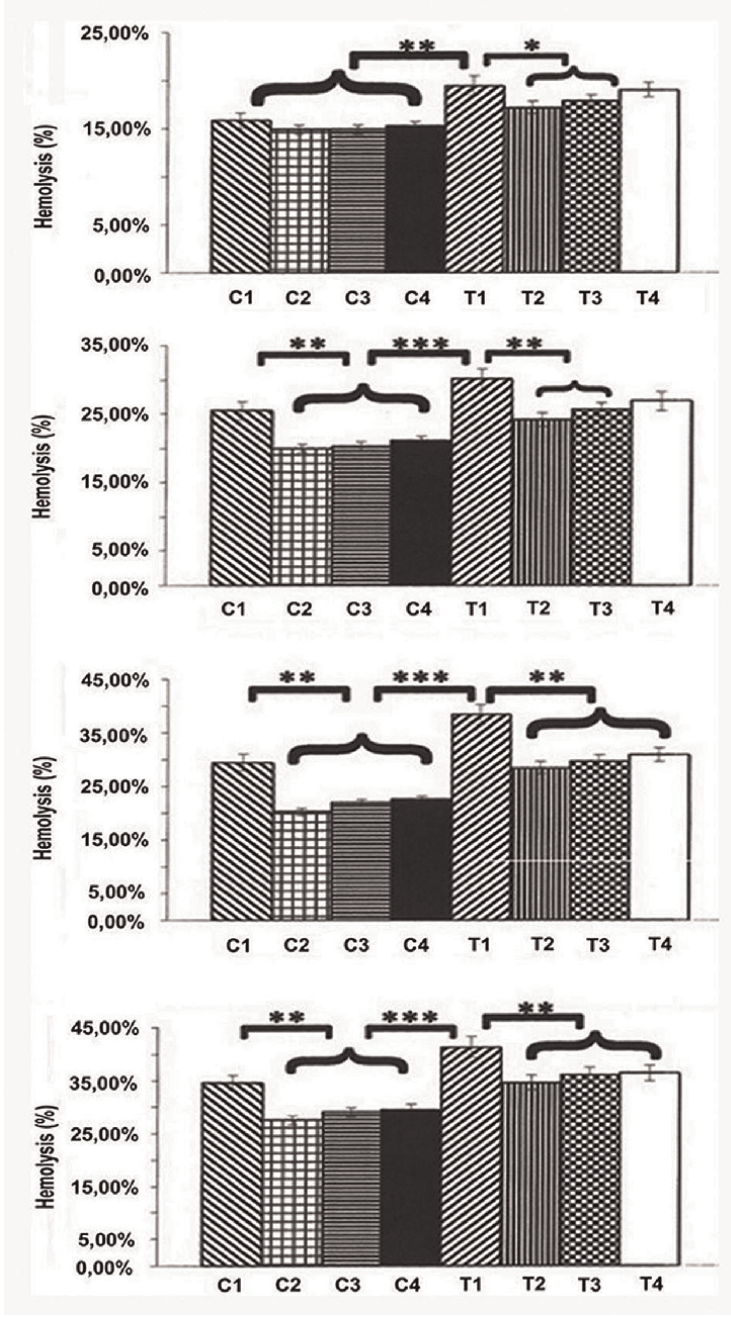


Figure 3.
Effect of 2.2′-azobis-2 amidinopropan dihydrochloride-induced hemolysis in sportive human RBCs at several times. Data are mean ± standard error of the mean on 10 determinations. Column labels are according to incubation times a = (60 min); b = (120 min); c = (180 min); d = (240 min). *P < 0.05, ** P < 0.01 *and* *** P < 0,001 *according to according to one-way analysis of variance and Bonferroni* post-hoc *test for coupled data at each time were performed (**Figure 1**). C = Control sample, T = Treated sample (Gallo et al., 2013) C1 = Control; C2 = Control + Red Wine 5, 2 mM (reducing power equivalents of gallic acid); C3 = Control +Resveratrol 5 μM; C4 = Control + Catechin 50 μM; T1 = AAPH 60 mM; T2 = AAPH 60 mM + Red Wine 5,2 mM (reducing power equivalents of gallic acid); T3 = AAPH 60 mM + Resveratrol 5 μM; T4 = AAPH 60 mM+ Catechin 50 μM. Of course C1 and T1 are a = (60 min). And so on, saying the others.*

induced hemolysis is more relevant than in other treated samples. As described in previous experiments (**Figures 1** and **2**) resveratrol and red wine polyphenol treatments are more efficient than catechin in preserving RBC membrane structural recovery at 60 and 120 min of incubation. In the present study, polyphenol (resveratrol and red wine) treatment is associated with an improvement in erythrocyte anion permeability for chloride-related with cell membrane derangements. The cell system exchange of Cl^- for HCO_3^- represents a target of membrane oxidative damage and is evaluated in RBCs by the specific C_M at 590 and 635 nm after treatment of heparinized blood with $NaNO_2$. According to our research, the in vitro action model of artificial agent AAPH on RBC plasma membranes shows that anion permeability for chloride (evaluated by C_M [9]) decreases after treatment and is partially recovered after administration of either resveratrol or red wine polyphenols extracts. Our data confirm those on LDL peroxidation [10] with AAPH by MDA, hemolysis and RBC morphology study from our laboratory [8, 11]. Moreover in the present work all previous results are compared, so that a relationship among them is evident and correlate each other, so that the action of red wine polyphenol extracts are demonstrated as effective reducing agents both alone and in mixture. MDA, hemolysis and RBC morphological study confirmed the resveratrol and red wine efficacy on improvement of RBC membrane integrity against in vitro oxidative stress damages. The described damages could be linked to the erythrocyte membrane framework, so a lack of adequate lipid organization can significantly influence ion permeability. The alteration of membrane fluidity consequent to lipid oxidation represented by MDA level increase can reflect the change of the structure and function of membrane lipids and proteins. The permeability is another important property of erythrocyte membrane. Depending on the exchanger ($Cl^- \leftrightarrow HCO_3^-$), that transports anions across the membrane, it is related to the framework of erythrocyte membrane. To sum up, the alteration of anion permeability can reflect the change of erythrocyte membrane protein framework. Moreover, our results could describe the overall effect of the three described alterations together.

2.2 Study on 4-hydroxytyrosol

The assay (ALDetectTM *Lipid Peroxidation* assay kit**)** is designed to measure either MDA alone (in hydrochloric acid) or MDA in combination with 4-hydroxyalkenals (in methanesulfonic acid).

So we have TOT = MDA + HNE and then TOT-MDA = HNE. The assay is based on the reaction of a chromogenic reagent, N-methyl-2-phenylindole (R1) with MDA and 4-hydroxyalkenals to yield a stable chromophore with maximal absorbance at 586 nm at 45°C. One molecule of either MDA or 4-hydroxyalkenal reacts with 2 molecules of reagent R1 to yield a stable chromophore with maximal absorbance at 586 nm. For simultaneous determination of MDA and 4-hydroxyalkenals, one must use the procedure utilizing methanesulfonic acid (MSA) as the acid solvent. The procedure in which HCl is used will only detect MDA, since the 4-hydroxyalkenals do not form a chromophore with reagent R1 under those conditions. Interestingly, in addition to the 586 nm chromophore, 4-hydroxyalkenals gave a second chromophore with maximal absorbance at 505 nm and alkanals produced a single chromophore with maximal absorbance at 505 nm. The concentration of reducing agent is chosen according to literature data [12]. Reducing agent is provided from Sigma Chemical Co. (St. Louis, MO, USA) and used at the final concentration of 80 μM for 3 h at 37°C.In vitro 4-hydroxytyrosol treatment to evaluate the membrane lipids reducible by this phenol.

As described in **Figure 4a** and **b** the level of oxidation products of membrane lipids are referred as μM of MDA or HNE. MDA levels are always ten times those of HNE as referred in right-hand scale. In **Figure 4a** and **b** the scales are quite different (for instance: 0.00 /0.85 in A and 0.000/0.033 in B for MDA). We can also observe that MDA levels of pathological samples are slightly higher than control ones but HNE levels of pathological samples are four times those of control ones. After the 4-hydroxytyrosol action both lipid metabolites substantially decrease. The mean values of total membrane protein levels in our casuistry are described in **Figure 4**. It is evident that for the two dosed substances each group of data is significantly different ($P < 0.001$) from the other ones. In **Figure 5** are reported the total levels of MDA + HNE per milligram of total membrane proteins of RBC ghosts. As described the total oxidized lipid levels in RBC ghost membranes almost doubled in pathological samples in comparison to control ones. This shows that even if total membrane proteins dramatically increase in hypertriglyceridemic patients, also total oxidized lipids sufficiently increase in such samples so that ratio between patients RBC oxidized lipids to membrane proteins is always doubled. Such partial compensation of lipids in comparison to proteins from RBC membrane could be explained as an attempt of RBC metabolism to partially compensate ghost structure damages by oxidative stress. The ratio of total oxidized lipids to membrane proteins before and after 4-hydroxytyrosol treatment are referred in **Figure 5**. After 4-hydroxytyrosol treatment, the ratio of oxidized lipids to total membrane proteins decreases even more, but the values in our controls are always lower than in hypertriglyceridemic patients. The mean values of ratio of oxidized lipids (MDA + HNE) and membrane proteins in our casuistry are described in **Figure 5**. It is evident that the two groups of data are significantly different ($P < 0.001$). Neither Vitamin E nor Vitamin C are used because their ultraviolet–visible (UV–Vis) spectra superimpose to HNE determination method interfering with their evaluation. The structures most exposed to the damaging action of radicals are the lipid structures constituting the cellular and nuclear membranes that are subjected to destructuring. One of the most sensitive sites to damage caused by ROS is the plasma membrane, in particular the target is at the level of polyunsaturated fatty acids. The oxidation by ROS of lipids is called peroxidation. Alkenals are products of lipid peroxidation while hydroxytyrosol is a powerful reducing agent. This explains the decrease in the parameters of alkenals.

According to our data in RBC, ghosts are present both HNE and MDA products [13]. As demonstrated by higher levels of HNE in comparison to MDA, according to Sommerburg *et al*. [14] The RBC membrane preparations are obtained increasing the efficiency of Fe^{2+} removal with an higher concentration of EDTA (100 mM). Spectrophotometric method [15] to measures free HNE in human RBCs is optimized for the determination of alkenals in membranes. Other kinds of reducing agents, such as Vitamin E, are not tested because their UV–Vis spectra superimpose to N-methyl-2-phenylindole product spectra. In membranes of hypertriglyceridemic patients, the levels (triglycerides, total membrane proteins, total aldehydes and their ratio) are higher than in control samples. All the data decrease relevantly ($P < 0.001$) after the 4-hydroxytyrosol treatment, especially those of alkenals. In summary, lipid peroxidation is divided into two stages. Lipid Hydroperoxide is produced in the Early stage while Aldehydes (4-HNE, MDA) is produced in the Late stage. Lipid peroxidation consists of the radical peroxidation of a polyunsaturated fatty acid, with the formation of the lipoperoxide radical and its subsequent fragmentation into short-chain aliphatic and malondialdehyde compounds. The oxidation of arachidonic acid occurs by means of two enzymes: lipooxygenase and cyclooxygenase. The reaction sequence can take

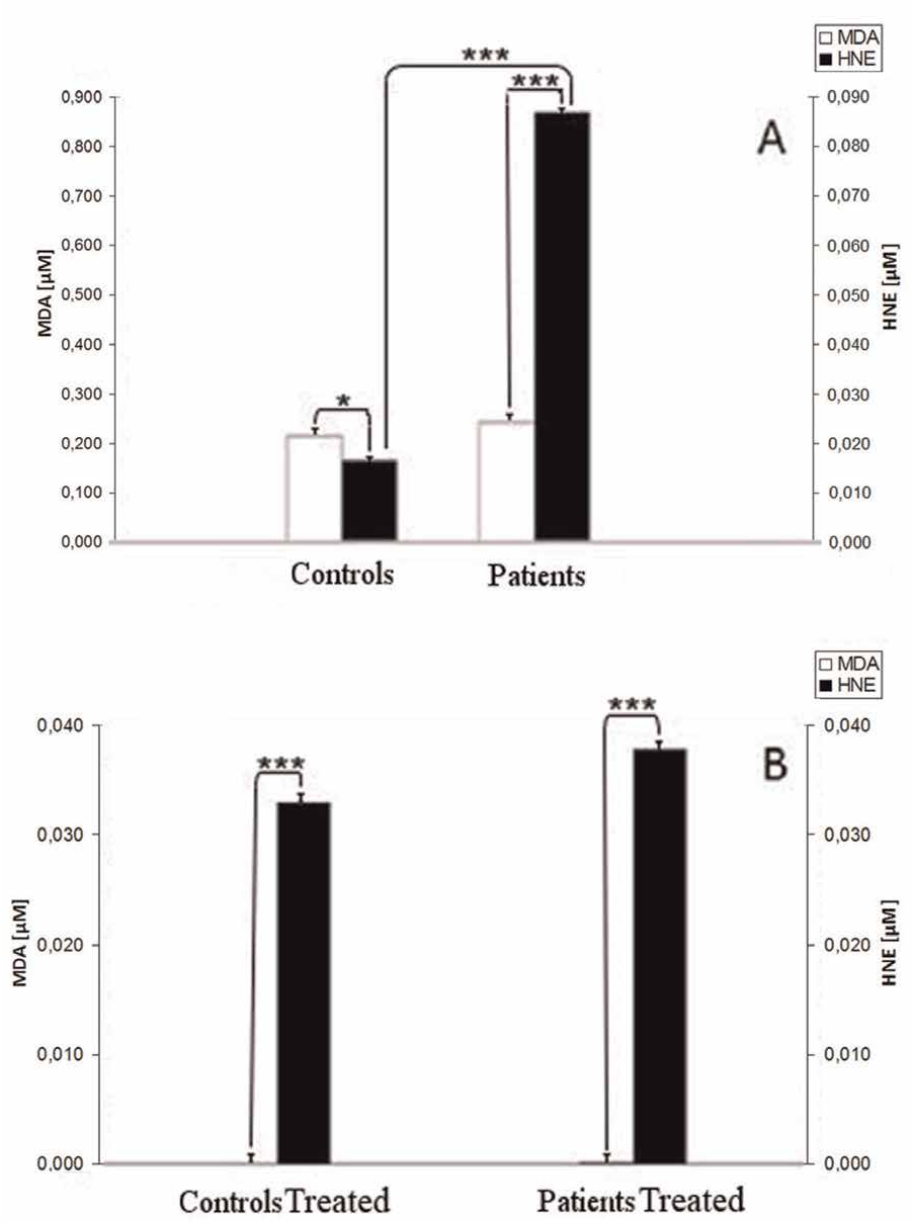


Figure 4.
Human RBC membrane concentration of MDA and HNE (µM absolute concentration in membrane samples) from controls and hypertriglyceridemic patients (A) and the same samples treated with 4-hydroxytyrosol (80 µM) (B). It is evident that for the two dosed substances each grouped data are significantly different for $P < 0.001$ *by two ways ANOVA and Bonferroni* post hoc *test.* *$P < 0.05$; **$P < 0.01$, *and* ***$P < 0.001$.

place under physiological conditions involving the arachidonic acid, contained in the membrane phospholipids, and is triggered by the superoxide anion, which is transformed into water producing the C-13 radical of arachidonic acid, which is followed by the radical in C-11. This, in the presence of oxygen, generates the radical peroxide or, by transferring the radical nature to another molecule, is transformed

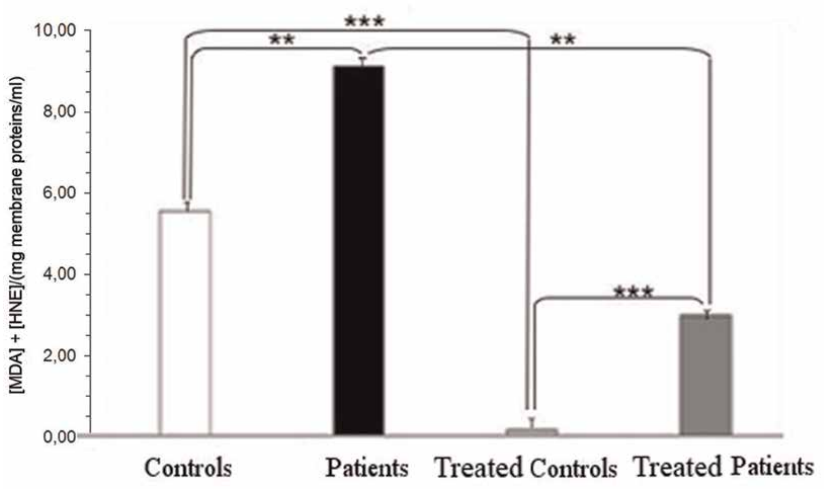


Figure 5.
Ratio of oxidized lipids (MDA + HNE) and membrane proteins. It is evident that all comparisons for the two dosed substances, each group of data is significantly different for $P < 0.001$ *by two-ways ANOVA and Bonferroni* post hoc *test.* $^{*}P < 0.05$; $^{**}P < 0.01$ *and* $^{***}P < 0.001$ *from the others.*

into stable hydroperoxide. The radical cycloperoxide, in the presence of oxygen and due to the effect of heat, can fragment, producing malondialdehyde (MDA) and aliphatic compounds of a radical nature. Peroxidation, like all chain reactions, consists of an "initiation", a "propagation" and a "termination" stage. The consequences of an intense peroxidation of biological membrane lipids are: loss of biological membrane fluidity, reduction of membrane potential, increased permeability to H^+ ions and other ions and rupture and release of cellular content to the outside.

3. Study on creatinine and uric acid

The studied population consisted of 10 patients with endogenous both hypercreatininemic hyperuricemic and ten normal subjects. The measurement of total proteins in RBC ghosts and HNE and MDA are conducted on blood samples of patients. The increase of MDA and HNE levels represent the elevated activities of oxidative stress in human body. It can be seen from **Figures 6** and **7** that the increase of protein concentration in the membrane of the blood samples is associated with the rise of MDA and HNE levels. From the ratio between either MDA levels (μM) or HNE levels and RBC membrane proteins (g/l), we deduced the following results on the basis of **Figures 8** and **9** in which we have divided samples into 3 groups according to their blood reactive oxygen species (ROS) levels. According to this kind of grouping, we can observe that both uric acid and creatinine levels have influence on the ratio either of MDA/protein or HNE/protein contents in RBC membranes, demonstrating their protective role against oxidative stress at low concentrations (lower than 5 mg/dL, for uric acid) and slight oxidizing power at high concentrations (higher than 1.1 mg/dL, for creatinine), as previously evaluated by Qasim and Mahmood [2] and even more powerful oxidant (HNE) could act similarly. In each plot, "a valley" was observed. In this valley, it is seen that there is a dramatically fall in μmole concentration of MDA per mg of RBC proteins which signifies low oxidative stress activity. Thus, it suggests

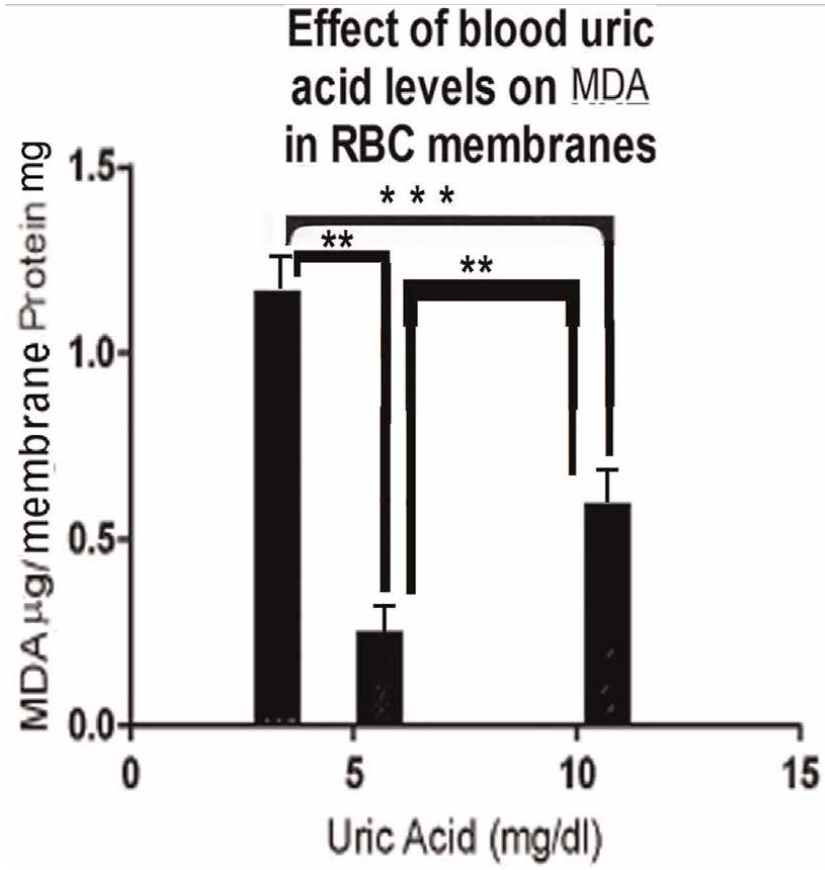


Figure 6.
Effect of blood uric acid levels on RBC membrane MDA. Each result is the main plus/minus standard error on the mean of ten independent experiments. Results are evaluated by one-way ANOVA and Bonferroni post-hoc test according to Graphpad prism 5.0. $^{*}p < 0.05$, $^{**}p < 0.01$ *and* $^{***}p < 0.001$.

that if the concentration of creatinine and uric acid is within medium range of uric acid concentration, each of these substances would strongly express their protective role toward oxidative stress. In **Figures 6–9** one can observe that the recovery of the curve is smooth and starts from the proximal area of Sample knot, suggesting that at this point both Creatinine and uric acid gradually loss their protective abilities as their concentration in the RBC membranes increase. The described results confirm the observation on MDA levels are similar for the action of uric acid and creatinine on ROS levels with almost parallel patterns [16–18]. An hypothetic explanation for this paradox could be that a rise in uric acid concentration represents an attempted protective response by the host [6]. Probably HNE data in the same experimental conditions should parallel the similar effects of ROS on the oxidation of longer chain lipids in human RBC membranes. The relationship between oxidation of long chain fatty acids and the concentration of both uric acid and creatinine blood levels according to their paradoxical action on oxidation of this kind of RBC constituents probably are attributable to multiple mechanisms of interaction of several constituents of ROSs mixture generated in human organisms with the molecular structures of RBC membranes as carbonylated proteins. This hypothesis shall be investigated in further

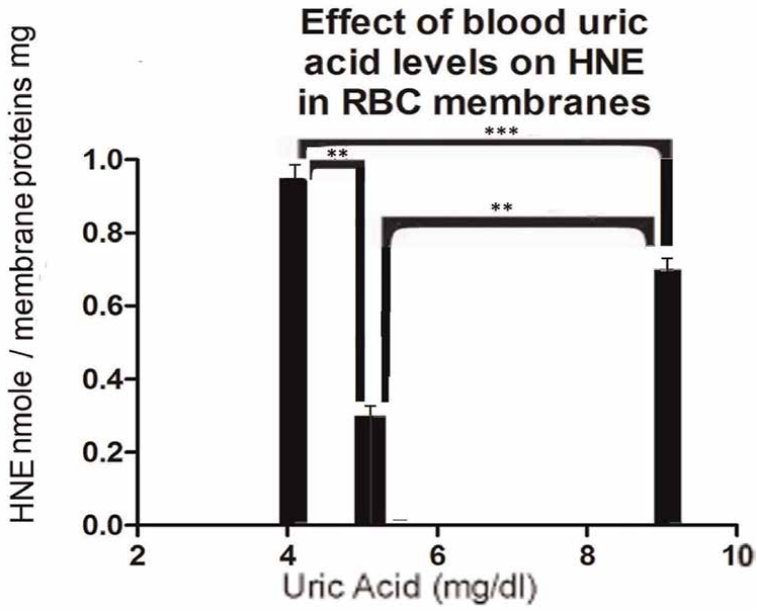


Figure 7.
Effect of blood uric acid levels on RBC membrane HNE. Each result is the mean plus/minus standard error on the main of ten independent experiments. Results are evaluated by one-way ANOVA and Bonferroni post-hoc test according to GraphPad prism 5.0. $^{*}p < 0.05$, $^{**}p < 0.01$ *and* $^{***}p < 0.001$.

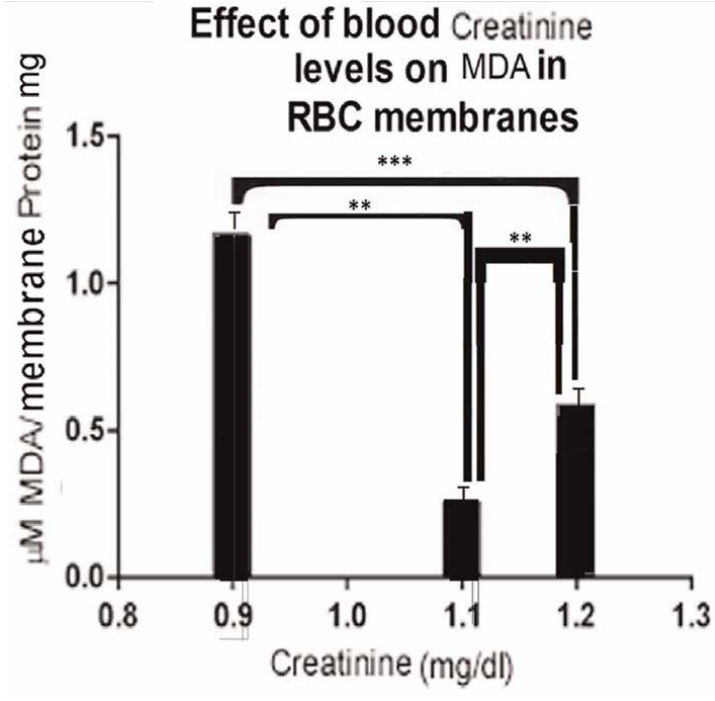


Figure 8.
Effect of blood creatinine levels on RBC membrane MDA. Each result is the mean plus/minus standard error on the main of ten independent experiments. Results are evaluated by one-way ANOVA and Bonferroni post-hoc test according to Graphpad prism 5.0. $^{*}p < 0.05$ $^{**}p < 0.01$ *and* $^{***}p < 0.001$.

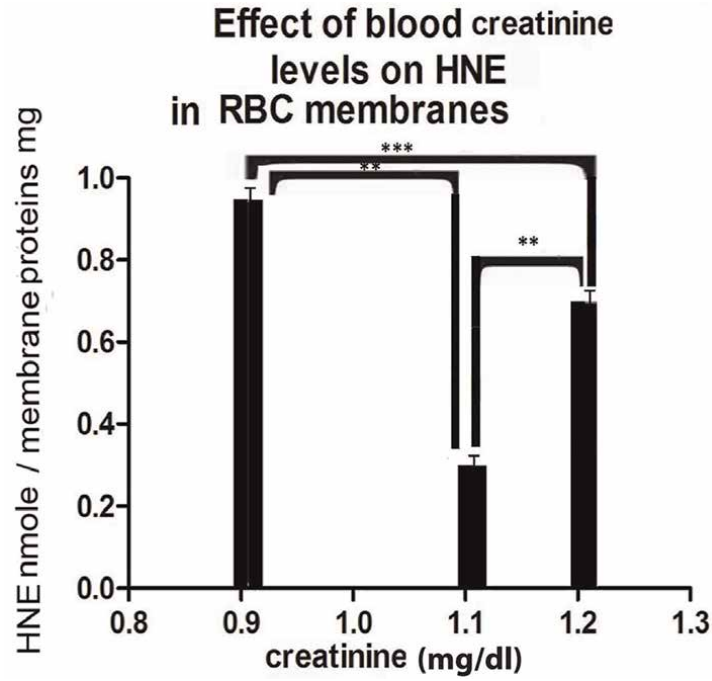


Figure 9.
Effect of blood creatinine levels on RBC membrane HNE. Each result is the mean plus/minus standard error on the main of ten independent experiments. Results are evaluated by one way ANOVA and Bonferroni post-hoc test according to GraphPad prism 5.0. * $p < 0.05$, ** $p < 0.01$ *and* $^{***}p < 0.001$.

research also on other structural components of membrane. At present, we have only data on total RBC membrane proteins.

The data from this research suggest that nitrogen metabolism, mainly creatinine, acts upon cellular lipid metabolism, as this chemical in itself is a reductant compound but at high intracellular concentrations it works as an oxidizing product, as described by Qasim and Mahmood [2]. Creatinine metabolism can interact with uric acid excretion by kidneys. Hyperuricemia damages kidneys where creatinine is excreted by humans. The interaction between concentrations of creatinine and uric acid, that is powerful scavenger of singlet oxygen, slows down the activity of oxidative stress in human erythrocyte membranes. The contemporaneous modulation of both creatinine and uric acid metabolism and their anatomical and functional consequences could modulate MDA and HNE levels. Only if the concentration of these 2 substances overcomes thresholds, they will begin to express their harmful both oxidative and reductive activities.

4. Study on PUFAs

Currently, in the literature, there are only partial discussions on the role of lipids and their oxidation products as intermediates of their membrane structural damage and/or on the protective role in the same structures [19, 20]. Elevated levels of triglycerides are associated with atherosclerosis and predispose to cardiovascular disease [21]. Oxidative stress, i.e., an altered balance between the production of free radicals

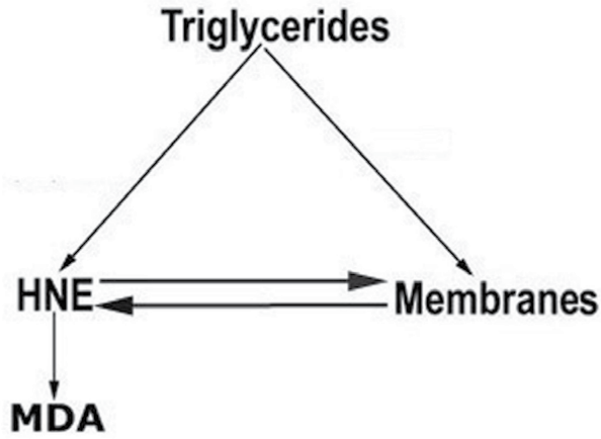


Figure 10.
Graphical resume of main metabolic steps of fatty acids in cell membrane.

and antioxidant defenses [22]. The peroxidation of n-3 and n-6 polyunsaturated fatty acids (PUFAs) and of their metabolites is a complex process. It is initiated by free oxygen radical-induced abstraction of a hydrogen atom from the lipid molecule followed by a series of nonenzymatic reactions that ultimately generate the reactive aldehyde species 4-hydroxyalkenals (HNE). Some authors show that high doses of dietary n-3 PUFAs, as well as long-time treatments, affect human RBC susceptibility to lipid peroxidation by changes in fatty acid composition content. According to experimental data, the accumulation of the alkenals in RBC membrane could be produced either by partial PUFA oxidation contained in glycerides and plasma glycerides and by glycerides into recycled plasma membrane in RBC neogenesis (**Figure 10**). According to these hypotheses, the increased charge of triglycerides in plasma forces its metabolism toward either incorporation in cell membrane of degradative oxidation. This last pathway induces increase of oxidative product such as alkenals and MDA. Furthermore, free alkenals can be dissolved in lipid membrane bilayer degrading their structures. This last process favors increased level of macromolecular assemblies in circulation that enhances microcirculation damage. Such last data could be studied in following works.

5. Conclusions

In the first study, it is evident that after *in vitro* oxidative damage of the membrane, red wine polyphenol extracts are as effective reducing agents also on C_M indicator of membrane protein damages. Consequently, both red wine and polyphenol extracts both alone and mixed among them efficiently relieve the effects of oxidative stress. Regarding the study on 4-hydroxytyrosol. All the data decrease relevantly ($P < 0.001$) after the 4-hydroxytyrosol treatment, especially those of alkenals. As for the creatinine and uric acid studies they this study suggest that at low concentrations of creatinine and uric acid there is oxidative stress in human erythrocyte membrane, but if creatinine and uric acid have slowed down to sufficient limits there is also oxidative stress, as demonstrated by MDA and HNE levels; only if the concentration of these two substances overwhelms a threshold. According to experimental data, the

accumulation of the alkenals in RBC membrane could be produced either by partial PUFA oxidation contained in glycerides and plasma glycerides and by glycerides into plasma membrane recycled RBC.

Acknowledgements

The authors would like to acknowledge Prof. Domenico Sturino for his mother language support and technical revision of manuscript.

Conflict of interest

There are no conflicts of interest.

Acronyms and abbreviations

ORAC	Oxygen Radical Absorbance Capacity, or the oxygen radical absorption capacity
AAPH	2, 2′-azobis (2-amidinopropane) dihydrochloride
RBC	red blood cell
MDA	malondialdehyde
HNE	4-hydroxynonenal
PUFAs	polyunsaturated fatty acids
EPA	eicosapentaenoic acid
DHA	docosahexaenoic acid
ROS	reactive oxygen species
C_M	the visible absorbance spectrum method evaluating the absorption of methemoglobin

Author details

Giuseppe Gallo
Department of Biology, Ecology and Earth Sciences, University of Calabria, Rende, CS, Italy

*Address all correspondence to: pino72@tiscali.it

References

[1] H VisioliH F, Galli C, Plasmati E, H ViappianiH S, H HernandezH A, Colombo C, Sala A. Olive phenol hydroxytyrosol prevents passive smoking-induced oxidative stress Circulation. 2000; 102(18):2169-2171.

[2] Qasim N, Mahmood R. Diminution of Oxidative Damage to Human Erythrocytes and Lymphocytes by Creatinine: Possible Role of Creatinine in Blood. PLoS One. 2015;10(11):e0141975.

[3] Mufidah M, Ermina P, Gemini A, Marianti AM, Lukman M, Rusdi M, *et al*. Lipid Peroxidation Inhibitory Activity *in vitro* of *Mezzetia parvi-flora* Becc. Wood Bark Polar Extract. Pharmacogn J. 2017;9(2):171-175.

[4] Gallo G, Martino G. *In vitro* action of 2.2'azobis2 amidinopropan dihydrochloride, red wine polyphenols, resveratrol and catechin on anion permeability for chloride in human red blood cell. Free Radicals and Antioxidants. 2014;4(2):13-17.

[5] Gallo G, Bruno R, Taranto A, Martino G. Are Polyunsaturated Fatty Acid Metabolites, the Protective Effect of 4-hydroxytyrosol on Human Red Blood Cell Membranes and Oxidative Damage (4-hydroxyalkenals) Compatible in Hypertriglyceridemic Patients?. Pharmacogn Mag. 2017;13 (Suppl 3):S561-S566.

[6] Sautin YY, Johnson RJ. Uric acid the oxidant–antioxidant paradox. Nucleosides Nucleotides Nucleic Acids. 2008;27(6-7):608-619.

[7] P Palozza , E Sgarlata, C Luberto, E Piccioni, M Anti, G Marra, F Armelao, P Franceschelli, G M Bartoli n-3 fatty acids induce oxidative modifications in human erythrocytes depending on dose and duration of dietary supplementation. Am J Clin Nutr 1996;64(3):297-304.

[8] Gallo G, Martino G, Carino AR. Spinning, oxidative damage and hemolysis in athletes. Free Radic Antioxid 2013;3:61-66.

[9] Coccia R, Spadaccio C, Foppoli C, Perluigi M, Covino E, Lusini M, *et al.* The effect of simvastatin on erythrocyte membrane fluidity during oxidative stress induced by cardiopulmonary bypass: A randomized controlled study. Clin Ther 2007;29:1706-1717.

[10] Frémont L, Belguendouz L, Delpal S. Antioxidant activity of resveratrol and alcohol-free wine polyphenols related to LDL oxidation and polyunsaturated fatty acids. Life Sci 1999;64:2511-2521.

[11] Gallo G, Mazzulla S, Martino G. Scavenger enzymes and natural reducing compounds roles in oxidative stress relieving of mammalians. Rec Res Dev Physiol 2012;5:159-173.

[12] Paiva-Martins F, Fernandes J, Rocha S, Nascimento H, Vitorino R, Amado F, *et al.* Effects of olive oil polyphenols on erythrocyte oxidative damage. Mol Nutr Food Res 2009;53: 609-616.

[13] Riahi Y, Cohen G, Shamni O, Sasson S. Signaling and cytotoxic functions of 4-hydroxyalkenals. Am J Physiol Endocrinol Metab 2010;299: E879-E886.

[14] Sommerburg O, Grune T, Hampl H, Riedel E, van Kuijk FJ, Ehrich JH, *et al.* Does long-term treatment of renal anaemia with recombinant erythropoietin influence oxidative stress in haemodialysed patients? Nephrol Dial Transplant 1998;13:2583-2587.

[15] McGowan MW, Artiss JD, Strandbergh DR, Zak B. A peroxidase-coupled method for the colorimetric determination of serum triglycerides. Clin Chem 1983;29:538-542.

[16] Rutkowski P, Słominska EM, Szołkiewicz M, Aleksandrowicz E, Smolenski RT, Wołyniec W, *et al*. Relationship between uremic toxins and oxidative stress in patients with chronic renal failure. *Scand* J Urol Nephrol. 2007; 41(3):243-248.

[17] Buranakarl C, Trisiriroj M, Pondeenana S, Tungjitpeanpong T, Jarutakanon P, Penchome R, *et al*. Relationships between oxidative stress markers and red blood cell characteristics in renal azotemic dogs. Res Vet Sci. 2009;86(2):309-313.

[18] Onyesom I. Synergistic effect of alcohol and fructose administration on blood urate and biochemical indices of insulin resistance in albino rabbits. Indian J Med Res. 2006;124(6):715-717.

[19] Richard D, Kefi K, Barbe U, Bausero P, Visioli F. Polyunsaturated fatty acids as antioxidants. Pharmacol Res 2008;57:451-455.

[20] Ambrozova G, Pekarova M, Lojek A. Effect of polyunsaturated fatty acids on the reactive oxygen and nitrogen species production by raw 264.7 macrophages. Eur J Nutr 2010;49:133-139.

[21] Pejic RN, Lee DT. Hypertriglyceridemia. J Am Board Fam Med 2006;19:310-316.

[22] Chang T, Wu L. Methylglyoxal, oxidative stress, and hypertension. Can J Physiol Pharmacol 2006;84:1229-1238.

Chapter 14

Environmental Particulate Air Pollution Exposure and the Oxidative Stress Responses: A Brief Review of the Impact on the Organism and Animal Models of Research

Pauline Brendler Goettems Fiorin, Mirna Stela Ludwig, Matias Nunes Frizzo and Thiago Gomes Heck

Abstract

Particulate matter (PM) is a mixture of solid particles and liquid droplets found in the air, and it is one of the most harmful air pollutants. When inhaled, it affects the pulmonary system, cardiovascular systems, and other tissues. The size, composition, and deposition of PM, mainly related to fine and ultrafine particulate matter, are factors that determine the harmful effects of exposure to particles. Among the main effects is the inducer of ROS production, and consequently oxidative tissue damage in target organs and other responses, mediated by inflammatory cytokines and cellular stress response. The main pathway through which particles are potent mediators of oxidative stress is the damage caused to DNA and lipid molecules, whereas the pro-inflammatory response involves an immune response against PM, which in turn, it is related to cell stress responses observed by heat shock proteins (HSPs) expression and release. Thus, the ability of an organism to respond to PM inhalation requires anti-oxidative, anti-inflammatory, and cellular stress defenses that can be impaired in susceptible subjects as people with chronic diseases as diabetes and obesity. In this chapter, we discuss the mechanistic aspects of PM effects on health and present some animal research models in particle inhalation studies.

Keywords: air pollution, fine particulate matter, oxidative stress, animal models

1. Introduction

Pollutant refers to chemical substances, particles, or toxic gases, introduced into the environment from various sources and that cause adverse effects to living beings and the ecosystem, compromising the soil, water, and atmospheric air. Atmospheric

pollution, in turn, can be defined as any form of matter in quantity, concentration, time, or other characteristics, which make or may make the air unsuitable or harmful to health, inconvenient to public welfare, harmful to fauna and flora, or environmental safety. Air pollution is one of the biggest environmental threats to human health, a condition related to human development activities and climate change agenda. In 2021, the World Health Organization (WHO) listed the improvement of air quality within the top 10 health challenges of the year.

The primary sources of air pollutants are anthropogenic, which originate from processes carried out by industries, mining, transport, and construction. Also, pollutants are classified as mobile sources, which include most forms of transport, such as automobiles, trucks, and planes, or fixed sources, such as industrial and housing facilities. Another form of classification is to determine the pollutants that are emitted directly by polluting sources, called primary pollutants, such as particulate matter (PM), sulfur dioxide (SO_2), nitrogen dioxide (NO_2), carbon monoxide (CO), or those that result from interactions with the environment, called secondary pollutants. Among the air pollutants mentioned, PM is the pollutant that has received greater emphasis in scientific research, both in epidemiological and experimental studies.

The term PM refers to a mixture of solids and liquid droplets suspended in the air [1] classified as large "coarse" particles (PM_{10}; particles with a diameter less than 10 μm). PM_{10} represents mainly the overall mass of PM and can be derived from numerous sources, such as silica-based crustal particles (e.g., soil, sand, and volcanic ash), burning of natural materials (e.g., woodsmoke), or wear of machinery (e.g., vehicle braking and tire erosion). "Fine" PM ($PM_{2.5}$: particles with a diameter less than 2.5 μm) and "ultrafine" PM ($PM_{0.1}$: particles with a diameter less than 100 nm) are generally derived from industrial burning of fossil fuels and traffic-related sources [2]. PM is primarily generated by fuel combustion in different sectors, including transport, energy, households, industry, and agriculture. In 2013, the outdoor air pollution and PM as carcinogenic by the WHO International Agency for Research on Cancer (IARC) [3].

The size of inhaled particles is inversely correlated to their potential to cause health problems. Small particles, less than 10 μm in diameter, are the most significant problems because they can penetrate deeply into the lungs, and some particles can reach the bloodstream [4]. The health risks associated with PM equal or smaller than 10 and 2·5μm in diameter (PM_{10} and $PM_{2.5}$, respectively) represent a particular public health relevance. Both $PM_{2.5}$ and PM_{10} can penetrate deep into the lungs, but $PM_{2.5}$ can even enter the bloodstream, primarily resulting in cardiovascular and respiratory impacts and affecting other organs.

In 2005, the guidelines established by the WHO for $PM_{2.5}$ concentrations went from 65 to 25 $\mu g/m^3$ during the 24-h period and from 15 to 10 $\mu g/m^3$ during the period one year [5]. However, guidelines may be different in each country. For example, in Brazil, the final air quality guidelines were established by CONAMA resolution No. 0^3/90, updated in Resolution No. 491, of November 19, 2018, which provides for air quality standards. Considering the levels of fine particulate matter ($PM_{2.5}$) of 25 $\mu g/m^3$ for the 24-h period and 10 $\mu g/m^3$ for the annual average, the same was indicated by the WHO [6].

In 2021, the WHO released the update of air quality guidelines based on the extensive scientific evidence currently available identifying the levels of air quality necessary to protect public health worldwide. The updated WHO Global Air Quality Guidelines (AQGs) provide recommendations on air quality guideline levels and interim targets for six key air pollutants. Moreover, for PM, there was a significant

reduction in the recommended levels. For $PM_{2.5,}$ the standard established for the annual average 15 years ago was reduced by half, from 10 to 5 μg/m^3, and concerning the exposure in the 24 h, the parameter was reduced from 25 to 15 μg/m^3. About PM_{10}, in the annual average, it reduced from 20 to 15 μg/m^3 and the 24-h period values were reduced from 50 to 45 μg/m^3 [7].

To achieve these goals, the WHO has set interim targets that serve as incremental steps in the progressive reduction of air pollution toward the air quality guideline levels and are intended for use in areas where air pollution is high. These levels could be used by authorities in highly polluted areas to develop pollution reduction policies that are achievable within realistic time frames [7].

According to the IQAIR website [8], the 15 most polluted cities in the world, considering only $PM_{2.5}$, are located in China or India (87% are Indian cities). The city of Hotan in China was the "champion" in 2020, with an annual average of 110.6ug/m^3 of pollution by $PM_{2.5}$. China and India are part of the BRICS, a group formed by five large emerging countries—Brazil, Russia, India, China, and South Africa—representing about 42% of the population, 30% of the territory, and 18% of trade worldwide. In the air pollution ranking, South Africa is classified in 49th place, Brazil in 68th and Russia in 86th, with an annual average of 18.0, 14.2, and 9.3 respectively in 2020, which is considered moderate (South Africa and Brazil) and good air quality for Russia, considering the new WHO guidelines [8].

The assessment of environmental data allows us to understand the economic, social, and environmental impact of pollution in both developed or developing countries, and it is crucial for planning to reduce air pollution activities. Since air pollution can affect our health in various ways, depending on the exposure time (short or long term), type of pollutant, concentration of polluting agents, age group, and prior health condition. In this way, a study published in 2017 described ambient $PM_{2.5}$ as the fifth-ranking mortality risk factor in 2015. Exposure to $PM_{2.5}$ caused 4·2 million deaths and 103·1 million -adjusted life-years (DALYs) in 2015, representing 7·6% of total global deaths and 4·2% of global DALYs, 59% of these in the east and south Asia. Deaths attributable to ambient $PM_{2.5}$ increased from 3·5 million in 1990 to 4·2 million in 2015. These deaths occur mainly due to stroke, heart disease, lung cancer, and chronic respiratory diseases [9]. Liu et al. [10], demonstrated in a study that evaluated particulate air pollution and daily mortality in 652 cities that an increase of 10 μg/m^3 in the 2-day moving average of PM_{10} concentration was associated with increases of 0.44% in daily all-cause mortality, 0.36% in daily cardiovascular mortality, and 0.47% in daily respiratory mortality. The corresponding increases in daily mortality for the exact change in $PM_{2.5}$ concentration were 0.68, 0.55, and 0.74%, respectively. Evidencing an independent association between short-term exposure to PM_{10} and $PM_{2.5}$ and daily all-cause, cardiovascular, and respiratory mortality in more than 600 cities across the globe, reinforcing the evidence of a link between mortality and PM concentration established in regional and local studies [10].

Studies have described particulate pollution exposure to various problems, including premature death in people with heart or lung disease; non-fatal heart attacks; cardiac arrhythmia; asthma; decreased lung function; increased respiratory symptoms such as airway irritation, coughing, or difficulty breathing. People with heart or lung disease, children, and older adults are most likely to be affected by exposure to particulate pollution [11]. In addition, sensitive groups (also called at-risk populations)—A term used for a category of people at increased risk of experiencing adverse health effects related to exposure to air pollution, may be at increased risk due to intrinsic (biological) factors, extrinsic factors (external, non-biological), increased

exposure and increased levels of air pollutants. Thus, the severity of the health effects experienced by the sensitive groups may be greater than in the general population [12]. Therefore, the establishment of air quality standards in large cities has contributed to estimates of data that helps environmental management and community health. In parallel, many laboratories are carrying out research that emphasizes the mechanisms by which exposure to air pollution impacts human health, thus alerting the need to develop public health policies and stricter air quality standards.

2. Particulate matter inhalation: characteristics and associated diseases

Among air pollutants, the one that has received greater emphasis in current research is fine particulate matter ($PM_{2.5}$), consisting of pollutants formed from combustion processes [13]. Particulate matter$<_{2.5}$ μm ($PM_{2.5}$) is the most commonly implicated constituent that causes a disproportionate number of global deaths and contributes significantly to global disability. The global burden of disease study report indicated that ambient outdoor air pollution, particularly $PM_{2.5}$, was the fifth leading risk factor for global mortality in 2015 [14].

The source of PM can be explained as either direct emission into the air or as conversion from gaseous precursors (such as sulfur dioxide, oxides of nitrogen, ammonia, and non-methane volatile organic compounds) released from both anthropogenic and natural sources. The chemical constituents in PM are commonly found to include inorganic ions (e.g., sulfates, nitrates, ammonium, sodium, potassium, calcium, magnesium, and chloride) and can be expanded further to include all varieties of constituents such as metals (including cadmium, copper, nickel, vanadium, and zinc), and polycyclic aromatic hydrocarbons (PAH) [15]. The harmful effects of $PM_{2.5}$ on health depend on the exposure time and its concentration in the environment.

Ambient particles contain many soluble metals, including transition metals that are capable of redox cycling [16]. Transition metals are thought to be very important in PM cellular toxicity. The bioavailability of transition metals in PM and their redox properties, which are considered very important for the toxic effects and the oxidative damage [17]. $PM_{2.5}$ can invade the respiratory tract and vascular system [18] and thus cause a systemic effect, acting as a potent inducer of ROS production and the release of pro-inflammatory cytokines (TNF- α, IL-6, IL-8) for circulation [19, 20].

Exposure to PM can occur in many forms, as described by Thompson [21]. As with any chemical contaminant, components of PM may enter the human body by three mechanisms: inhalation, dermal absorption, and ingestion. PM can be ingested by the direct consumption of contaminated beverages and food and during the clearance of particles removed from the airways via mucociliary transport. Another possible route of exposure to airborne PM is impaction or deposition to the skin. Deposition velocities are influenced by substrate surface properties (e.g., roughness, wetness, temperature, surface charge) and particle size-dependent. Inhalation is a rather obvious route of exposure, but to better understand the effects, it is crucial to consider the anatomy of the human airway, patterns of particle size-dependent deposition, and the fate of particles that have been inhaled.

Nevertheless, mainly, the size is determinant for the deposition characteristics and effects of the PM. During a pollution episode, each lung acinus could receive on average 30-million particles and each alveolus about 1500 particles (for 24 h exposure), of which 50% are being deposited. Lung airways and alveoli retain mostly $PM_{2.5}$ rather than PM_{10} since the last one is frequently stopped in upper airway anatomic

structures. Also, analytical electron microscopy measurements showed that 96% of effectively retained particles in the lung parenchyma were $PM_{2.5,}$ and only 5% were ultrafine particles (0.1 μm), which means translocation to the pulmonary and systemic circulation, affecting other organs [17]. Thus, the size of PM and their retention play an essential role in the PM cytotoxic effects, including the site of deposition (upper or lower airways), bio-persistence solubility, and its composition [14].

In the USA, studies involving residual oil fly ash (ROFA), one of the components of $PM_{2.5}$, have helped develop and refine this theory. ROFA, or residual oil fly ash, is the term used to refer to the primarily inorganic residues that remain after the incomplete oxidation of carbon compounds. ROFA contains about 10% by weight of water-soluble Fe, Ni, and V, and its intratracheal instillation in rats leads to aldehyde generation [16, 22].

ROFA particles are generally smaller than 2.5 μm in size and are chemically considered complex compared to other air pollution particles, as they contain sulfates, silicates, carbon, and nitrogen. In addition to the elements mentioned, it also has a large number of metals that are naturally present in fuels (petroleum, paraffin, and diesel oil) and remain when the volatile fraction is distilled [23]. This pollutant has been used in experimental studies as a surrogate particle to investigate the mechanisms of responses to PM inhalation in animals [24, 25]; as it is mainly made up of different metals, it promotes Fenton reactions, producing reactive oxygen species [22].

The harmful effects of $PM_{2.5}$ on human health depend on the time of exposure and particles concentration in the environment. As expected, repetitive and long-duration exposure to higher doses of $PM_{2.5}$ induces cumulative and persistent effects. However, subchronic exposure to low doses (5 μg/day, intranasally) of $PM_{2.5}$ in animal models is sufficient to potentiate metabolic dysfunction in high-fat diet-fed mice, promoting glucose intolerance and increasing fasting glycemia and triglyceride levels [26].

3. The role of oxidative stress induced by PM inhalation in cells and tissues

Oxidative stress is described as a biochemical imbalance, which occurs when the production of pro-oxidants such as free radicals or reactive oxygen species exceeds the body's natural antioxidant capacity, resulting in oxidative damage [27, 28] and consequently leading to the development of a broad spectrum of human diseases.

The main pro-oxidant agents are the reactive oxygen species (ROS), such as radicals, superoxide ($O2^{-}$) and hydroxyl (OH^{-}) radicals, and also some non-radical species derived from O_2, such as the hydrogen peroxide (H_2O_2) [27]. Antioxidants can be classified as exogenous, obtained mainly through the diet, or endogenous, being produced by our body to avoid oxidative stress and consequent tissue destruction. Antioxidant defense systems can inhibit the oxidation of other molecules in the organism, transferring electrons from a substance to an oxidizing agent (ROS), acting in two lines: enzymatic antioxidant defenses [e.g., superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)] and non-enzymatic antioxidant defenses (e.g., vitamins A, C, and E; glutathione, α-lipoic acid; carotenoids, ubiquinone or coenzyme Q10).

ROS production is inherent in all aerobic species, primarily as a product of mitochondrial electron transport. At physiological levels, ROS are essential for the regulation of critical signaling pathways involved in cell growth, proliferation, differentiation, and survival, but excess ROS, mainly resulting from imbalanced antioxidant defense and detoxification, can lead to harmful (i.e., pathological) oxidative stress [29].

The most important pathophysiological mechanism that has been proposed to explain the association of PM exposure and the occurrence of respiratory infections, lung cancer, and chronic cardiopulmonary diseases is oxidative stress through the generation of ROS [17]. Recent studies using different methods have consistently demonstrated that peroxides and ROS are the critical mediators of particle toxicity [29].

The molecular events by which pulmonary oxidative stress occurs in response to particle inhalation involve the inflammatory process. ROS (e.g., superoxide, hydroxyl radical, nitric oxide, and peroxynitrite) generated in air pollution exposure induces oxidative tissue damage in target organs, with contributions of non-immune and immune cells in the inflammatory response. The role of protective proteins (e.g., surfactant, proteins, and antioxidants) in this process is highly complex and may differ depending on experimental models, especially in concomitant disease states [30].

The pieces of evidence, to date, suggest that ROS generation in response to $PM_{2.5}$ could either involve disruption of cellular redox signaling and upregulation of endogenous ROS production resulting in exaggerated responses, as described in details in some reviews [14, 30].

Wang et al. [31] evaluated two classical mechanisms of oxidative stress and intracellular calcium overload to explore their roles in PM-induced endothelial cell apoptosis from the perspective of subcellular levels in endothelial cells. They showed that internalization of particles induces oxidative stress, followed by the disorder of subcellular structures, including endoplasmic reticulum (ER) stress, mitochondrial dysfunction, activated caspase pathways, which cause endothelial cell apoptosis. They also highlighted that antioxidants and calcium inhibitors confer protective effects. Also, ROS are generated during phagocytosis of the particles, leading to enhancement of oxidative stress and triggering the inflammatory response. Consequently, the activation of inflammatory signaling pathways results in the release of cytokines and other mediators that can further induce ROS production by activating endogenous enzymes, leading to a positive feedback loop, which can aggravate the effects triggered by PM exposure [32].

In summary, it is well known that in the lungs, exposure to PM triggers inflammation, endothelial activation, and oxidative stress, caused by the deposition of PM into the alveolar space in the lung, inducing the release of cytokines from alveolar macrophages. The probable sequence of events for PM-induced lung inflammation involves the following: injury to epithelial cells by ROS, possibly enhanced in the presence of metals via Haber-Weiss and Fenton chemistry; and activation of vascular endothelium and circulating leukocytes, circulating leukocytes; emigration of inflammatory cells from blood to tissue sites, promoting pro-inflammatory condition [32]. Moreover, even at low doses, it is also able to demonstrate early changes in the elastic and viscoelastic pulmonary mechanical components, such as worsening impedance, alveolar collapse, and histological changes, in addition to oxidative stress and inflammation [33].

An epidemiological study conducted by Hu et al. [34] exposed 768 participants to environmental levels of $PM_{2.5}$, and assessed urinary levels of PAH metabolites and metals, and evaluated urinary 8-OHdG, a biomarker of endogenous oxidative damage to DNA, 8-iso-PGF2α a biomarker of excessive chemical lipid peroxidation in humans, and MDA for determination of lipid peroxidation levels. They discuss that particulate matter alone, its bound polycyclic aromatic hydrocarbons, and heavy metals induce increased oxidative stress on DNA and lipid [34].

Since PM can invade the bloodstream, particles exposure may also impact cardiovascular health. Air pollution increases the risk of myocardial infarction, stroke,

and acute heart failure. Since ROS act mainly inducing endothelial dysfunction, monocyte activation, and some proatherogenic changes in lipoproteins, which initiate plaque formation, this context favors thrombus formation because of an increase in coagulation factors and platelet activation [2, 35]. In this way, animal models of acute exposure to higher levels of particles in chambers showed that PM oxidative effects are associated with autonomic nervous system imbalance, which is avoided by the pre-treatment with drugs acting as sympathetic or parasympathetic blockers by antioxidant pre-treatment [36]. In addition, exposure to particles may induce electric alterations on the heart [37], suggesting an explanation of the increased risk of myocardial infarct under inadequate air quality for susceptible subjects [38].

In the lungs and heart, but also in other organs, oxidative stress may induce a cell stress response, characterized by the increased expression and release to the bloodstream of heat shock proteins (HSPs). High plasma levels of these proteins are simultaneously correlated with impaired energy balance, with an alteration in the pro/anti-inflammatory status and with an imbalance in the body's pro/antioxidant systems, and also has been used as a biomarker of cell stress response in diseases as obesity, hypertension, and diabetes [26, 39, 40].

HSPs are highly conserved proteins during species evolution and are found in eukaryotic and prokaryotic organisms. HSPs can be grouped according to their molecular weights into families (HSP110, HSP100, HSP90, HSP70, HSP60, and HSP30). Specifically, proteins from the 70 kDa HSPs family (HSP70) are highly conserved [41] and have a cytoprotective role in cells, as well as present anti-apoptotic and anti-inflammatory proprieties in various stressful conditions [42]. The increase in the synthesis of HSPs may show an increase in stress tolerance, preventing protein damage related to oxidative stress [43].

In this way, a study carried out by Kido et al. [44] suggested that inhalation of air pollution induces an increase in HSP70 in lung macrophages and also a systemic increase in blood HSP70 levels (a.k.a eHSP70 since it is located in extracellular fluids). These stress responses marked by alterations in both intracellular (iHSP70) and extracellular (eHSP70) suggest these proteins as a relevant immunological mediator that contributes to other aggravating factors (vascular dysfunction and cardiovascular events). For example, exposure to ROFA suspension for three consecutive days (750 μg) promoted an increase in plasma levels of eHSP70 associated with plasma oxidative stress, showing that HSP70 represents a potential inflammatory and indirectly an oxidative biomarker [45].

Furthermore, a study conducted by Goettems-Fiorin et al. [26] highlights that exposure to the $PM_{2.5}$ potentiates metabolic dysfunction in mice treated with HFD, associated with altered cellular stress response, assessed by the ratio [eHSP70]/[iHSP70], called H-index, a biomarker of the low grade chronic inflammatory state, increasing the risk of type 2 diabetes development. The study shows a positive correlation between adiposity, increased body weight and glucose intolerance, and increased glucose and triacylglycerol plasma levels. And when evaluating the H-index in the pancreas, demonstrated that the pancreas exhibited lower iHSP70 expression, accompanied by a 3.7-fold increase in the plasma to pancreas [eHSP72]/[iHSP70] ratio, highlighting that exposure to PM2.5 markedly enhances metabolic dysfunction in HFD-treated mice.

Thus, at least in animal models, HSP70 has been used recently as a biomarker for early assessments of harmful health effects, such as those caused by exposure to air pollution [26, 45, 46]. In humans, the study of Chao et al. [47] with drivers showed an increase in pro-inflammatory activity, with an increase in plasma levels of TNF-α related to the level of pollution exposure. In terms of inflammation, exposure to air pollutants

also promotes responses by cytokine signaling, and PM exposure is associated with the expression of IL1, IL6, and TNF-α [48], and the imbalance pro-inflammatory response in immune cells is also associated with unbalanced eHSP70/iHSP70 ratio [49, 50].

In this way, the intensity of $PM_{2.5}$ exposure and its association with other risk factors might reduce HSP70 levels [51–53]. Independently, metabolic impaired condition and PM exposure may increase the eHSP70/iHSP70 ratio [26, 50, 53–55]. Since eHSP70 is related to increase pro-inflammatory signaling, while iHSP70 is had anti-inflammatory roles by inhibiting NF-kB signaling for inflammatory mediators [42], the unbalance in eHSP70/iHSP70 ratio (increased values in favor of eHSP70) indicates an organism under stress condition without an effective stress response [39, 50, 56].

4. Animal models of PM studies

Experimental studies to evaluate air pollution-induced oxidative stress in humans are challenging to perform for ethical and population heterogeneity reasons [29]. Thus, several experimental studies have shown the effects of exposure to air pollution [18, 20, 57–60], for which many models are used to assess the degree of exposure versus the effect produced. Also, the studies of exposure to pollutants in animal models, which mimic exposure to air pollution in the human population, enable the study of pathophysiological mechanisms by which the body develops numerous diseases related to air pollution.

In this way, many in vivo exposure studies models in animals have been published, with different exposure models to atmospheric pollution. Inhalation methods are closer to mimicking the reality of human exposure, such as inhalation of concentrated PM (CAP) or inhalation of environmental air particulates.

In the CAP method, the exposure system uses the principle of the condensational growth of the ambient particles followed by virtual impaction to concentrate the aerosol and allows the particles larger than 2.5 μm in aerodynamic diameter removed at the concentrator inlet, and the remaining aerosol to be concentrated by inertial separation techniques that dispose of most of the carrier air, which enables delivery of concentrated streams of real-world particles to human subjects or laboratory animals via whole-body exposure [60].

The environmental air particles propose, the exposure of the animals to PM in a chamber, where the air is propelled from the environment to the inner area of the chamber, is frequently used [61–63]. In this model, the experimental groups are divided into filtered air (with a series of filters to prevent pollution from entering) or unfiltered (exposed/polluted group). Alternatively, this model can be performed by uptake urban air using a concentrator of atmospheric pollution particles [64–66]. The last one was used early in toxicological studies, using an atmospheric pollution particle concentrator developed by Harvard University [66]. The advantage of the chamber protocols is to submit the organism to a "real word" air pollution context. On the other hand, since the urban pollutants may present a significant variability between the days of the exposure, at least in terms of concentration and composition, the animals are exposed to days of higher levels and days with low levels of PM in the chamber [61, 62]. As a result, the biochemical and molecular outcomes in this model may also present a significant variability and the interpretation of the results can be specific for urban areas.

Other studies used intratracheal and intranasal instillation methods [26, 67, 68]. Intratracheal instillation should generally be considered a method for a single exposure of the lungs to characterize potential toxicity [69]. The intratracheal instillation

method is often used to expose animals to both soluble and insoluble particles, and it is a relatively inexpensive method that allows instantaneous administration directly into the trachea of known concentration of the pollutant under test directly to the lung [70]. In the intranasal instillation model, the handler restrained the animal (suspended by the cervical region, held with the hand on the back). Then the suspension in liquid (with a known concentration of pollutant) is administered directly into the animal's nostril, that by apnea reflex, inhales the suspension [70, 71]. Thus, the advantage of the intratracheal or intranasal protocols is to submit the organism to a controlled pollution context in terms of dose and frequency of exposure, adequate for toxicological studies and mechanistic studies. Also, since the particles may present a significant variability in terms of the composition, dependent on the source of particles used in the study (e.g., metal composition variability), intratracheal and intranasal protocols allow that, even if the animals are exposed to the same dose, but with different source particles, the studies may reveal different responses. However, as limitations, these procedures may not represent a "real world condition".

When compared to inhalation procedures, instillation is also easier, less expensive, and incurs less health risks to the lab staff. Nonetheless, inhalation is a more realistic physiological approach since it better represents the natural route for PM exposure, and CAP inhalation remains the main one, as it is closer to simulating reality environmental conditions and physiological animal responses in laboratory PM administration [72].

A study by Curbani et al. [72] highlights that studies with intratracheal or intranasal instillation models PM concentrations were three orders of magnitude higher than the environmental ones found in megacities through acute and sudden exposures. And inhalation exposure protocols were closer to ambient PM concentrations, being one or two orders of magnitude higher than the PM concentrations found in megacities. Researchers must be aware, since, the discrepancy is a result of the experimental conditions, where most protocols are planned to reduce the exposure time and increase the PM concentration, thereby attempting to achieve the same result as long time of exposure and low PM concentrations [72].

The models described above are complementary in the information about environmental pollution's impact on health. The development of models to assess exposure to air pollution within cities, to attribute the health risks produced is a priority for future research. The mechanisms of harmful health effects related to exposure to pollution are not fully elucidated in clinical and epidemiological studies, raising questions about how pathologies develop and why exposed individuals become more susceptible to certain conditions. The integrative view of epidemiological, clinal, and animal studies is required to elucidate the fundamental problem of air pollution. To reach this goal, animal models that mimic exposure to air pollution effects are appropriate to elucidate the pathophysiological mechanisms by which the body develops numerous diseases. These studies are complemented by in vitro, in silico models to explain toxicological aspects. Studies using these exposure models have effectively related the damage caused by air pollution to the development of chronic diseases, whether respiratory, cardiometabolic, or neurodegenerative, highlighting mainly the physiological effects produced for the aggravation of these conditions [73].

5. Conclusion

Experimental and epidemiological studies have shown evidence of the harmful effects of exposure to air pollution, especially by PM. The effects caused by such

particles depend on their composition, the exposure time, and the particle size, with emphasis on fine and ultrafine particles due to their multisystemic action. Animal models of PM exposure revealed clearly the pathophysiological mechanisms at the systemic and cellular levels, and the choice of the experimental protocol needs to be clear in terms of the source of the pollutant, dose, and representability of toxicological or real word aspects. Complementarily, animal studies highlighted that the increase of air pollution levels induces responses by oxidative stress, which is an initial mediator of inflammatory processes and helps to elucidate such mechanisms and contribute to the establishment of stricter policies to control emissions and air quality.

Author details

Pauline Brendler Goettems Fiorin, Mirna Stela Ludwig, Matias Nunes Frizzo and Thiago Gomes Heck*
Research Group in Physiology, Postgraduate Program in Integral Attention to Health, Regional University of Northwestern Rio Grande do Sul State (UNIJUI), Ijuí, RS, Brazil

*Address all correspondence to: gomesheck@yahoo.com

IntechOpen

References

[1] EPA. Glossary for Air Pollution Terms in Particle Pollution and Your Patients' Health [Internet]. 2021. Available from: https://www.epa.gov/pmcourse/glossary-air-pollution-terms-particle-pollution-and-your-patients-health

[2] Miller MR, Shaw CA, Langrish JP. From particles to patients: Oxidative stress and the cardiovascular effects of air pollution. Future Cardiology. 2012;**8**:577-602

[3] WHO. WHO Global Air Quality Guidelines [Internet]. 2021. Available from: https://www.who.int/news-room/q-a-detail/who-global-air-quality-guidelines

[4] EPA. What is Particulate Matter? [Internet]. 2021. Available from: https://www3.epa.gov/region1/eco/uep/particulatematter.html

[5] World Health Organization. WHO Air Quality Guidelines for Particulate Matter, Ozone, Nitrogen Dioxide and Sulfur Dioxide: Global Update 2005. Geneva, Switzerland: WHO; 2005. pp. 1-21

[6] Conselho Nacional do Meio Ambiente (CONAMA). Resolução Nº 491/2018. Diário Of da República Fed do Bras [Internet]. 2018. Available from: http://www2.mma.gov.br/port/conama/legiabre.cfm?codlegi=740

[7] WHO. WHO Global Air Quality Guidelines. Particulate Matter ($PM_{2.5}$ and PM_{10}), Ozone, Nitrogen Dioxide, Sulfur Dioxide and Carbon Monoxide. Geneva, Switzerland: WHO; 2021. p. 300

[8] IQAir. World's Most Polluted Countries 2020 ($PM_{2.5}$) [Internet]. 2020. Available from: https://www.iqair.com/world-most-polluted-countries

[9] Cohen AJ, Brauer M, Burnett R, Anderson HR, Frostad J, Estep K, et al. Estimates and 25-year trends of the global burden of disease attributable to ambient air pollution: An analysis of data from the Global Burden of Diseases Study 2015. Lancet. 2017;**389**(10082):1907-1918

[10] Liu C, Chen R, Sera F, Vicedo-Cabrera AM, Guo Y, Tong S, et al. Ambient particulate air pollution and daily mortality in 652 Cities. The New England Journal of Medicine. 2019;**381**(8):705-715

[11] EPA. Health and Environmental Effects of Particulate Matter (PM) [Internet]. 2021. Available from: https://www.epa.gov/pm-pollution/health-and-environmental-effects-particulate-matter-pm

[12] EPA. Course Outline and Key Points for Particle Pollution. 2021. Available from: https://www.epa.gov/pmcourse/course-outline-and-key-points-particle-pollution

[13] EPA. Particulate Matter (PM) Basics [Internet]. 2021. Available from: https://www.epa.gov/pm-pollution/particulate-matter-pm-basics

[14] Gangwar RS, Bevan GH, Palanivel R, Das L, Rajagopalan S. Oxidative stress pathways of air pollution mediated toxicity: Recent insights. Redox Biology. 2020;**34**(May):101545. DOI: 10.1016/j.redox.2020.101545

[15] Kim KH, Kabir E, Kabir S. A review on the human health impact of airborne particulate matter. Environment International. 2015;**74**:136-143. DOI: 10.1016/j.envint.2014.10.005

[16] Kelly FJ. Oxidative stress: Its role in air pollution and adverse health effects.

Occupational and Environmental Medicine. 2003;**60**(8):612-616

[17] Valavanidis A, Fiotakis K, Vlachogianni T. Airborne particulate matter and human health: Toxicological assessment and importance of size and composition of particles for oxidative damage and carcinogenic mechanisms. Journal of Environmental Science and Health, Part C: Environmental Carcinogenesis and Ecotoxicology Reviews. 2008;**26**(4):339-362

[18] Pearson JF, Bachireddy C, Shyamprasad S, Goldfine AB, Brownstein JS. Association between fine particulate matter and diabetes prevalence in the U.S. Diabetes Care. 2010;**33**(10):2196-2201

[19] Lodovici M, Bigagli E. Oxidative stress and air pollution exposure. Journal of Toxicology. 2011;**2011**:1-9

[20] Xu Z, Xu X, Zhong M, Hotchkiss IP, Lewandowski RP, Wagner JG, et al. Ambient particulate air pollution induces oxidative stress and alterations of mitochondria and gene expression in brown and white adipose tissues. Particle and Fibre Toxicology. 2011;**8**(1):20

[21] Thompson JE. Airborne particulate matter: Human exposure & health effects. Journal of Occupational and Environmental Medicine. 2018;**60**(5): 392-423

[22] Ghio AJ, Silbajoris R, Carson JL, Samet JM. Biologic effects of oil fly ash. Environmental Health Perspectives. 2002;**110**(SUPPL. 1):89-94

[23] Domenico M Di. Efeitos da exposição subcrônica de residual oil fly ash (ROFA) associada à administração de resveratrol Efeitos da exposição subcrônica de residual oil fly ash (ROFA) associada à administração de resveratrol. Pós-Graduação Em Ciências Da Saúde. 2015;1-73

[24] Arantes-Costa FM, Lopes FDTQS, Toledo AC, Magliarelli-Filho PA, Moriya HT, Carvalho-Oliveira R, et al. Effects of residual oil fly ash (ROFA) in mice with chronic allergic pulmonary inflammation. Toxicologic Pathology. 2008;**36**(5):680-686

[25] Biselli PJC, Lopes FDTQS, Moriya HT, Rivero DHRF, Toledo AC, Saldiva PHN, et al. Short-term exposure of mice to cigarette smoke and/or residual oil fly ash produces proximal airspace enlargements and airway epithelium remodeling. Brazilian Journal of Medical and Biological Research. 2011;**44**(5):460-468

[26] Goettems-Fiorin PB, Grochanke BS, Baldissera FG, dos Santos AB, Homem de Bittencourt PI, Ludwig MS, et al. Fine particulate matter potentiates type 2 diabetes development in high-fat diet-treated mice: Stress response and extracellular to intracellular HSP70 ratio analysis. Journal of Physiology and Biochemistry. 2016;**72**(4):643-656

[27] Halliwell B, JMC G. Free Radicals in Biology and Medicine. 3rd ed. New York: New York Oxford Univ Press; 2007

[28] Sies H. Strategies of antioxidant defense. European Journal of Biochemistry. 1993;**215**:213-219

[29] Leni Z, Künzi L, Geiser M. Air pollution causing oxidative stress. Current Opinion in Toxicology. 2020; **20-21**:1-8. DOI: 10.1016/j.cotox.2020. 02.006

[30] Rao X, Zhong J, Brook RD, Rajagopalan S. Effect of particulate matter air pollution on cardiovascular oxidative stress pathways. Antioxidants Redox Signal. 2018;**28**(9):797-818

[31] Wang Y, Wu T, Tang M. Ambient particulate matter triggers dysfunction of subcellular structures and endothelial cell apoptosis through disruption of redox equilibrium and calcium homeostasis. Journal of Hazardous Materials. 2020;**394**(December 2019): 122439. DOI: 10.1016/j.jhazmat.2020.122439

[32] Valacchi G, Magnani N, Woodby B, Ferreira SM, Evelson P. Particulate matter induces tissue oxinflammation: From mechanism to damage. Antioxidants Redox Signal. 2020;**33**(4): 308-326

[33] Riva DR, Magalhães CB, Lopes AA, Lanças T, Mauad T, Malm O, et al. Low dose of fine particulate matter ($PM_{2.5}$) can induce acute oxidative stress, inflammation and pulmonary impairment in healthy mice. Inhalation Toxicology. 2011;**23**(5):257-267

[34] Hu W, Wang Y, Wang T, Ji Q, Jia Q, Meng T, et al. Ambient particulate matter compositions and increased oxidative stress: Exposure-response analysis among high-level exposed population. Environment International. 2021;**147**:106341. DOI: 10.1016/j.envint.2020.106341

[35] Bourdrel T, Bind M-A, Béjot Y, Morel O, Argacha JF. Cardiovascular effects of air pollution. Archives of Cardiovascular Diseases. 2017;**110**(11): 634-642

[36] Rhoden CR, Wellenius GA, Ghelfi E, Lawrence J, González-Flecha B. PM-induced cardiac oxidative stress and dysfunction are mediated by autonomic stimulation. Biochimica et Biophysica Acta—General Subjects. 2005;**1725**(3):305-313

[37] Wellenius GA, Saldiva PHN, Batalha JRF, Murthy GGK, Coull BA, Verrier RL, et al. Electrocardiographic changes during exposure to residual oil fly ash (ROFA) particles in a rat model of myocardial infarction. Toxicological Sciences. 2002;**66**(2):327-335

[38] Damaceno-Rodrigues NR, Veras MM, Negri EM, Zanchi ACT, Rhoden CR, Saldiva PHN, et al. Effect of pre- and postnatal exposure to urban air pollution on myocardial lipid peroxidation levels in adult mice. Inhalation Toxicology. 2009;**00**(00):090505075116058-090505075116059

[39] Krause M, Heck TG, Bittencourt A, Scomazzon SP, Newsholme P, Curi R, et al. The chaperone balance hypothesis: The importance of the extracellular to intracellular HSP70 ratio to inflammation-driven type 2 diabetes, the effect of exercise, and the implications for clinical management. Mediators of Inflammation. 2015;**2015**:1-12

[40] Cangeri Di Naso F, Rosa Porto R, Sarubbi Fillmann H, Maggioni L, Vontobel Padoin A, Jacques Ramos R, et al. Obesity depresses the anti-inflammatory HSP70 pathway, contributing to NAFLD progression. Obesity. 2015;**23**(1):120-129

[41] Smock RG, Blackburn ME, Gierasch LM. Conserved, disordered C terminus of DnaK enhances cellular survival upon stress and DnaK in vitro chaperone activity. The Journal of Biological Chemistry. 2011;**286**(36): 31821-31829

[42] Heck TG, Schöler CM, de Bittencourt PIH. HSP70 expression: Does it a novel fatigue signalling factor from immune system to the brain? Cell Biochemistry and Function. 2011;**29**: 215-226

[43] Kalmar B, Greensmith L. Induction of heat shock proteins for protection

against oxidative stress. Advanced Drug Delivery Reviews. 2009;**61**(4):310-318. DOI: 10.1016/j.addr.2009.02.003

[44] Kido T, Bai N, Yatera K, Suzuki H, Meredith A, Mukae H, et al. Diesel exhaust inhalation induces heat shock protein 70 expression in vivo. Inhalation Toxicology. 2011;**23**(May):593-601

[45] Baldissera FG, Santos B, Machado M, Goettems PB. Subacute exposure to residual oil fly ash (ROFA) increases eHSP70 content and extracellular-to-intracellular HSP70 ratio: A relation with oxidative stress markers. Cell Stress and Chaperones. 2018;**23**(6):1185-1192

[46] Mukhopadhyay I, Nazir A, Saxena DK, Kar CD. Heat shock response: HSP70 in environmental monitoring. Journal of Biochemical and Molecular Toxicology. 2003;**17**(5):249-254

[47] Chao HR, Hsu JW, Ku HY, Wang SL, Huang HB, Liou SH, et al. Inflammatory response and $PM_{2.5}$ exposure of urban traffic conductors. Aerosol and Air Quality Research. 2018;**18**(10):2633-2642

[48] Chen R, Li H, Cai J, Wang C, Lin Z, Liu C, et al. Fine particulate air pollution and the expression of microRNAs and circulating cytokines relevant to inflammation, coagulation, and vasoconstriction. Environmental Health Perspectives. 2018;**126**(1):1-7

[49] Schöler CM, Marques CV, da Silva GS, Heck TG, de Oliveira Junior LP. Homem de Bittencourt PI. Modulation of rat monocyte/macrophage innate functions by increasing intensities of swimming exercise is associated with heat shock protein status. Molecular and Cellular Biochemistry. 2016;**421**(1-2):111-125

[50] Heck TG, Scomazzon SP, Nunes PR, Schöler CM, da Silva GS, Bittencourt A, et al. Acute exercise boosts cell proliferation and the heat shock response in lymphocytes: Correlation with cytokine production and extracellular-to-intracellular HSP70 ratio. Cell Stress & Chaperones. 2017;**22**(2):271-291

[51] Costa-Beber LC, Goettems-Fiorin PB, dos Santos JB, Friske PT, Frizzo MN, Heck TG, et al. Ovariectomy enhances female rats' susceptibility to metabolic, oxidative, and heat shock response effects induced by a high-fat diet and fine particulate matter. Experimental Gerontology. 2021;**145**:1-12

[52] Costa-Beber LC, Goettems-Fiorin PB, dos Santos JB, Friske PT, Heck TG, Hirsch GE, et al. Ovariectomy reduces the cardiac cytoprotection in rats exposed to particulate air pollutant. Environmental Science and Pollution Research. 2021;**28**(18):23395-23404

[53] Beber LCC, da Silva MOAF, dos Santos AB, Mai AS, Goettems-Fiorin PB, Frizzo MN, et al. The association of subchronic exposure to low concentration of PM 2.5 and high-fat diet potentiates glucose intolerance development, by impairing adipose tissue antioxidant defense and eHSP72 levels. Environmental Science and Pollution Research. 2020;**27**:32006-32016

[54] Bruxel MA, Tavares AMV, Zavarize Neto LD, de Souza BV, Schroeder HT, Bock PM, et al. Chronic whole-body heat treatment relieves atherosclerotic lesions, cardiovascular and metabolic abnormalities, and enhances survival time restoring the anti-inflammatory and anti-senescent heat shock response in mice. Biochimie. 2019;**156**:33-46

[55] Qin F, Cui S, Dong Y, Xu M, Wang Z, Qu C, et al. Aerobic exercise ameliorates particulate matter-induced lung injury in aging rats. Environmental Pollution. 2021;**280**:116889. DOI: 10.1016/j.envpol.2021.116889

[56] Heck TG, Ludwig MS, Frizzo MN, Rasia-Filho AA, De Bittencourt PIH. Suppressed anti-inflammatory heat shock response in high-risk COVID-19 patients: Lessons from basic research (inclusive bats), light on conceivable therapies. Clinical Science. 2020;**134**(15):1991-2017

[57] Fajersztajn L, Saldiva P, Pereira LAA, Leite VF, Buehler AM. Short-term effects of fine particulate matter pollution on daily health events in Latin America: A systematic review and meta-analysis. International Journal of Public Health. 2017;**62**(7):729-738

[58] Rajagopalan S, Al-Kindi SG, Brook RD. Air pollution and cardiovascular disease: JACC state-of-the-art review. Journal of the American College of Cardiology. 2018;**72**(17):2054-2070. DOI: 10.1016/j.jacc.2018.07.099

[59] Sun Q, Wang A, Jin X, Natanzon A, Duquaine D, Brook RD, et al. Long-term air pollution exposure and acceleration of atherosclerosis and vascular inflammation in an animal model. Journal of the American Medical Association. 2005;**294**(23):3003-3010

[60] Yanan Shang QS. Particulate air pollution: Major research methods and applications in animal models. Environmental Disease. 2018;**3**(3):57-62

[61] Pereira CEL, Heck TG, Saldiva PHN, Rhoden CR. Ambient particulate air pollution from vehicles promotes lipid peroxidation and inflammatory responses in rat lung. Brazilian Journal of Medical and Biological Research. 2007;**40**(10):1353-1359

[62] Heck TG, Petry MR, Maslinkiewicz A, Goettems-Fiorin PB, Ludwig MS, Saldiva PHN, et al. Effects of ambient particles inhalation on lung oxidative stress parameters in exercising rats. Journal of Exercise Physiology Online. 2014;**17**(3):58-69

[63] Camargo Pires-Neto R, Júlia Lichtenfels A, Regina Soares S, Macchione M, Hilário Nascimento Saldiva P, Dolhnikoff M. Effects of São Paulo air pollution on the upper airways of mice. Environmental Research. 2006;**101**(3):356-361

[64] Chuang HC, Lin YJ, Chou CCK, Hwang JS, Chen CC, Yan YH, et al. Alterations in cardiovascular function by particulate matter in rats using a crossover design. Environmental Pollution. 2017;**231**:812-820. DOI: 10.1016/j.envpol.2017.08.082

[65] Sun Q, Yue P, Deiuliis JA, Lumeng CN, Kampfrath T, Mikolaj MB, et al. Ambient air pollution exaggerates adipose inflammation and insulin resistance in a mouse model of diet-induced obesity. Circulation. 2009;**119**(4):538-546

[66] Rhoden CR, Lawrence J, Godleski JJ, González-Flecha B. N-acetylcysteine prevents lung inflammation after short-term inhalation exposure to concentrated ambient particles. Toxicological Sciences. 2004;**79**(2):296-303

[67] Goettems-Fiorin PB, Costa-beber LC, Borges J, Friske PT. Ovariectomy predisposes female rats to fine particulate matter exposure's effects by altering metabolic, oxidative, pro-inflammatory, and heat-shock protein levels. Environmental Science and Pollution Research. 2019;**26**(20):20581-20594

[68] Zanchi AC, Venturini CD, Saiki M, Nascimento Saldiva PH, Tannhauser Barros HM, Rhoden CR. Chronic nasal instillation of residual-oil fly ash (ROFA) induces brain lipid peroxidation and behavioral changes in rats. Inhalation Toxicology. 2008;**20**(9):795-800

[69] Driscoll KE, Costa DL, Hatch G, Henderson R, Oberdorster G, Salem H, et al. Intratracheal instillation as an

exposure technique for the evaluation of respiratory tract toxicity: Uses and limitations. Toxicological Sciences. 2000;**55**(1):24-35

[70] Osier M, Oberdörster G. Intratracheal inhalation vs intratracheal instillation: Differences in particle effects. Fundamental and Applied Toxicology. 1997;**40**(2):220-227

[71] Medeiros N, Rivero DHRF, Kasahara DI, Saiki M, Godleski JJ, Koutrakis P, et al. Acute pulmonary and hematological effects of two types of particle surrogates are influenced by their elemental composition. Environmental Research. 2004;**95**(1): 62-70

[72] Curbani F, de Oliveira BF, do Nascimento MM, Olivieri DN, Tadokoro CE. Inhale, exhale: Why particulate matter exposure in animal models are so acute? Environmental Pollution. 2019;**251**:230-237

[73] Brook RD, Franklin B, Cascio W, Hong Y, Howard G, Lipsett M, et al. Air pollution and cardiovascular disease: A statement for healthcare professionals from the expert panel on population and prevention science of the American Heart Association. Circulation. 2004;**109**(21):2655-2671

Chapter 15

Advanced Oxidation Processes (AOPs) – Utilization of Hydroxyl Radical and Singlet Oxygen

Pavel Krystynik

Abstract

Considering the nature of organic contaminants in water, methods of their oxidative decomposition seem to be most appropriate for their removal from contaminated water. There are a lot of methods of chemical oxidation, however, Advanced Oxidation Processes (AOPs) seem to be the most suitable technologies for organic contaminants removal. AOPs belong to a group of processes that efficiently oxidize organic compounds towards harmless inorganic products such as water or carbon dioxide. The processes have shown great potential in treatment of pollutants of low or high concentrations and have found applications for various types of contamination. The hydroxyl radical (•OH) is oxidizing agent used at AOPs to drive contaminant decomposition. It is a powerful, non-selective chemical oxidant, which reacts very rapidly with most organic compounds. Another strong oxidizing agent, singlet oxygen, can be generated by photosensitization of phthalocyanines. Phthalocyanines are molecules based on pyrrol structures connected mainly with methionine groups (–CH=) having a metallic central atom. Illumination upon specific wavelengths initiates formation of singlet oxygen that attack organic contaminants.

Keywords: hydroxyl radicals, singlet oxygen, advanced oxidation processes, photosensitization, oxidation

1. Introduction

Considering the nature of contaminants, chlorinated hydrocarbons and other groups of organic compounds belong to the group of chemicals that have widely been used in the past due to their environmental persistence and toxicity their use has been prohibited and became greatly controlled [1–7]. They can be decomposed by many chemical oxidation processes among which Advanced oxidation processes (AOPs) prevail. AOPs represent a group of methods of chemical oxidation in liquid phase which are employed to destroy organic compounds. AOPs were developed in order to oxidize organic compounds that can be resistant or which are able to deactivate traditionally used biological stage at sewage disposal plants (these compounds are non-biodegradable) including also pharmaceutical residues [8, 9]. If the final results of chemical oxidation are just inorganic products, such as CO_2, H_2O and other

harmless inorganic compounds, we talk about complete mineralization or total oxidation. AOPs are employed to replace standard oxidation technologies, such as oxidation with $KMnO_4$, $K_2Cr_2O_7$ and $Na_2S_2O_8$ because they can oxidize many organic compounds only partially [10]. Those oxidants can also serve as secondary source of pollution, e.g. hexavalent chromium ions are environmentally non-friendly. Some of the AOPs can also be based on sulphate chemistry combined with UV irradiation or photochemical processes combined with electrochemical processes [11, 12]. The effectiveness of oxidation agents is given by their standard oxidation potentials that were listed in **Table 1** in Section 2.1.3. AOPs comprise several common features that can be briefly described as follows: [13–17].

- Hydroxyl radicals OH• are employed as reactive oxidizing agent without any restrictions to reaction mechanisms.
- OH• attack is very fast; Rate constant reaches values of 10^9 mol^{-1} dm^3 s^{-1}.
- OH• attack reveals very low selectivity; It gives very high assumption for wide utilization of AOPs for water treatment containing non-biodegradable organic compounds.
- AOPs can effectively work at ambient pressure and temperature; It brings savings in process cost.
- OH• can be generated by various methods; It gives flexibility to utilization of AOPs for solving of various practical problem.

The main disadvantages of AOPs are relatively high treatment costs and special safety requirements because of the use of very reactive chemicals (ozone, hydrogen peroxide), etc. and high-energy sources (UV lamps, electron beams, etc.). Attention is also paid to low energy sources, such as UV LED [18]. Among AOPs the following

Oxidative species	Reaction	E°[V]
Fluorine	$F_2 + 2H^+ + 2e^- \rightleftharpoons 2\ HF\ (aq)$	3.03
Hydroxyl radical	$OH\bullet + H^+ + e^- \rightleftharpoons H_2O$	2.8
Singlet oxygen	$^1O_2 + 2H^+ + 2e^- \rightleftharpoons H_2O$	2.42
Ozone	$O_3 + 2H^+ + 2e^- \rightleftharpoons H_2O + O_2$	2.07
Sodium persulfate	$S_2O_8^{2-} + 2e^- \rightleftharpoons 2\ SO_4^{2-}$	2.01
Hydrogen peroxide	$H_2O_2 + 2\ H^+ + 2\ e^- \rightleftharpoons 2\ H_2O + O_2$	1.78
Hydro peroxide radical	$2\ HO_2\bullet + 2\ H^+ + 2\ e^- \rightleftharpoons 2\ H_2O + O_2$	1.7
Potassium manganite	$MnO_4^- + 8H^+ + 5e^- \rightleftharpoons Mn^{2+} + 4H_2O$	1.68
Chloride (IV) oxide	$ClO_2 + 4H^+ + 5e^- \rightleftharpoons Cl^- + 2H_2O$	1.57
Potassium dichromate	$Cr_2O_7^{2-} + 14H^+ + 6e^- \rightleftharpoons 2\ Cr^{3+} + 7\ H_2O$	1.38
Chlorine	$Cl_2 + 2e^- \rightleftharpoons 2\ Cl^-$	1.36
Dissolved oxygen	$O_2\ (g) + 4H^+ + 4e^- \rightleftharpoons 2\ H_2O$	1.22

Table 1.
Standard redox potentials of some typical oxidative species [13].

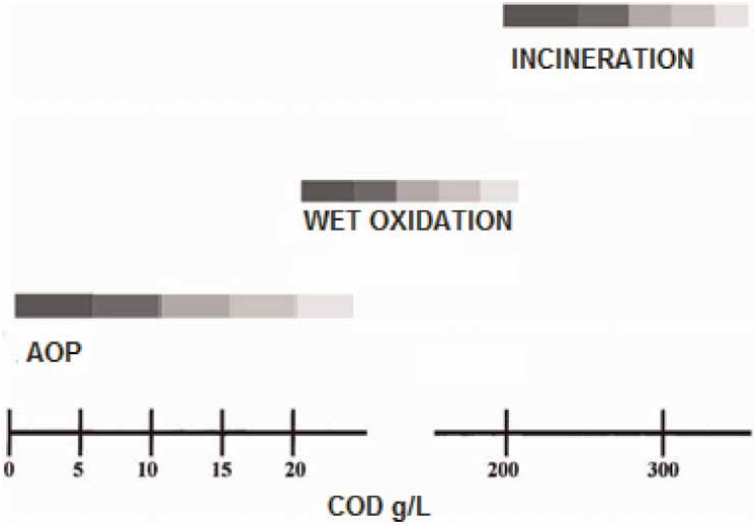


Figure 1.
Suitability of water treatment technologies according to COD contents [12].

processes can be categorized: Fenton oxidation (Fe^{2+}/H_2O_2); Fenton-like oxidation (Fe^{3+}/H_2O_2); photo assisted Fenton ($Fe^{2+/3+}/H_2O_2/UV$); photocatalysis ($TiO_2/hv/O_2$); ozone systems (O_3/H_2O_2, O_3/UV), UV photolysis (UV/H_2O_2). It is favorable to treat wastewaters with maximum content of COD = 10 to 15 g/L (chemical oxygen demand) [19]. For higher values of COD, other oxidation methods are usually applied as can be seen in **Figure 1**.

Significance of the AOPs' usage in water treatment is supported by existing registered trademarks like ULTROX®, RAYOX®, UVOX®, ECOCLEAR®. Trademarks ULTROX®, RAYOX®, UVOX® are *ex-situ* water remediation technologies utilizing ultraviolet irradiation and ozone used by company Ultrox International in California, US [20]. ECOCLEAR® is a heterogeneous catalytic ozonation process [21].

A brief summary of oxidative species used for chemical oxidation is briefly given in **Table 1**. The oxidative species are arranged according to their standard redox potentials. Standard redox potential describes capability of certain oxidizing agents for oxidation reaction. The higher the redox potential is revealed, the stronger the oxidizing agent is.

2. Fenton oxidation

Fenton oxidation is the most traditional method of AOPs. It was invented by Henry John Horstman Fenton in 1890 [22]. He discovered oxidation with reagent containing Fe^{2+} and hydrogen peroxide. The OH• production occurs by means of H_2O_2 addition to the solution containing Fe^{2+} salts:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH + OH\bullet \quad (1)$$

This is a very simple way of producing OH• requiring neither special reactants nor special reaction apparatus. Iron is naturally very abundant and non-toxic element to the environment. Hydrogen peroxide is also environment friendly chemical and easy

to store and handle. It was pointed recently that at low values of pH = 2,5–3 Fe^{3+} salts are reduced to Fe^{2+} and reaction becomes Fenton-like [23]. It is described by Eqs. (2) and (3):

$$Fe^{3+} + H^2O^2 \rightleftharpoons H^+ + FeOOH^{2+} \quad (2)$$

$$FeOOH^{2+} \rightarrow HO_2\bullet + Fe^{2+} \quad (3)$$

Fenton oxidation also exists in several modifications. One of them is the photo assisted Fenton reaction. It is the classical Fenton reaction enhanced by presence of UV–VIS irradiation [24, 25]. It utilizes a product yielding from reaction (2) and upon irradiation it yields Fe^{2+} ions and OH• as described by reaction (4).

$$Fe(OH)^{2+} + h\nu \rightarrow Fe^{2+} + OH\bullet \quad (4)$$

There are also other modifications of Fenton-like reactions, e.g. electro-Fenton, nano-Fenton utilizing graphene oxide wrapped nanoparticels of Fe3O4 [26, 27] and various modifications.

3. Photocatalytic processes

Photocatalytic processes employing semiconductor metal oxides have been the target of research interest for more than 30 years [28]. Many types of catalysts were tested and TiO_2 revealed many interesting properties (high stability, good performance and low cost) for organic compounds degradation [29, 30]. Triggering mechanism of TiO_2 photocatalysis is ultraviolet light absorption resulting in formation of electron–hole pairs.

$$TiO_2 + h\nu \rightarrow \bar{e} + h^+ \quad (5)$$

Electrons are capable of interaction with dissolved oxygen yielding superoxide radicals, and the holes are capable of oxidizing water molecules or hydroxyl anions adsorbed on the TiO_2 surface to reactive OH• radicals.

$$TiO_2\left(^{h+}\right) + H_2O \rightarrow TiO_2 + OH\bullet + H^+ \quad (6)$$

$$TiO_2\left(^{h+}\right) + OH^- \rightarrow TiO_2 + OH\bullet \quad (7)$$

Reactions 6 and 7 are of great importance in oxidative degradation of processes due to high concentration of H_2O and OH^- adsorbed on the catalyst surface. Great interest is also put on the exploitation of solar spectrum with TiO_2 but overlap between TiO_2 absorption spectra and solar spectrum is quite low [31, 32]. The emphasis is also laid on doping of TiO_2 with various metals [33, 34]. TiO_2 phtoocatalysis is also combined with SiO_2 or Al_2O_3 for enhancing photodegradability of organic compounds utilizing adsorption as synergistic effect [35, 36].

4. Ozone water system

Ozone utilization for water purification is another well-known technology [37]. Ozone is understood as one of the most efficient oxidizing agents. As can be seen in

Table 1, it is on the fourth position right after fluorine, hydroxyl radical and singlet oxygen. Ozone utilization can be intensified by addition of hydrogen peroxide and/or exploiting UV irradiation. Ozone water systems with H_2O_2 and/or UV require alkaline environment [14, 16].

$$O_3 + H_2O + h\nu \rightarrow O_2 + H_2O_2 \quad (8)$$

$$2\ O_3 + H_2O_2 \rightarrow 2\ OH\bullet + 3\ O_2 \quad (9)$$

Another oxidation process utilizing ozone is $Mn^{2+}/(COOH)_2/O_3$ that is capable of hydroxyl radical production under gently acidic conditions (at pH > 4). Under acidic conditions $Mn^{(III)}$ dioxalate and $Mn^{(III)}$ trioxalate are formed. Hydroxyl radicals are produced by reaction between manganese complexes and ozone [16, 38]. The decomposition of ozone is accompanied with hydroxyl radical generation and this decomposition is initiated by the presence of OH^-.

5. Hydrogen peroxide photolysis

Hydrogen peroxide photolysis by ultraviolet light (UV-C/H_2O_2) is one of the most effective AOPs. The UV-C/H_2O_2 system is based on the decomposition of hydrogen peroxide towards hydroxyl radicals using ultraviolet irradiation with wavelengths below 280 nm [39]. The mechanism of hydroxyl radical formation is understood as homolytic cleavage of hydrogen peroxide molecule yielding two radicals from one hydrogen peroxide molecule. On the contrary hydrogen peroxide has a small absorption coefficient (18.6 $M^{-1}\ cm^{-1}$ at 254 nm) and consequently the utilization of UV-C light source is decreased when organic compounds act as optical filters[8]. The cage effect of water molecules also decreases the efficiency of hydroxyl radical generation [15, 16]. There are also studies reporting on hydrogen peroxide photolysis upon 308–465 nm [40].

5.1 Hydrogen peroxide

It is a colorless, odorless, weakly acidic liquid similar to water having higher density and viscosity. It is miscible with water in all proportions. Hydrogen peroxide is a versatile oxidant that is effective over the whole pH range with relatively high oxidation potential (E_0 = 1.763 V at pH = 0, E_0 = 0.878 V at pH = 14) and water is the only co-product. [41].

Hydrogen peroxide can oxidize a broad variety of inorganic and organic substrates in liquid-phase reactions under mild reaction conditions. In industry H_2O_2 is used as an efficient bleaching agent in place of chlorine containing agents. Owing to its low molecular weight hydrogen peroxide is a more efficient oxidizing agent than other oxidants, such as nitric acid and sodium hypochlorite. H_2O_2 is an environment friendly compound since it decomposes towards water and oxygen (10). That makes it one of the cleanest and most versatile chemical oxidants available [42]. Hydrogen peroxide decomposition towards oxygen and water is intensified by increasing the temperature. Decomposition also occurs when hydrogen peroxide is exposed to metals.

$$2\ H_2O_2 \rightarrow 2\ H_2O + O_2 \quad (10)$$

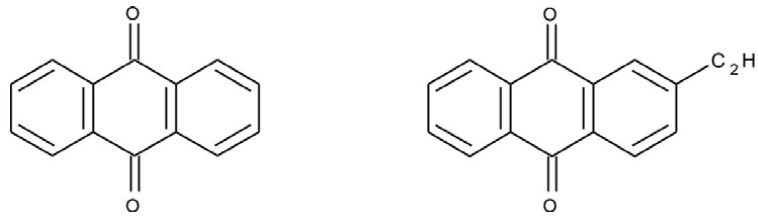


Figure 2.
Anthraquinone and 2-ethylanthraquinone.

The first historical preparation of hydrogen peroxide was performed by Luis Jacques Thenard in July, 1818 [43]. In the 1950's production of hydrogen peroxide in industrial scale started to be performed by anthraquinone way [44]. The main stages of the process are (**Figure 2**):

- Hydrogenation of anthraquinone.
- Oxidation of the resulting anthraquinol.
- Extraction of hydrogen peroxide solution.
- Purification and concentration of hydrogen peroxide.

The 2-ethyl derivate of anthraquinone is generally used in the process. A solution of the 2-ethylanthraquinone in a multi-compound mixed solvent system containing a non-polar hydrocarbons and polar solvents is hydrogenated using hydrogen. A suspension of a nickel or palladium based catalyst on a solid support is used. The reaction yields 2-ethylanthraquinol. Level of hydrogen feeding must be carefully controlled otherwise hydrogenation of aromatic rings occurs and undesired products are obtained. The catalyst is separated from the reaction mixture by filtration and it is recycled. The solution of 2-ethylanthraquinol is then oxidized by air to hydroperoxide, which reacts in water to reform 2-ethylanthraquinone and it produces a dilute solution of hydrogen peroxide. Thus the overall equation of hydrogen peroxide production is very simple:

$$H_2 + O_2 \rightarrow H_2O_2 \qquad (11)$$

The demonstrative reaction scheme for anthraquinone process is shown in **Figure 3**: Illustrative reaction scheme of hydrogen peroxide production:

Water is added to extract the peroxide as an aqueous solution containing 35% w/w solution of hydrogen peroxide. The organic solvent layer is recycled to the hydrogenation unit for further reaction. The aqueous hydrogen peroxide solution is treated with organic solvents and by air being pumped through to remove residual 2-ethylanthraquinone and solvents and produce the standard 35% product. Some applications require higher concentration product which is produced by vacuum concentration or distillation to concentrations of up to 70%.

Hydrogen peroxide in its purest form is inherently stable. However, if brought into contact with certain contaminants (mainly heavy metals) it can decompose according to Eq. (10). Typical additives enhancing stability of produced H_2O_2 are sodium compounds like stannate (IV) (which hydrolyzes to tin(IV) hydroxide), sodium citrate,

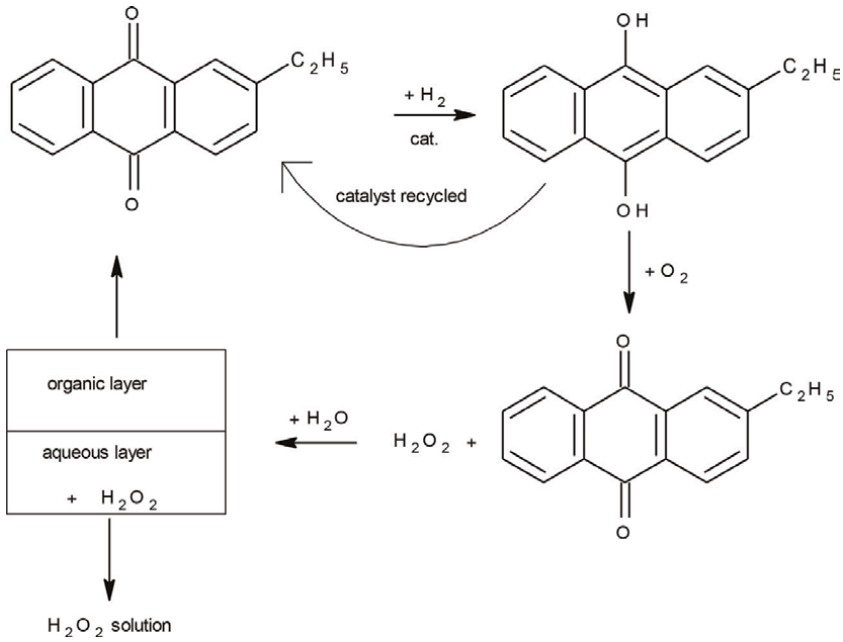


Figure 3.
Illustrative reaction scheme of hydrogen peroxide production from anthraquinone process [45].

sodium dihydrophosphate and various phosphates [46]. They act by complexing with any traces of transition metal ions present which would otherwise catalyze the decomposition of hydrogen peroxide. Acetone hydrogenation yielding propan-2-ol is an alternative to the use of 2-ethylanthraquinone. Oxidation of propan-2-ol yields hydrogen peroxide and acetone that is brought back to the beginning of the process.

5.2 Mechanism of hydrogen peroxide photolysis

The simplified mechanism of hydrogen peroxide decomposition is described as follows: [47].

$$H_2O_2 + h\nu \rightarrow 2\ OH\bullet \tag{12}$$

$$OH\bullet + H_2O_2 \rightarrow HO_2\bullet + H_2O \tag{13}$$

$$HO_2\bullet + H_2O_2 \rightarrow OH\bullet + H_2O + O_2 \tag{14}$$

$$2\ HO_2\bullet \rightarrow H_2O_2 + O_2 \tag{15}$$

$$HO_2\bullet \rightarrow O_2\bar{\bullet} + H+ \tag{16}$$

$$1/2\ O_2\bar{\bullet} + H_2O \rightarrow H_2O_2 \tag{17}$$

$$1/2\ O_2\bar{\bullet} + H_2O \rightarrow 2OH\bullet \tag{18}$$

The homolytic cleavage of hydrogen peroxide molecule yielding two hydroxyl radicals is described by Eq. (12). A certain part of hydroxyl radicals reacts with the hydrogen peroxide molecule yielding hydroperoxide radicals (Eq. 13). Hydroperoxide

radical then reacts with hydrogen peroxide yielding the desired hydroxyl radicals (Eq. 14). Eq. (15) shows radical recombination that can lead back to the hydrogen peroxide. Also superoxide radicals can be produced from hydroperoxide radicals. Superoxide radicals can be then transformed into hydrogen peroxide or hydroxyl radicals in water environment (Eqs. 17 and 18). However, superoxide radicals appear in the reaction system utilizing hydrogen peroxide decomposition in much lower extent [48, 49].

Hydroxyl radicals produced by reactions (12) to (14) can attack organic molecules and decompose them through various reaction intermediates towards inorganic products, such as CO_2, H_2O and relevant mineral acids. The formation of hydroxyl radicals increases when more alkaline conditions are used. Presence of hydroxyl anions causes formation of peroxide anion which reveals the molar absorption coefficient of 240 M^{-1} cm^{-1}. Photolysis of HO_2^- anions leads to additional formation of hydroxyl radicals [50].

$$H_2O_2 + OH^- \rightarrow H_2O + HO_2^- \tag{19}$$

$$2HO_2^- + h\nu \rightarrow 2\,OH\bullet + O_2 \tag{20}$$

5.3 Reaction mechanism of hydroxyl radicals with organic molecules

The hydroxyl radical is a particle with very low lifetime period but very high reactivity. Mechanism of organic molecule attack is dependent on the type of organic molecule. In general, there are three possible simplified mechanisms: [13].

- Attack accompanied with hydrogen abstraction.
- Attack accompanied with electron transfer.
- Attack in terms of electrophilic addition.

The hydroxyl radical generated by direct hydrogen peroxide photolysis reacts with aliphatic organic compounds to produce an organic radical (R•). These radicals react quickly with dissolved oxygen yielding the organic peroxyl radical (RO_2•) initiating subsequent thermal oxidation reactions.

$$RH + OH\bullet \rightarrow H_2O + R\bullet TiO_2 + h\nu \rightarrow \bar{e} + h^+ \tag{21}$$

$$R\bullet + O_2 \rightarrow RO_2\bullet \tag{22}$$

Subsequent thermal reactions are of three types: [13, 51].

- Heterolysis and generation of organic cations as well as superoxide anion.
- 1,3-Hydrogen shift and homolysis into hydroxyl radicals and carbonyl compounds.
- Back reaction to R• and O_2.

The second mechanism of reaction is accompanied by electron transfer. Reduction of hydroxyl radicals to hydroxide anions by an organic compound is of particular interest in the decomposition of halogenated hydrocarbons.

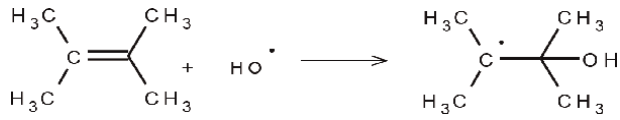


Figure 4.
Reaction scheme of electrophilic addition.

$$RX + OH\bullet \rightarrow RX\bullet^{+} + OH^{-} \quad (23)$$

The third possible mechanism is employed when organic molecules with multiple bounds react with hydroxyl radicals. This mechanism works either with aliphatic or aromatic hydrocarbons and runs similarly as electrophilic addition (**Figure 4**).

Electrophilic addition is of particular interest for an interpretation of the rapid dechlorination of chlorinated phenols yielding chloride ions. One possible pathway could in fact consist of an electrophilic addition of the hydroxyl radical to the aromatic ring and of subsequent fragmentation of the chlorohydrol intermediate followed by the ring opening (**Figure 5**).

5.4 Advantages and limits

The use of hydrogen peroxide as an oxidant brings a number of advantages in comparison to other methods of chemical or photochemical water treatment: [14–16].

- Infinite solubility in water.
- No mass transfer problems associated with gases (for example ozonation).
- Two hydroxyl radicals are formed for each molecule of H_2O_2 photolyzed.
- Peroxyl radicals are generated after HO• attack on most organic substrates, leading to subsequent oxidation reactions.
- Minimal capital investment, very cost-effective source of hydroxyl radicals, and simple operation procedure.
- UV irradiation is capable of photolytic degradation of some compounds; Only addition of hydrogen peroxide leads to total oxidation.

There are, however, also obstacles encountered with the UV-C/H_2O_2 process: [52, 53].

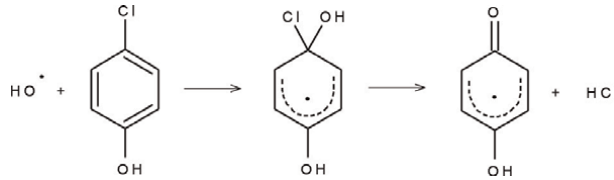


Figure 5.
Example of 4-chlorophenol attack with hydroxyl radical.

- The rate of chemical oxidation of the contaminant is limited by the rate of formation of hydroxyl radicals.
- Molar absorption coefficient of H_2O_2 at 254 nm is only 18,6 $l \cdot mol^{-1} \cdot cm^{-1}$. It causes decrease of irradiation in cases where organic substrates act as inner filters.
- Higher rates of HO• radical formation may, nevertheless, be realized by the use of Xe-doped Hg lamps having a strong emission spectra in the region of 210–240 nm, where H_2O_2 reveals a higher molar absorption coefficient.
- Post-treatment of residual H_2O_2 might be required.
- UV light penetration is negatively affected by increasing turbidity.

The main disadvantage of oxidative processes using hydroxyl radicals as an oxidizing agents can be found in efficient trapping of HO• radicals by HCO^{3-} and CO^{3-} ions [54]. Hydroxyl radical is nonselective and, thus, can be exhausted by the presence of organic or inorganic compounds other than the contaminants of the main concern.

$$HO\bullet + HCO_3^- \rightarrow H_2O + CO_3^{\bullet-} \quad (24)$$

$$HO\bullet + CO_3^{2-} \rightarrow HO^- + CO_3^{\bullet-} \quad (25)$$

Carbonate and hydrocarbonate ions act then as scavengers of hydroxyl radicals. Similar observations were also made for ammonia [55]. Although, the generated carbonate radical anion has been shown to be an oxidant itself. Its oxidation potential is, however, significantly lower than that of the HO• radical [56].

5.5 Reactors for UV-C/H_2O_2 oxidations

Most conventional UV reactors are often available in tubular arrangements and can either be an open channel or a closed vessel [57]. For large scale operation of these reactors, multistage arrangement (reactors in series) is recommended [58]. Ideal characteristic features of the tubular reactor arrangements are uniform flow through the inside of the reactor, high length-to-width ratio, flow from the bottom to the top[48]. Production of UV irradiation requires energy to supply UV lamps. Low and medium pressure mercury vapor lamps are considered to be the suitable option [59]. Another possible option is to use pulsed UV xenon arc lamps [60]. Low pressure lamps emit their maximum energy photons at wavelength of 254 nm, while medium pressure lamps emit photons with wavelengths ranging from 180 to 1370 nm with maximum between 200 and 400 nm [61].

Typically, low-pressure lamps are closed in a quartz sleeve to separate the water stream from the lamp surface or alternatively the treated water can flow through the quartz tube surrounded by UV lamps [62, 63]. Although Teflon sleeves are also an alternative to quartz sleeves, quartz sleeves absorb only 5% of the UV radiation, while Teflon sleeves absorb 35% [59]. The UV lamps can be arranged in different ways in the reactor, depending on the required scale. A system designed for large scales (water flows over 1000 m^3/h), would typically consist of one single reactor vessel equipped

with several UV lamps arranged perpendicularly or parallel to the water flow [64]. The more water needs to be treated, the more lamps and possibly more reactors in series are recommended. Generally, no or simple cooling system is required for systems handling large volumes of effluent, since heat transfers from the lamps are usually low (<1°C) [65].

Hydrogen peroxide introduction into the treated water is one of the most critical points of the oxidation process. It must not come to the contact with any metallic parts otherwise undesired decomposition of H_2O_2 is initiated. The reaction rate between hydrogen peroxide and organic compound determines the point of the H_2O_2 addition [66]. A good homogenization of reaction mixture must be ensured. The simplest method of hydrogen peroxide dosing is the gravity feed through mixing valve but this method is rather difficult to control the rate of dosing. Another easy option is the pump feeding which is very easy to maintain and to control the rate of hydrogen peroxide dosing. For water treatment 30–50% H_2O_2 solutions are recommended. Higher concentrations (≈ 70%) would increase the reaction rates, but these are not already safe because they can produce detonable mixtures upon storage [67].

5.6 Perspectives of AOPs

Many AOP processes use UV radiation, but alone UV/O_2 photooxidation is generally effective when using ionizing (energy>5 eV) or polychromatic UV radiation. The energy of UV-A and UV-B radiation is for degradation of most organic substances by direct photolysis insufficient. The low-pressure mercury lamp is a source of UV-C radiation with a wavelength of 253.7 nm, which is itself for removal most organic pollutants only partially effective. Therefore, this source is often combined with H_2O_2, O_3 and also with catalysts Fe^{2+}, Fe^{3}+ Mn^{2+} or TiO_2 in practice. The use of photolysis in application of polychromatic radiation, such as available medium-pressure mercury lamp (emitting radiation in the area 254–400 nm) can also be applied. This approach reveals advantages of both generations of radicals OH•, as well as for the direct excitation of organic molecules absorbing in UV region, such as benzene, toluene, xylene, dinitrotoluene or chlorophenols. However, the oxidation reaction rate of the pollutant tends to increase with increasing concentration of hydrogen peroxide, which is a source of OH radical. The H_2O_2 concentration must be carefully optimized because reaction system overdosed with H_2O_2 will result in undesired decomposition towards H_2O and O_2. Also, natural presence of scavenging ions may decrease applicability of the process.

6. Phthalocyanines

Phthalocyanines are macro-cyclic compounds that are derived from the group of heterocyclic compounds containing four pyrrol structures connected mainly with methionine groups (–CH=). A typical structure of metallic phthalocyanine is shown in **Figure 6**. Unlike porphyrines, phthalocyanines contain four benzene rings bounded on basic pyrrol structures. Phthalocyanines are, due to their photochemical properties, often labeled as photosensitizers [68]. Photosensitizers are compounds containing chromophores. Chromophore is a part of molecule responsible for light absorption. Absorption of light photon causes excitation to higher energy layer and reaction with molecular oxygen yields singlet oxygen species [69].

It must be reminded that solubility of phthalocyanine in water is very low. Modification by halogenation, chlorine methylation, sulphonation or sulphochlorination influences their solubility in both polar and non-polar solvents [70].

There are two major methods of phthalocyanine productions. The first is based on reaction of phthaloimide with relevant metal, metal chloride or metal alkoxide in quinoline solvent. Presence of urea enhances the reaction pathway with use of metal chlorides because it blocks chlorination of phthalocyanine structure [71]. Second method is based on the reaction of urea and phthalanhydride yielding di-iminophthaloimide. Di-iminophthaloimide then reacts with relevant metal chloride with sodium molybdate as catalyst [72]. First method is mostly performed as batch process; the second is carried out continuously.

Phthalocyanine produced by both batch and continuous methods reveals similar structure to the one showed in **Figure 6**. A characteristic metallic atom is bounded in the center of phthalocyanine molecule. However, as mentioned before, this raw phthalocyanine is insoluble in water. To enhance the solubility, benzene rings are modified by sulphonation or sulphochlorination [70]. The main area of phthalocyanine utilization covers dyes and pigments. Their share on the market is 30% of all produced organic dyes.

6.1 Mechanisms of singlet oxygen generation

It is known that phthalocyanines are capable to generate singlet oxygen when interact with visible light [73]. Singlet oxygen is a form of oxygen molecule in its excited state where all electrons are coupled. Singlet oxygen evinces high lifetime period. Stability of singlet oxygen species is dependent on reaction environment [74].

The general mechanism of the phthalocyanine interaction with light is usually described by two types of reaction mechanisms [75–77]. For both mechanisms, interaction of phthalocyanine with visible light photons occurs, mostly at wavelength of 670 nm. **Figure 7** depicts absorption spectra of zinc phthalocyanine (ZnPC). Strong absorption area in visible part of spectra enables photon absorption followed by excitation. The double peak in visible area indicates a presence of both mono- and

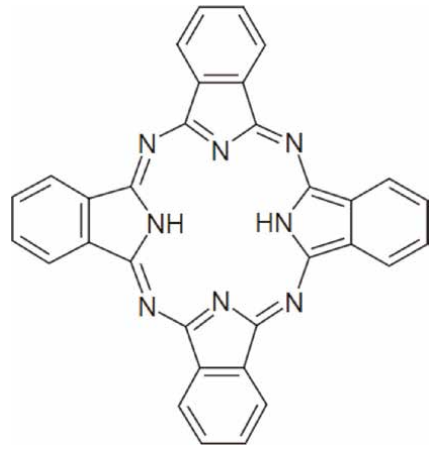


Figure 6.
Basic structure of the phthalocyanine molecule.

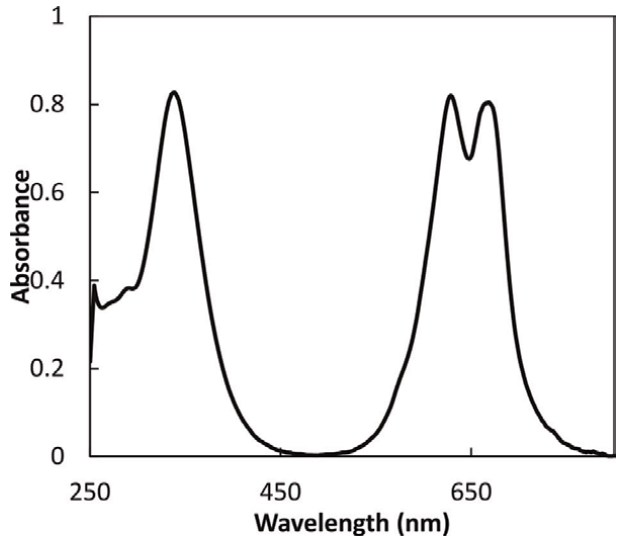


Figure 7.
Absorption spectra of ZnPC.

disulphonated forms of ZnPC. Absorption of photons at 670 nm causes excitation of phthalocyanine molecule towards the excited singlet state.

It starts with its absorption of photon(s) followed by intersystem crossing from the excited singlet state to the low-lying triplet state.

$$PC + h\nu \rightarrow {}^{1}PC* (isc) \rightarrow {}^{3}PC* \tag{26}$$

The triplet state of phthalocyanine transfers its energy to an oxygen molecule. The first mechanism involves hydrogen-atom abstraction or electron-transfer between the excited phthalocyanine and another molecule (e.g. solvent, air oxygen), forming free radicals and ion-radicals [78, 79]. These radicals can then react with ground-state oxygen to form an active species such as the superoxide radical anion.

$$^{3}PC* + O_2 \rightarrow PC^{\bullet} + O_2^{\bar{\bullet}} \tag{27}$$

$$PC^{\bar{\bullet}} + O_2 \rightarrow PC + O_2^{\bar{\bullet}} \tag{28}$$

$$½\ O_2^{\bar{\bullet}} + H_2O \rightarrow H_2O_2 \tag{29}$$

$$½\ O_2^{\bar{\bullet}} + H_2O \rightarrow 2\ OH^{\bullet} \tag{30}$$

$$O_2^{\bar{\bullet}} \rightarrow {}^{1}O_2 \tag{31}$$

This mechanism causes an electron transfer from phthalocyanine to oxygen molecule yielding superoxide anion-radical (Eqs. 27 and 28). Superoxide anion-radical tends to hydrogen peroxide formation in water environment and further produces hydroxyl radicals as active species (Eqs. 29 and 30) or it can be transferred towards singlet oxygen (Eq. 31) [80].

The second mechanism comprises interaction of triplet phthalocyanine state with molecular oxygen. Energy from triplet phthalocyanine transfers to the oxygen and the upcoming release of energy causes formation of singlet oxygen and basic state of phthalocyanine [81].

$$^{3}PC* + O_2 \rightarrow PC + {}^{1}O_2 \quad (32)$$

6.2 Mechanisms of singlet oxygen interactions with organic molecules

As already mentioned singlet oxygen is a particle of long lifetime depending on the environment where it currently exists. And also the reaction mechanism depends on type of organic molecule which enters the reaction with singlet oxygen. It is supposed that singlet oxygen interacts with organic compounds upon yielding endoperoxides and superoxide radicals or hydrogen peroxide via mechanisms resulting in one- or two-electron transfer [82].

In case of substituted aromatic hydrocarbon (e.g. 4-chlorophenol) chlorine cleavage can be observed yielding either *p*-benzoquinone or *p*-hydroquinone. Following reactions with singlet oxygen lead to aromatic ring opening (**Figure 8**).

6.3 Advantages and limits

The use of phthalocyanines as catalysts producing singlet oxygen reveals several advantages to other methods of chemical or photochemical water treatment: [83–86].

- Thermal stability of the phthalocyanines.
- Solubility in water is possible to enhance by substitution of aromatic rings.
- No mass transfer problems.
- Possibility of artificial visible light utilization.

Difficulties related with the processes utilizing phthalocyanines as photosensitizers can also be found: [87].

- The rate of chemical oxidation of the contaminant is limited by the rate of formation of singlet oxygen.
- Crude form of phthalocyanine is insoluble in water thus modification has to be performed.
- Too high concentrations cause dark color of the treated water and light photons cannot reach the entire irradiated volume.

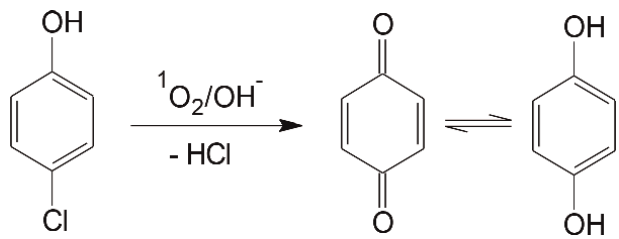


Figure 8.
Simplified reaction scheme of 4-chlorophenol attack by singlet oxygen.

Phthalocyanine catalyzed oxidations have been widely described in the laboratory in cuvette arranged on optical bench [88–90]. Also performance of such catalyzed reaction in micro reactor arrangement is coming to the fore [91, 92]. Immobilization of phthalocyanines on various supports is also well known process but it does not work sufficiently [93, 94]. All these processes and many others are performed only at laboratory scale. First quarter scale experiments performed with phthalocyanines acting as photosensitizers have been described recently [95]. However, if the phthalocyanines successfully mineralize all organic contaminants in water, phthalocyanine removal needs to be applied as in treated water it would act as coloring contaminats. For such purposes, activated carbon might be effective.

7. Summary

Advanced oxidation processes for hydroxyl radicals and singlet oxygen species were introduced and discussed according to their mechanisms and applicability for water treatment. The emphasis was put on hydrogen peroxide photolysis in UV-C region but other AOPs were also introduced. Phthalocyanine utilization as photosensitizer for generation of singlet oxygen, as another strong oxidizing agent was also discussed. It has been demonstrated that applicability potential of such technologies exists. AOPs based on hydroxyl radicals' generation reveal higher potential for application because they usually don't require addition of coloring chemicals (as phthalocyanines).

Acknowledgements

Financial support of Technology Agency of the Czech Republic (Project No. SS02030008) and Ministry of Education, Youth and Sports of the Czech Republic (Project No. CZ.02.1.01/0.0/0.0/17_048/0007435) is gratefully acknowledged. Part of the work was provided by research infrastructure NanoEnviCz (Project No. LM2018124).

Conflict of interest

The author declares no conflict of interest.

Author details

Pavel Krystynik[1,2]

1 Faculty of Environment, University of J.E. Purkyne, Usti nad Labem, Czech Republic

2 Institute of Chemical Process Fundamentals, Academy of Sciences of the Czech Republic, Prague, Czech Republic

*Address all correspondence to: krystynik@icpf.cas.cz

IntechOpen

References

[1] A. Moretto, Comprehensive Toxicology (Second Edition), Volume 13, 2010, 445-457.

[2] Tsygankov, V., Lukyanova, O., Boyarova M.: Marine Pollution Bulletin 2018, 128, 208-213.

[3] Campillo, J.A., Fernández, B., García, V., Benedicto, J., León, V.M.: Chemosphere 2017, 182, 584-594.

[4] Bapayeva G., Issayeva, R., Zhumadilova, A., Nurkasimova, R.: Reproductive Toxicology 2016, 65, 67-75.

[5] Muzyed, S. K. I., Kucuksezgin, F., Tuzmen N.: Marine Pollution Bulletin 2017, 119, 247-252.

[6] Trojanowicz M., Bojanowska_ Czajka A., Bartosiewitz I., Kulisa K.: Chem. Eng. J. 2018, 336, 170-199.

[7] Milun, V., Lušić, J., Despalatović M.: Chemosphere 2016, 153, 18-27.

[8] Taoufik N., Boumya W., Achak M., Sillanpää M., Barka N.: J. Environ. Manag. 2021, 288, 112404-112425.

[9] Rivero-Huguet, M.; Marshall, W. D. Chemosphere 2011, 83, 668-673.

[10] Siegriest R. L., Urynowicz M. A., West O. R., Crimi M. L., Lowe K. S.: Principles and practices of In situ chemical oxidation using permanganate. Battelle press, Columbus, Ohio 2001, ISBN-13: 978-1574771022.

[11] Ghime D., Ghosh P.: dvanced Oxidation Processes: A Powerful Treatment Option for the Removal of Recalcitrant Organic Compounds. InTech Open 2019. ISBN: 978-1-83880-971-3.

[12] Dewil R., Mantzavinos D., poulios I., Rodrigo M.A.: J. Environ. Manag. 2017, 195(2), 93-99.

[13] Dušek, L. Chem. Listy 2010, 104, 846-854.

[14] Badawya, M. I.; Ghalyb, Y.; Gad-Allaha, T. A. Desal. 2006, 194, 166-175.

[15] Legrini, O.; Oliveros, E.; Braun, A. M. Chem. Rev. 1993, 93, 671-698.

[16] Andreozzi, R.; Caprio, V.; Insola, A.; Marotta, R. Catal. Today 1999, 53, 51-59.

[17] M'Arimi M.M., Mecha C.A., Kiprop A.K., Ramkat R.: Renew. Sustain. Energy Rev. 2020, 121, 109669-109686.

[18] Jamalia, A.; Vanraes, R.; Hanselaer, P.; Van Gerven, T. Chem. Eng. Process.: Process Intens. 2013, 71, 43-50.

[19] Mishra, V. S.; Mahajani, V. V.; Joshi, J. B. Ind. Eng Chem. Res. 1995, 34, 2-48

[20] Lehr, J. H. Wiley's Remediation Technologies Handbook: Major Contaminant Chemicals and Chemical Groups. John Wiley & Sons, 2004, ISBN: 978-0-471-45599-8.

[21] Kaptijn, J. P. Ozone Sci. Eng. 1997, 19, 297-305.

[22] Fenton, H. J. H. J. Chem. Soc. 1894, 65, 899-911.

[23] Pignatello, J. J. Environ. Sci. Technol. 1992, 26, 944-951.

[24] Kiwi, J.; Pulgarin, C.; Peringer, P.; Gratzel, M. Appl. Catal. B: Environ. 1993, 3, 85-99.

[25] Pulgarin, C.; Kiwi, J. Chimia 1996, 50, 50-55.

[26] Zheng M., Xing C., Zhang W., Cheng Z., Liu X., Zhang Q.: Inorg. Chem. Commun 119, 2020, 108040-108047.

[27] Liu Ch., liu Y., Zeng S., Li Ch.: Appl. Surf. Sci. 544, 2021, 148886-148898.

[28] Pignatello, J. J.; Liu, D.; Huston, P. Environ. Sci. Technol. 1999, 33, 1832-1839.

[29] Ollis, D.; Al-Ekabi, H.; Eds. Photocatalytic Purification of Water and Air, Elsevier, New York, 1993.

[30] Zhang, Y.; Crittenden, J. C.; Hand, D. W.; Perram, D. L. Environ. Sci. Technol. 1994, 28, 435-442.

[31] Muruganandham, M.; Wu, J. J. Appl. Catal. B: Environ. 2008, 80, 32-41.

[32] Duran, A.; Monteagudo, J. M. Water Res. 2007, 41, 690-698.

[33] Muruganandham, M.; Swaminathan, M. Sep. Purif. Technol. 2006, 56, 101-107.

[34] Emamdoust A., La Parola V.,. Pantaleo G, Testa M. L., Farjami Shayesteh S., Venezia A. M.: J. Energ. Chem. 2020, 47, 1-9.

[35] Ramesh A., Tamizhdurai P., Mangesh V.L., Palanichamy K., Gopinath S., Sureshkumar K., Shanthi K.: J. Hydrogen Energ. 2019, 44 (47), 25607-25620.

[36] Fernández-Catalá J., Cazorla-Amorós D., Berenguer-Murcia Á.: Appl. Catal. A 2018, 564, 120-126.

[37] Rice, R. G. Ozone Sci. Eng. 1996, 18, 477-515.

[38] Song Y., Zhao Ch., Wang T., Kong Z., Zheng L., Ding H., Liu Y., Zheng H.: Water Res. 2021, 189, 116623-116632.

[39] Baxendale, J. H.; Wilson, J. A.; Trans. Farad. Soc. 1957, 16, 344-356.

[40] Song Y.L., Cui Ch.X., Liu Y.J.: J. Photochem. Photobiol. A Chem. 2016, 317, 68-71.

[41] Campus-Martin J. M., Blanco-Brieva G., Fierro J. L. G., Angew. Chem. Int. Ed. 2006, 45, 6962-6984.

[42] Dittmeyer, R.; Grunwaldt J. D.; Pashkova, A. Catal. Today 2014, 248, 149-155.

[43] Thenard, L. J. Ann. Chim. Phys. 1818, 8, 306-312.

[44] Goor G., Glenneberg J., Jacobi S.: Hydrogen Peroxide: Manufacture and Industrial Use for Production of Organic Chemicals, Springer, Netherlands 1992, ISBN 978-94-017-0984-2.

[45] Ranganathan S., Sieber V: Catalysts 2018, 8, 379-400.

[46] Apyari V.V., Terenteva E.A., Kolomnikova A.R., Garshev A.V., Dimitrienko S.G., Zolotov Y.A.: Talanta 2019, 202, 51-58.

[47] Ogata, Y.; Tomizawa, K.; Takagi, K. Can. J. Chem. 1981, 59, 14-18.

[48] Gogate, P. R.; Pandit, A. B. Adv. Environ. Res. 2004, 8, 553-597.

[49] Paul, S. M. M.; Aravind, K. U.; Pramod, G.; Aravindakumar, C. T. Chemosphere 2013, 91, 295-301.

[50] Guittonneau, S.; de Laat, J.; Dore, M.; Duguet, J. P.; Bonnel, C. Rev. Sci. Edu. 1988, 1, 85-91.

[51] Tuazon, E. C.; Aschmann, S. M.; Nishino, N.; Arey, J.; Atkinson, R. Phys. Chem. Chem. Phys. 2005, 7, 2298-2304.

[52] Glaze, W. H.; Kang, J.-W.; Chapin, D. H. Ozone Sci. Eng. 1987, 9, 335-352.

[53] Suty, H.; De Traversay, C.; Cost, M. Wat. Sci. Technol. 2004, 49, 227-233.

[54] Pignatello J. J., Oliveros E., MacKay A. Crit. Rev. Environ. Sci. Technol. 2006, 36, 1-84.

[55] Balachandran R., Zhao M., Dong B., Brown I., Raghavan S., Keswani M.: Microel. Eng. 2014, 130, 82-86.

[56] Bhattacharjee, S. Shah, Y. T. Rev. Chem. Eng. 1998, 14, 1-46.

[57] Shishida, K.; Echigo, S.; Yamada, H.; Matsui, S. J. Jpn. Soc. Water Environ. 1999, 22, 199-205.

[58] Gogate, P. R.; Pandit, A. B. Adv. Environ. Res. 2004, 8 501-551.

[59] EPA (US).: Ultraviolet Light Disinfection Technology in Drinking Water Application – An Overview. EPA 811-R96-002, 1996.

[60] Kosaka, K.; Yamada, H.; Shishida, K.; Echigo, S.; Minear, R. A.; Tsuno, H.; Matsui, S. Water Res. 2001, 35, 3587-3594.

[61] Combs, R.; McGuire, P. Ultrapure Water J. 1989, 6, 62-68.

[62] Sindelar, H. R.; Brown, M. T.; Boyer, T. H.; Chemosphere 2014, 105, 112-118.

[63] Krystynik, P.; Kluson, P.; Hejda, S.; Masin, P.; Tito, D. N. Water Environ. Res. 2014, 86, 2212-2220.

[64] Hofman-Caris, R. C. H. M.; Harmsen, D. J. H.; Beerendonk, E. F.; Knol, T. H.; Houtman, C. J.; Metz, D. H.; Wols, B. A. Chem. Eng. J. 2012, 210, 520-528.

[65] Flores M.J., Brandi R.J., Cassano A. E., Labas M.D.: Chem. Eng. J. 2012, 198-199, 388-396.

[66] Siegrist R. L., Crimi M., Simpkin T J., Eds. In Situ Chemical Oxidation for Groundwater Remediation, Springer, LLC 2011, ISBN: 978-1-4419-7825-7.

[67] Ayling G. W., Castrantas H. M.: Chem.Eng. 1981, 88, 79-82.

[68] Günsel A., Güzel E., Bilgicli A.T., Atmaca G.Y., Erdogomus A., Yarasir M. N.: J. Luminis. 2017, 192, 888-892.

[69] Ghazal B., Husain A., Ganesan A., Durmus M., Zhang X.F., Makhseed S.: Dyes and Pigments 2019, 164, 296-304.

[70] Trousil V., Cerny J., Korinkova R., Pummerova M., Mikulcova V., Herynkova M.: J. Photochem. Photobiol. A Chem. 2021, 405, 112985-112993.

[71] Kluson, P.; Drobek, M.; Strasak, T.; Krysa, J.; Karaskova, M.; Rakusan, J. J. Mol. Catal. A: Chem. 2007, 272, 213-219.

[72] Moser F. H., Thomas A. L.: Phthalocyanine Compounds. Reinhold Publ. Co., New York, Chapman and Hall, London, 1963, ISBN: 978-0278915954.

[73] Lang, K.; Mosinger, J.; Wagnerova, M. D. Chem. Listy. 2005, 99, 211-221.

[74] Derosa, C. M.; Crutchley, J. R. Coordin. Chem. Rev. 2002, 233, 351-371.

[75] Ogunbayo, T. B.; Antunes, E.; Nyokong, T. J. Mol. Catal. A: Chem. 2011, 334, 123-129.

[76] Gerdes, R.; Wohrle, D.; Spiller, W.; Schneider, G.; Schulz-Ekloff, G. J.

Photochem. Photobiol. A: Chem. 1997, 111, 65-74.

[77] Oldham, T. C.; Phillips, D. J. Photochem. Photobiol. B: Biol. 2000, 55, 1619.

[78] Cerny, J.; Karaskova, M.; Rakusan, J; Nespurek, S. J. Photochem. Photobiol. A: Chem. 2010, 210, 82-88.

[79] Ishii, K. Coordin. Chem. Rev. 2012, 256, 1556-1568.

[80] Rychtarikova, R.; Kuncova, G. Chem. Listy. 2009, 103, 800-813.

[81] Dilber G., Durmus M., Kantekin H.: Dyes and Pigments 2019, 160, 267-284.

[82] Czaplicka, M. J. Hazard. Mater. 2006, 134, 45-59.

[83] D'Alessandro, N.; Liberatore, L.; Tonucci, L.; Morvillo, A.; Bressan, M. J. Mol. Catal. A: Chem. 2001, 175, 83-90.

[84] Tonucci, L.; Nicastro, M.; D'Alessandro, N.; Bressan, M.; D'Ambrosio, P.; Morvillo, A. Green Chem. 2009, 11, 816-820.

[85] Sharma, V. B.; Jain, S. L.; Sain, B. Tetrahedron Lett. 2003, 44, 383-386.

[86] Sorokin, A. B. Chem. Rev. 2013, 113, 8152-8191.

[87] Kluson, P.; Drobek, M.; Kalaji, A.; Zarubova, S.; Krysa, J.; Rakusan, J. J. Photochem, Photobiol. A: Chem. 2008, 199, 267-273.

[88] Kluson, P.; Drobek, M.; Krejcikova, S.; Krysa, J.; Kalaji, A.; Cajthaml, T.; Rakusan, J. Appl. Catal. B: Environ. 2008, 80, 321-326.

[89] Kluson, P.; Drobek, M.; Zsigmond, A.; Baranyi, J.; Bata, P.; Zarubova, S.; Kalaji, A. Appl. Catal. B: Environ. 2009, 91, 605-609.

[90] Kluson, P.; Drobek, M.; Kalaji, A.; Karaskova, M.; Rakusan, J. Res. Chem. Intermediat. 2009, 35, 103-116.

[91] Drhova, M.; Hejda, S.; Kristal, J.; Kluson, P. Proced. Eng. 2012, 42, 1365-1372.

[92] Hejda, S.; Drhova, M.; Kristal, J.; Buzek, D.; Krystynik, P.; Kluson, P. Chem. Eng. J. 2014, 255, 178-184.

[93] Bartels, O.; Wohrle, D.; Caro, J.; Wark, M. Stud. Surf. Sci. Catal. 2004, 154, 2884-2892.

[94] Mohamed, R. M.; Mohamed, M. M. Appl. Catal. A: Gen. 2008, 340, 16-24.

[95] Krystynik, P.; Kluson, P.; Hejda, S.; Buzek, D.; Masin, P.; Tito, D. N. Appl. Catal. B: Environ. 2014, 160, 506-513.

Chapter 16

Antioxidants from Plant Sources and Free Radicals

Nurhayat Atasoy and Ufuk Mercan Yücel

Abstract

Today, many factors such as advancing technology, environmental pollution, radiation, contaminated water, pesticides, heavy metals, stress and oxygen metabolism in living cells inevitably cause the formation of free radicals in the human body. Free radicals are very reactive forms of oxygen that destroy the cells of the organism. This calls for cardiovascular disease, cancer, cataracts, diabetes and many more diseases. To provide solutions to these diseases, firstly, we can eliminate the negative effects of free radicals and prevent the formation of diseases. While there is an antioxidant defense system in the human body that can prevent this, the environmental factors encountered break down this defense resistance and sometimes make it inadequate. We can strengthen our weakened antioxidant defense systems by eating a natural and balanced diet and consuming fruits and vegetables containing antioxidants, thus preventing illness. Research shows that free radicals have a significant effect on aging, free radical damage can be controlled with adequate antioxidant defense, and optimal antioxidant nutrient intake can contribute to improved quality of life. This review is intended to highlight once again the importance of alternative antioxidants in the body to eliminate free radicals and their harmful effects.

Keywords: Oxidative stress, free radicals, antioxidant vegetables and fruits, metabolic activities

1. Introduction

1.1 Free radicals and oxidative stress

Free radicals are short-lived, reactive molecules with unpaired electrons in their outer orbits. The most important of the free radicals are superoxide radical (O2 • −), hydroxyl radical (OH), singlet oxygen ($^1O_2$) and non-radical hydrogen peroxide (H_2O_2) and peroxynitrite (ONOO-) and they are known as "reactive oxygen species (ROS)". ROS can respond easily to biological molecules such as fats, nucleic acids, proteins and carbohydrates in the body. Therefore, they are held responsible for many diseases such as aging, cancer, cardiovascular diseases, immune system diseases, cataracts, diabetes, kidney and liver diseases [1, 2]. The main reason for the harmful effects of reactive species is that they are radicals, which may lead to the formation of radicals or have a higher oxidation potential [3]. Since the reaction between reactive oxygen species and

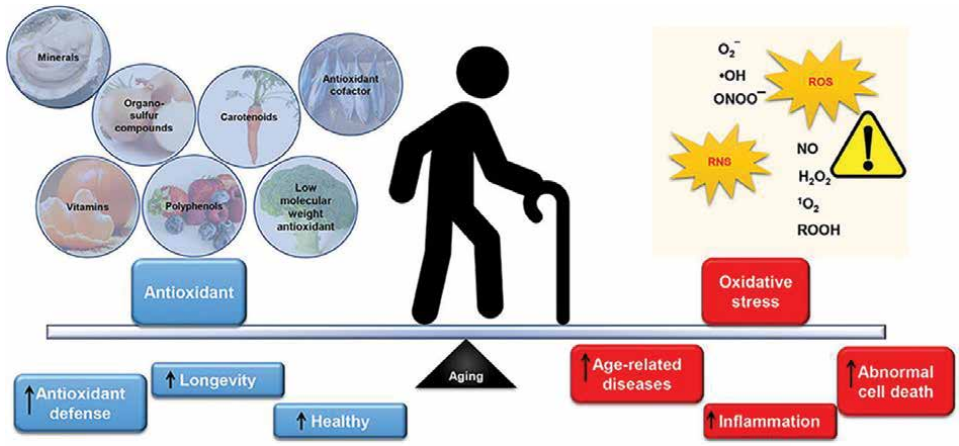


Figure 1.
Indicates the effect of oxidative stress and how aging interacts with age-related illnesses. Summarizes the dietary intake of anti-oxidants on oxidative stress in aging [4, 5].

bio-molecules is in the form of a radical chain reaction, oxidative damage is also in the form of chains. This chain reaction produces new reactive species that, in turn, damage other biomolecules (**Figure 1**) [1, 2].

Free radicals; It can be caused by activating phagocytes, antineoplastic agents, radiation, habit-forming substances, environmental agents and stress, autooxidation of small molecules, enzymes and proteins, mitochondrial electron transport systems, peroxisomes, plasma membrane and oxidative stressors [6]. Free radical formation happens continuously in cells due to enzymatic and non-enzymatic reactions. Enzymatic reactions that serve as sources of free radicals include reactions involved in the respiratory chain, phagocytosis, prostaglandin synthesis, and the cytochrome P-450 system [5, 7, 8]. Free radicals can also occur in non-enzymatic reactions of oxygen.

Some self-generated sources of free radicals [9].

- mitochondria
- xanthine oxidase
- Peroxisomes
- Inflammation
- Phagocytosis
- Arachidonate roads
- For exercise (depending on its intensity, length and type, exercise, metabolic processes and oxygen consumption increase, leading to free radical formation).
- Ischaemia/reperfusion injury.

Sources of external-produced free radicals include:

- Exposed to cigarette smoke
- Environmental pollutants
- Radiation
- Some medicines, pesticides
- Industrial solvents
- Ozone

Research shows that free radicals have a significant effect on aging, free radical damage can be controlled with adequate antioxidant defense, and optimal antioxidant nutrient intake can contribute to improved quality of life. Recent studies show that antioxidants can positively influence life expectancy. Numerous experiments have shown that DNA and RNA are susceptible to oxidative lesions. It has been reported that DNA is considered as the main target, especially in aging and cancer [10]. It has been found that oxidative nucleotides such as glycol, ditch and 8-hydroxy-2-deoxyguanosine increase during oxidative damage to DNA under UV radiation or It has been suggested that 8-hydroxy-2-deoxyguanosine can be used as a biomarker for oxidative stress [11].

1.2 Oxidative changes in DNA

DNA oxidation, along with DNA hydrolysis and DNA methylation, is a major contributor to genome instability and degradation. Spontaneous mutagenesis under aerobic conditions is greater than under anaerobic conditions, and deletion of the OxyR regulon, which prevents DNA damage in bacteria, significantly increases spontaneous mutations [11]. Among the DNA bases, guanine is the most susceptible to oxidative damage. The major mutagenic lesion is 8-Oxo-7,8-dihydroguanine (also called 8-oxoguanine or 8-hydroxyguanine), which forms base pairs with adenine instead of cytosine and therefore produces transversion mutations after replication [12]. Numerous DNA damage reactions and their degradation products have been studied [13]. The accumulation of 8-oxoguanine causes mitochondrial dysfunction and is oncogenic [14] and the enzyme human mutt homolog (MTH1), which detoxifies oxidized nucleotides, is a potential target in cancer therapy [15, 16] (**Figure 2**).

Reactive oxygen and nitrogen species such as superoxide anion, hydrogen peroxide, hydroxyl radical and nitric oxide and their biological metabolites also play an important role in carcinogenesis. The reaction of free radicals with DNA induces ROS DNA damage as it involves chain breakage, base modification and DNA protein cross-links. Numerous researchers have suggested the involvement of free radicals in carcinogenesis, mutation and transformation; their presence in the biosystem can lead to mutation, transformation and ultimately cancer [12]. The induction of mutagenesis, the best known of the biological effects of radiation, occurs mainly by DNA damage by H2O. Radical and other species are produced by radiolysis, and also direct radiation action on DNA, reaction effects on DNA. The radicals are mainly added to the double bond of the pyrimidine bases and hydrogen is abstracted from the sugar portion of the DNA

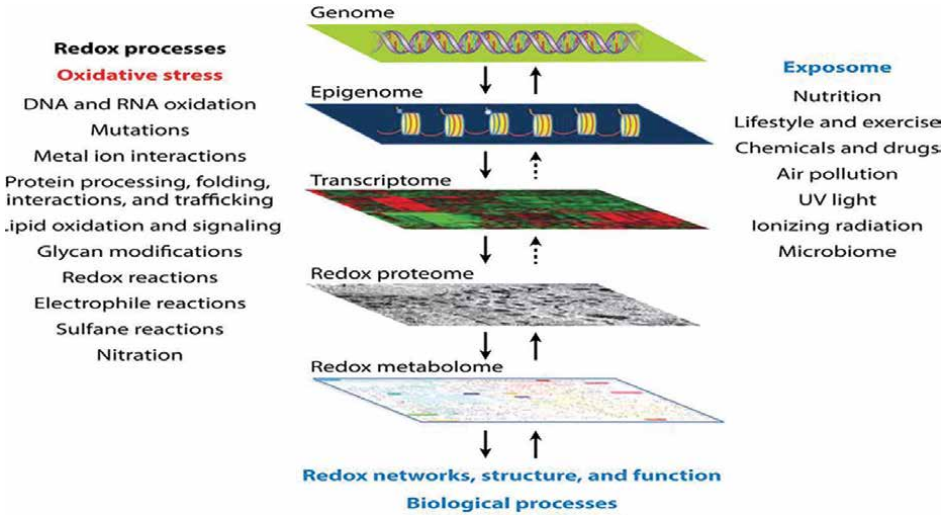


Figure 2.
Redox processes have fundamental implications in biology. Oxidative stress, either as reversible (oxidative distress) pervades all principal levels, from genome integrity and maintenance to the redox metabolism [12].

resulting in a chain reaction. These effects cause cell mutagenesis and carcinogenesis, and lipid peroxides are responsible for the activation of carcinogens [11, 17–19].

Free radicals and antioxidant balance in healthy people, this balance in unhealthy people in terms of free radicals. However, when antioxidant mechanisms are activated or this balance is shifted towards antioxidants, it becomes easier to cope with the complications of diseases caused by imbalance. They stand out as compounds that are effective in preventing diseases. Today, it has been a subject of interest, especially in the determination of the antioxidant capacity of dietary foods. In this review, some important antioxidant foods and their properties, which are consumed extensively in our country and especially in our region, will be explained [20, 21].

2. Antioxidants

There are many defense mechanisms to prevent the formation of reactive oxygen species (ROS) and the damage they cause. These mechanisms are known as "antioxidant defence systems" or "antioxidants" for short.

Antioxidants act in four ways. (1) It is the scavenging effect by affecting the free oxygen radicals, keeping them or converting them to weaker new molecules. Antioxidants, tracheobronchial mucus and small molecules exert this type of effect. (2) Reducing their activity or inactivating them by interacting with free oxygen radicals and transferring hydrogen to them is the suppressive effect. Vitamins, flavonoids have such an effect. (3) The chain-breaking effect is the effect of binding free oxygen radicals and breaking their chains and preventing their functions. Hemoglobin, ceruloplasmin and minerals show chain-breaking effects. (4) Repairing the damage caused by free radicals is a restorative effect [22].

Antioxidants can be of endogenous or exogenous origin. Antioxidants react with free radicals (bonding with them) and prevent them from damaging cells. These features increase the chance of living a healthier life with minimal effects on aging, as

they reduce the risk of cells becoming abnormal and eventually forming tumors, as well as reducing cell destruction [23].

The overload of free radicals poses a danger to the body. However, they are also necessary for the body to see its functions and to be protected from diseases. Free radicals are controlled in a very delicate balance in the body. Some mechanisms prevent, destroy or reduce oxidative damage in cells. Substances that inactivate oxidants by direct action are called antioxidants. All antioxidants exert their effects in four main ways:

1. Collector effect

2. suppressive effect

3. Chain braking effect

4. Restorative effect

Antioxidants can be divided into two main groups: natural (endogenous) and unnatural (exogenous) antioxidants [24].

2.1 Endogenous antioxidants

Endogenous antioxidants are divided into two classes, enzyme and non-enzymatic.

Endogenous antioxidants that are enzymes are: (1) Superoxide dismutase (SOD), (2) Glutathione peroxidase (GSH-PX), (3) Glutathione S-Transferases (GST), (4) Catalase (CAT), (5) Mitochondrial cytochrome oxidase system, (6) Hydroperoxidase [25, 26].

Non-enzyme endogenous antioxidants are: (1) Melatonin, (2) Ceruloplasmin, (3) Transferrin, (4) Myoglobin, (5) Hemoglobin, (6) Ferritin, (7) Bilirubin, (8) Glutathione, (9) Cysteine, (10) Methionine, (11) Urate, (12) Lactoferrin, (13) Albumin [26].

2.2 Exogenous antioxidants

They can be classified as exogenous antioxidants, vitamins, drugs, and food antioxidants.

Vitamin exogenous antioxidants are: (1) α-tocopherol (vitamin E), (2) β-carotene, (3) Ascorbic acid (vitamin C), (4) Folic acid (folate).

Exogenous antioxidants used as drugs are: (1) Xanthine oxidase inhibitors (allopurinol, oxypurinol, tearing aldehyde, tungsten), (2) NADPH oxidase inhibitors (adenosine, local anesthetics, calcium channel blockers, nonsteroidal anti-inflammatory drugs, diphenylamine iodonium), (3) Recombinant superoxide dismutase, (4) Trolox-C (vitamin E analogue), (5) Those that increase endogenous antioxidant activity (ebselen and acetylcysteine, which increase GSH-PX activity), (6) Nonenzymatic free radical scavengers (mannitol, albumin), (7) Iron redox cycle inhibitors (desferrioxamine)), (8) Neutrophil adhesion inhibitors, (9) Cytokines (TNF and IL-1), (10) Barbiturates, (11) Iron chelators [27].

Exogenous antioxidants in foods are: (1) Butylated hydroxytoluene (BHT), (2) Butylated hydroxyanisole (BHA), (3) Sodium benzoate, (4) Ethoxyquin, (5) Propylgalate, (6) Fe-superoxide dismutase [28].

2.3 Antioxidants from plant sources

2.3.1 Herbs, fruits, and vegetables

The body produces some antioxidants, which it uses to neutralize free radicals. These antioxidants are called endogenous antioxidants. However, the body relies on external (external) sources, primarily diet, to obtain the rest of the antioxidants it needs. These exogenous antioxidants are often called dietary antioxidants. Fruits, vegetables, and grains are rich dietary sources of antioxidants. Some dietary antioxidants are also available as dietary supplements [19, 29].

Examples of dietary antioxidants include beta-carotene, lycopene, and vitamins A, C, and E (alpha-tocopherol). The mineral element selenium is generally thought to be a dietary antioxidant, but the antioxidant effects of selenium are most likely due not to selenium but to the antioxidant activity of proteins containing this element as an essential component (i.e., proteins containing selenium) itself [30].

Antioxidants can reduce oxidative stress-induced carcinogenesis by inhibiting cell proliferation secondary to direct clearance of ROS and/or protein phosphorylation. Beta-carotene may be protective against cancer through its antioxidant function because oxidation products can cause genetic damage. Thus, the photoprotective properties of β-carotene may protect against ultraviolet light-induced carcinogenesis. Strengthening the immunity of β-carotene may contribute to cancer protection. B-carotene may also have an anticarcinogenic effect by altering the hepatic metabolism effects of carcinogens [31]. Vitamin C may help in preventing cancer [32]. Possible mechanisms by which vitamin C can affect carcinogenesis include antioxidant effects, blocking nitrosamine formation, strengthening the immune response, and accelerating detoxification of liver enzymes. Takes place. Vitamin E, an important antioxidant, plays a role in immune competence by increasing humoral antibody protection, resistance to bacterial infections, cell-mediated immunity, tumor necrosis factor production by T-lymphocytes, inhibition of mutagen formation, repair and blocking of membranes in DNA. microcell line formation. [25]. Therefore, vitamin E may be beneficial in the prevention of cancer and may inhibit carcinogenesis by stimulating the immune system. Administration of a blend of the three antioxidants above revealed the greatest reduction in the risk of developing heart cancer.

Oxidative stress has been postulated to play a role in many conditions, including antersclerosis, inflammatory state, certain cancers, and the aging process. Oxidative stress is now associated with all inflammatory diseases (arthritis, vasculitis, glomerulonephritis, lupus erythematous, adult respiratory diseases syndrome), ischemic diseases (heart diseases, stroke, intestinal ischemia), hemochromatosis, acquired immunodeficiency syndrome, emphysema, organ transplantation, gastric ulcer, hypertension and preeclampsia, a neurological disorder (Alzheimer's disease, Parkinson's disease, muscular dystrophy), alcoholism, smoking-related diseases and others [34]. Excessive oxidative stress can lead to oxidation of lipids and proteins in association with changes in their structure and function. Heart disease remains the biggest killer, responsible for about half of all deaths. Oxidative events can affect cardiovascular diseases, therefore; has the potential to provide enormous benefits to health and lifespan. Polyunsaturated fatty acids are found as an important part of low-density lipoproteins (LDL) in the blood, and the oxidation of these lipid components of LDL plays a vital role in atherosclerosis [35]. The three most important cell types in the vessel wall are endothelial cells.; smooth muscle cells and macrophages can release free radicals that affect lipid peroxidation [36]. With continued

highly oxidized lipids, blood vessel damage continues during the reaction process and can cause foam cells to form and plaque atherosclerosis symptoms. Oxidized LDL is atherogenic and is thought to be important in the formation of anterscleroticplaques. Also, oxidized LDL is cytotoxic and can directly damage endothelial cells. Antioxidants such as B-carotene or vitamin E play a vital role in preventing various cardiovascular diseases.

This situation becomes more evident in the later stages of the organism. Reactive species produced during oxidative stress are known to cause aging as mentioned above. Because with aging, there is an increase in the oxidative damage of reactive oxygen species on biomolecules [37, 38]. There is an antioxidant defense system against various oxidants that cause oxidative stress in the organism. This antioxidant defense system; shows its effect by preventing the excessive production of free radicals, reducing the effect of the formed free radicals or reducing or repairing the oxidative damage that occurs. These systems include endogenous antioxidant enzymes such as SOD, CAT and GPX, GSH, metal-binding proteins such as ceruloplasmin and transferrin, some antioxidant elements such as Zn and Cu, and antioxidant vitamins such as A, C, and E [7]. Aerobic organisms with antioxidant defense systems prevent the formation of reactive oxygen species (ROS) produced as a result of aerobic respiration and substrate oxidation. Small amounts of reactive oxygen species, including hydroxyl radicals (OH), superoxide anions (O2 • –), and hydrogen peroxide (H2O2), are constantly produced by aerobic organisms in response to both internal and external stimuli [39, 40].

Antioxidants are divided into primary and secondary antioxidants, according to their reaction mechanism. Some antioxidants show more than one mechanism of action and they are called multifunctional antioxidants. Primary antioxidants (type-1 or chain-breaking antioxidants) are free radical receptors that delay or inhibit the initial stage of autoxidation or interrupt the advanced stage of autoxidation [41]. In addition to these, primary antioxidants; slow down the rate of new radical generation, initiating new oxidation chains. They can act by reducing hydroperoxides (such as glutathione, peroxidase, catalase) or by removing transition metal ion catalysts (transferring) [42]. Secondary (type-2 or protective antioxidants) antioxidants have a wide variety of reaction mechanisms. These antioxidants slow down the oxidation rate but do not convert free radicals into more stable products. Secondary antioxidants can cheat and deactivate prooxidant metals, decompose hydroperoxides to non-radical species, deactivate singlet oxygen, absorb ultraviolet radiation, or act as deoxidizers.

These antioxidants generally increase the activity of primary antioxidants. Citric acid, ascorbic acid, ascorbyl palmitate, lecithin, tartaric acid, EDTA and β-carotene can be given as examples of secondary antioxidants [43]. Plants play an important role in human nutrition and life. Many plants, especially the leaves or various parts of which are consumed as vegetables, are also used for therapeutic purposes among people. As in all countries of the world, medicinally measures of plants are being researched in Turkey and these plants are being developed as a remedy for many diseases.

2.3.2 Polyphenols, flavonoids, anticyanides

Many antioxidant compounds naturally found in plant sources have been identified as free radicals or active oxygen scavengers [44]. Attempts have been made to examine the antioxidant potential of a wide variety of vegetables, such as potatoes, spinach, tomatoes, and legumes [45]. Several reports are showing the antioxidant potential of fruits [45]. Strong antioxidant activities have been found in strawberries. Cherries, citrus, prunes and olives. Green and black teas have been extensively

studied in the recent past for their antioxidant properties, as they contain 30% of the dry weight as phenolic compounds [46].

Besides dietary sources, Indian herbs also provide antioxidants, including (with common/Ayurvedic names in parentheses) *Acacia catechu* (chair), *Aegle marmelos* (Bengal quince, Bel), *Allium cepa* (Onion), *A. Sativum* (Garlic) is found., Lahasuna), *Aloe vera* (Indain aloe, Ghritkumari), Amomum subulatum (Great cardamom, Bari elachi), *Andrographis paniculata* (Kiryat), Asparagus recemosus (Shatavari), *Azadirachta indica* (Neem, Nimba), Bacopa monniera (Brahmi), *Butea monosperma* (Palas, Dhak), *Camellia sinensis* (Green tea), Cinnamon acupuncture (Cinnamon), Cinnamon tamala (Tejpat), Curcma longa (turmeric, Haridra), *Emblica officinalis* (Inhian gooseberry, Amlaki), Glycyrrhiza glapra), (Yashtim) Hemidesmus indicus (Indian Sarasparilla, Anantamul), *Indigofera tinctoria*, *Mangifera indica* (Mango, Amra), *Momordica charantia* (Bitter gourd), *Murraya koenigii* (Curry leaf), *Nigella sativa* (*Nigella sativa*), *Ocimum sanctum* (Holy basil), *Onosma echioides* (Ratanjyot), Picrorrhiza kurroa (Katuka), Piper beetle, Plumbago zeylancia (Chitrak), *Sesamum indicum*, *Sida cordifolia*, Spirulina fusiformis (Alga), Swertia decursata, Syzigium cumini (Jamun), Terminalia ariuna (Arjunica), Terminalia ariuna (Arjunica) (Beheda), Tinospora cordifolia (Heart leaf moon seeds, Guduchi), Trigonella foenum- graecium (Fenugreek), Withania somifera (Winter cherry, Ashwangandha) and Zingiber officinalis (Ginger) [47].

Indian systems of medicine believe that complex diseases, unlike western ones, can be treated with complex combinations of botanicals with single medicines. This is why in India whole foods are used as functional foods rather than supplements. Spices such as onions, garlic, mustard, paprika, turmeric, cloves, cinnamon, saffron, curry leaves, fenugreek and ginger are some medicinal plants and dietary components with functional properties. Some herbs, such as *Bixa Orellana*, and vegetables such as Emilia, wheatgrass, soybeans, and Garcinia Cambodia have antitumor effects. Other medicinal plants with functional properties include *A. marmelos* [47].

Polyphenols are plant metabolites containing various phenol groups. In this group, there are about 4000 compounds gathered in 13 classes (flavonoids, phenolic acid, anthocyanin, catechins, flavones, flavonol, flavanone, isoflavones, lignans, proanthocyanidin, procyanidin, Resveratrol, tannin). They are abundant in green tea, grapes, and soy. The most important polyphenol group is phenolic acids. It has anti-inflammatory, antiallergic, antiviral, anti-aging, anticarcinogen and antioxidant properties. It has been reported to have positive effects on the cardiovascular system [48].

Some polyphenol hydroxyls are highly reactive: I. Neutralizing free radicals by donating a hydrogen atom or an electron, II. Chelation with metal ions in aqueous solutions, III. It has the properties of binding to proteins and forming precipitation. Polyphenols can increase the antioxidant activity of oral fluids. It has been reported that tea polyphenols increase the antioxidant capacity of saliva by keeping green or black tea in the mouth for 2–5 minutes25, and consumption of 2 grapefruits per day for 2 weeks increases the phagocytic capacity of gingival sulcus fluid (DOS) neutrophils has been shown to affect [49].

Flavonoids belong to the group of Polyphenols. According to their chemical structure, flavonoids are divided into subgroups of flavanones (Ex: Luteolin), flavonols (Ex: Quercetin, kaempferol), flavan-3-ols (Ex: Catechin), anthocyanins and isoflavones. Flavonoids have attracted the attention of researchers because they are free radical scavengers, regulate enzyme activities, inhibit cell proliferation, and act as antibiotics, antiallergic, antidiarrheal, antiulcer and anti-inflammatory drugs. It has antioxidant, antiviral, antibacterial properties. They are found in vegetables, fruits,

grains, tea and some spices. It is present in high amounts in citrus fruits, blueberries, blackberries, onions, peppers, tea and parsley [50].

Antioxidants are mainly represented by vitamin C and polyphenols such as anthocyanins, phenolic acids, flavanols, flavonols and tannins. These fruits are known as natural antioxidants because of their high antioxidant concentration, berry fruits are increasingly mentioned in the literature as natural functional foods. The biological value of berries is due to the presence of components such as vitamins, provitamins and related compounds, minerals, phytosterols and phenolic compounds [50]. The health benefits of these fruits are attributed to their high antioxidant properties and phenolics, which are the main bioactive components of berry fruits [50, 51]. Blackberry (Rubus sp.), blueberry (*Vaccinium myrtillus*), red currant (Ribes rug rum), blueberry (*V. Corymbosum*), Aronia (*Aronia melanocarpa*), cranberry (*V. macrocarpon*), laurel berry (Myrica sp.), raspberry (R. Ideas), black raspberry (R. Occidentalise) and strawberry (Fragaria ananassa) are an important source of bioactive compounds that are generally consumed as fresh or processed products in human nutrition [52, 53]. Some Foods Containing Polyphenols; Green Tea has been reported to have protective effects against cancer development and cardiovascular diseases due to its catechin content. It has been shown that catechins inhibit periodontal pathogens and prevent periodontal tissue destruction [54]. Contrary to these results; In a study conducted with Korean adults, consumption of less than one cup of green tea per day was associated with a decrease in the prevalence of periodontal disease; It has been reported that consumption of green tea once or more per day increases the prevalence of moderate to severe periodontitis [55–58].

Curcumin shows antioxidant properties by reducing the oxidizing effects of free radicals as a result of the interaction of the phenolic and methylone groups in its structure with free radicals [59]. Curcumin has a scavenging effect on reactive oxygen radicals such as superoxide anion, hydroxyl radical, singlet oxygen, nitric oxide, and peroxynitrite. It can protect lipids, hemoglobin and DNA from oxidative destruction. It can also play an antioxidant role with its ability to increase the intracellular GSH release and bind to iron. Bakır et. al. administered curcumin to rats orally and observed that it increased the intracellular release of GSH, played an antioxidant role with its iron binding capacity, and reduced cellular bone loss [60].

The antioxidant activities of carotenoids are primarily increased by their conjugated double bond structures that de-localize unpaired electrons. This is related to the ability of α-carotene to physically block singlet oxygen without degradation, and to the chemical reactivity of α-carotene to radicals such as proxy, hydroxyl, and superoxide radicals. Sufficient concentrations of carotenoids protect lipids from peroxidative damage. In addition, it has antiproliferative effects on cancerous cells. Foods containing vitamin C include strawberries, papaya, red and green hot peppers, citrus fruits, fresh spices, broccoli, kiwi, melon and cauliflower. Vitamin C plays an important role in a strong immune system, collagen production, regeneration of skin, cartilage, tendons and blood vessels. Its deficiency increases the risk of various diseases, including serious diseases such as heart diseases, arthritis, and cancer [61]. Vitamin C cooperates with vitamin E to regenerate α-tocopherol from α-tocopherol radicals found in cell membranes and lipoproteins [62].

Vegetable oils such as wheat germ oil, sunflower oil, corn and soybean are very rich in vitamin E. Almond, spinach, chard, kale, ground hot pepper, asparagus, garlic and peanuts contain vitamin E. Vitamin E is a tool in chemical structure. Different compounds of tools (tocopherol and tocotrienol) show vitamin E activity. The most active form of vitamin E in humans is α-tocopherol. Its main antioxidant function

is its protective feature from lipid peroxidation. During the antioxidant reaction, αtocopherol is converted to α-tocopherol radical with the transition of the mobile hydrogen atom to the lipid or lipid peroxyl radical. The alpha-tocopherol radical can be reduced to its original form of ascorbic acid. Vitamin E acts as an antioxidant by disrupting free radical chain reactions [63].

The body produces some antioxidants, which it uses to neutralize free radicals. These antioxidants are called endogenous antioxidants. However, the body relies on external (external) sources, primarily diet, to obtain the rest of the antioxidants it needs. These exogenous antioxidants are often called dietary antioxidants. Fruits, vegetables, and grains are rich dietary sources of antioxidants. Some dietary antioxidants are also available as dietary supplements [30, 31].

Examples of dietary antioxidants include beta-carotene, lycopene, and vitamins A, C, and E (alpha-tocopherol). The mineral element selenium is generally thought to be a dietary antioxidant, but the antioxidant effects of selenium are most likely due not to selenium but to the antioxidant activity of proteins containing this element as an essential component (i.e., proteins containing selenium) [33].

Many plants with antioxidant properties belong to the Labiatae family. The genera belonging to the Labiatae family include plants with important physiological activities (antioxidant and antimicrobial) especially because they contain terpenic compounds (mono-, di-, triterpenes), flavonoids, and phenolic acids [64].

Colored flavonoids are responsible for the color formation of many fruits and vegetables. However, various colorless flavonoids can also be found in nature. Anthocyanins, which are in the flavonoid subgroup, are pigment substances that give the leaves, flowers and fruits of plants, their characteristic clear blue, red, purple, violet color. They also contain a group of important antioxidant substances. The findings are showing the in-vitro antioxidant activity of anthocyanins and anthocyanin compounds. In some of the studies, it has been determined that anthocyanins reduce the risk of cardiovascular disease and cancer, and have analgesic and antidiabetic effects [55].

Flavonoids have attracted the attention of researchers because they are free radical scavengers, regulates enzyme activities, act as antibiotics, intelligence, antidiarrheal, antiulcer and anti-inflammatory drugs [56]. The role of phenolic compounds as antioxidants has enabled natural components containing phenolic compounds to be included in various product formulations and to be widely used in functional food development studies. Many studies have reported that these compounds reduce blood cholesterol levels, have osteoporosis and anticarcinogenic effects, and have antioxidant activity. There are also opinions that these substances can inhibit unwanted bacterial infections [56].

It is estimated that there are more than 600 anthocyanins in nature and this number is expected to increase with new studies. These natural compounds are commonly found in the human diet. They are normally observed at concentrations between 0.1% and 1.0% dry weight, especially in red, blue or purple fruits and vegetables. Due to their pigmentation and structural properties, anthocyanins are also used in industry as natural colorants. Anthocyanins have been observed to reduce chronic disease risks by enhancing antioxidant defense and modulating antioxidant and inflammatory signaling pathways. In addition, it has been determined that these compounds alleviate oxidative damage and inflammation, repair DNA damage, trigger apoptosis in cancer cells, reduce lipoprotein oxidation, normalize lip profiles, improve vascular endothelial function, decrease platelet reactivity and contribute to the improvement of neurotoxicity. The majority of anthocyanins (~90%) are composed of six

common anthocyanidin glycosides: pelargonidin (Pg), cyanidin (Cy), delphinidin (Dp), peonidin (Pn), petunidin (Pt), and malvidin (Mv) [57]. People's daily anthocyanin intake is highly variable depending on their eating habits [7, 11]. The richest resources to meet this requirement are flowers and fruits. However, it is found in significant concentrations in the stems, leaves and storage organs [58]. Colorful fruits such as peaches, strawberries, pomegranates, cherries, plums, and grapes, as well as many dark vegetables (black beans, red radishes, red onions, eggplant, red cabbage, purple corn, and purple sweet potatoes), are all rich in anthocyanins [58, 59]. These molecules have been found not only in natural sources but also in processed forms in foods and beverages such as red wine, fruit juices, yoghurt and jelly [58, 59].

The high content of anthocyanins found in fruits indicates that a daily intake can be achieved with regular consumption of fruits and fruit drinks. Anthocyanins have higher antioxidant capacities (AC) than other antioxidants with free radical scavenging potential. Anthocyanins have also been observed to reduce the risk of chronic disease by enhancing antioxidant defense and modulating antioxidant and inflammatory signaling pathways [61]. The beneficial cardiovascular protective effects of polyphenols, including anthocyanins, have been demonstrated in human studies [62]. Anthocyanins are thought to exert their cardiovascular protective effects through anti-inflammation and antiplatelet activity reported to be mediated by their colonic metabolites [61]. Various studies suggest that the intake of anthocyanin-rich fruits provides some beneficial effects against age-related neurodegeneration and cognitive decline. Studies in animal models have found that the intake of freeze-dried fruits or, anthocyanin fruit extracts (plum and blackberry) delays the decline of nerve functions and improves cognitive and motor performance [62]. However, anthocyanins have been shown to alleviate oxidative damage and inflammation [63], repair DNA damage [63], trigger apoptosis in cancer cells, reduce lipoprotein oxidation [64], normalize lip profiles, improve vascular endothelial function, reduce platelet reactivity, as demonstrated in cell and animal studies. And contribute to the improvement of neurotoxicity [64]. However, anthocyanins are considered to be anti-inflammatory, anti-cancer [63], anti-hemostatic and antiobesity agents that, when taken together, can reduce cancer risk, cardiovascular diseases, and neurological disorders including Alzheimer's disease [61, 65]. The anticancer activity of anthocyanins is also demonstrated in different animal models.

For example, in mouse models of bowel cancer fed cherry extract anthocyanins, the researchers observed a 74% reduction in cecum tumors in the treated animals relative to the control group [61]. In mouse models of colon cancer fed bilberry, Aronia, or grape anthocyanins, a 26–29% reduction of abnormal crypts obtained by reducing cell proliferation and COX-2 gene expression has been observed [61]. However, in a randomized, double-blind, and placebo-controlled study of 120 dyslipidemic participants consuming 320 mg/day anthocyanin supplement (containing 17 purified anthocyanins from black currant and blueberry) for 12 weeks, circulating high-density lipoprotein (HDL) increased, cholesterol and low-density lipoprotein (LDL) cholesterol concentrations were found to decrease [66–68]. Inflammation is a complex biological response to tissue damage, associated with the onset, development and progression of cancer or tumors, provided there is a favorable microenvironment. Flavonoids have been found to have immunomodulatory properties in vitro. The antioxidant activity of tea, in which anthocyanins have several anti-inflammatory effects, is mainly due to the phenolic substances it contains.

Langley-Evans [69] stated that 35–45% of dietary antioxidants originate from tea flavonoids, and as the temperature increases during brewing, the number of

antioxidants in the brew increases. Dillard and German [70] in a study conducted on 805 men aged 65–84, reported that the average daily flavonoid intake was 25.9 mg per day, 61% of which was due to tea.

Trevisanato and Young-In Kim [71] investigated the relationship between tea consumption and some types of cancer, and in oral cancer: 37.9% partial reduction in lesions after 6 months of green tea administration, in gastric cancer: green and black tea In those who consume 7 or more cups of green tea per day. Up to 31% reduction in cancer risk, in pancreatic cancer: 12% cancer risk in men consuming green tea up to 200 g/month, 53% in women; Those who consumed more than 200 g/month saw a 43% reduction in men, 47% reduction in women and a 4% reduction in the risk of colon cancer in those who consumed 2 or more cups of black tea per day [72].

It has been reported that green tea has high antioxidant activity due to the high flavanols it contains, while black tea has high antioxidant activity due to its flavanol content as well as secondary phenolic substances formed in the enzymatic oxidation stage [71]. Berries are known to contain a wide variety of bioactive compounds such as phenolic compounds, organic acids, tannins, anthocyanins and flavonoids. The chemical structure of phenolic compounds is characterized by one or more aromatic rings with hydroxyl groups. These compounds are classified into 5 main groups according to their structural properties: phenolic acids, Stevens, flavonoids (flavonols or catechins, flavonols, flavones, flavonols, isoflavonoids, anthocyanins), tannins and lignans [71].

It has been tried to be proven by studies that anthocyanin-rich raspberry, strawberry, cherry and blueberry are very effective in preventing the emergence of some diseases that cause premature death such as some types of cancer, vascular and heart diseases [72]. Phenolic compounds in the studies (mg/100 g fresh fruit); Blueberry (*Vaccinium myrtillus*) 525 mg/100 g, [73], Blackberry (*Rubus fruticosus*) 361 mg/100 g, 417-555 mg/100 g [74, 75], Black Currant (Ribes nigrum) 318.15 mg/100 g, 498-1342 mg/100 g [76, 77]. Blueberry (Vaccinium corymbosum) 181.1-473 mg /100 g, 261-585 mg/100 g [73, 75], Raspberry (Rubus ideaus) 113.73-177.6 mg/100 g, 192-359 mg/100 g [78–80], 20 Strawberry (Fragaria ananassa) 317.2 mg/100 g [71], 443.4 102 mg/100 g [81–85].

Researching ways to lead a healthy life and prevent diseases is one of the most studied research in the medical world. For this reason, the effects of natural vegetables, plants and fruits on the human body are gaining more and more importance every day. The fact that antioxidant substances taken with plants create a protective shield against the effects of oxygen and other harmful substances entering the body, which causes the cells to deform, increases the interest in such natural products [86].

3. Result

The danger of oxidative stress manifests itself when reactive oxygen species damage cellular molecules. Damage to proteins, lipids, and RNAs is relatively reversible, but damage to DNA can lead to irreversible problems. By using more foods with high antioxidant capacity, your body's cellular damage (oxidative stress) caused by free radicals can be reduced. In addition, many factors such as environmental pollution, radiation, contaminated water, pesticides and oxygen metabolism in living cells inevitably cause the formation of free radicals [87].

Research shows that free radicals have a significant effect on aging, free radical damage can be controlled with adequate antioxidant defense, and optimal antioxidant nutrient intake can contribute to improved quality of life. Antioxidants are

natural substances found in vegetables and fruits, protecting cells from aging and rusting, preventing cancer and weakening of immunity. The antioxidant capacities of vegetables and fruits vary depending on the amount and type of antioxidant compounds they contain. Because not all foods contain these compounds in the same amount. Fruits with red-purple content such as strawberries, blueberries, cranberries, raspberries and blackberries contain a significant amount of antioxidants. Tomatoes to be consumed in their season contain high levels of vitamins A, C and folic acid. In addition, Lycopene, which increases in amount when cooked, is a very powerful antioxidant. By using more foods with high antioxidant capacity, you can reduce your body's cellular damage (oxidative stress) caused by free radicals. Studies have shown that foods with high antioxidant capacity have a high capacity to absorb free oxygen radicals that damage cells and life of man. Many plants, in particular leaves or various parts that are consumed as vegetables, are also used for therapeutic purposes amongst people. As in all countries of the world, medical measurements of plants are being researched in Turkey and these plants are under development as a remedy for many diseases [88]. There is safe data that foods with higher antioxidant capacity, protect cells better and lead to longer and healthier lives. To protect our health, we should consume plenty of fresh fruits and vegetables in the season, pay attention to the variety of foods and improve our eating habits in the light of healthy eating principles.

Author details

Nurhayat Atasoy[1*] and Ufuk Mercan Yücel[2]

1 Faculty of Science, Department of Chemistry/Biochemistry Section, Yuzuncu Yil University, Van, Turkey

2 Faculty of Veterinary Medicine, Department of Pharmacology, Van Yuzuncu Yil University, Van, Turkey

*Address all correspondence to: nurhayatatasoy@ymail.com

IntechOpen

References

[1] Diplock AT, Charleux JL, Crozier-Willi G, et al, Functional food science and defence against reactive oxygen species. British Journal of Nutrition 1998; 80:77-112.

[2] Dogra V., Kim C. Singlet Oxygen Metabolism: From Genesis to Signaling. Front. Plant Sci. 2020;10:1640

[3] Valko M, Leibfritz D, Moncol J, et radicals and antioxidants in normal functions and human pathway. International Journal of Biochemistry and Cell Biology 2007; 39(1):44-84.

[4] Tan B. L., Norhaizan M. E., Liew W.P.P., Rahman H. S. Antioxidant and Oxidative Stress: A Mutual Interplay in Age-Related Diseases. Front Pharmacol. 2018; 9:1162

[5] Fimognari C. Role of oxidative RNA damage in chronic-degenerative diseases. Oxide. Med. Cell. Longe. 2015; 2015 (26078805): 358713, Doi:10.1155/2015/358713

[6] Akkuş İ. Free Radicals and Physio-pathological Effects. Mimosa Publications, Konya. 1995;157.

[7] Liu TZ, Stern A, Morrow JD. The isoprostanes: Unique bioactive products of lipid peroxidation. J Biomed Sci 1998;5:415-420.

[8] Ebadi M. Antioxidants and free radicals in health and disease: Reactive oxygen species, oxidative damage, neuronal cell death and an introduction to therapy in neurodegenerative diseases. Arizona: Prominent Press; 2001.

[9] Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev. 2010; 4(8): 118-126.

[10] Wu X, Beecher GR, Holden JM, et al. Concentrations of anthocyanins in common foods in the United States and estimation of normal consumption. J Agric Food Chem. 2006; 54(11): 4069-4075.

[11] Woo H D, Fernandez-Jimenez N, Ghantous A, Esposti1 DD, Cuenin C, Calais V, et al. Genome-wide profiling of normal gastric mucosaidentifies Helicobacter pylori- and cancer-associated DNA methylome changes. International Journal of Cancer. 2018; 143(3).

[12] Sies H., Berndt C., Jones D.P. Oxidative Stress. Annu. Rev. Biochem. 2017; 86:715-748.

[13] Storz G, Christman MF, Sies H, Ames BN. Spontaneous mutagenesis and oxidative damage to DNA in Salmonella typhimurium. Pans. 1987; 84:8917-8921.

[14] Freudenthal BD, Beard WA, Perera L, Shock DD, Kim T, et al. Uncovering the polymerase-induced cytotoxicity of an oxidized nucleotide. Nature. 2015; 517:635-639.

[15] Cadet J, Loft S, Olinski R, Evans MD, Bialkowski K, et al. Biologically relevant oxidants and terminology, classification and nomenclature of oxidatively generated damage to nucleobases and 2-deoxyribose in nucleic acids. Free Radic. Res. 2012;46:367-381.

[16] Leon J, Sakumi K, Castillo E, Sheng Z, Oka S, Nakabeppu Y. 8-Oxoguanine accumulation in mitochondrial DNA causes mitochondrial dysfunction and impairs

neuritogenesis in cultured adult mouse cortical neurons under oxidative conditions. SCI. Rep. 2016; 6:22086.

[17] Bräutigam L, Pudelko L, Jemth AS, Gad H, Narwal M, et al. Hypoxia signalling and the cellular redox tumour environment determines sensitivity to MTH1 inhibitory. Cancer Res. 2016;76:2366-2375.

[18] Wang XJ, Gao J, Ding SL, Wang K, Jiao JQ, et al. Oxidative modification of miR-184 enables it to target Bcl-XL and Bcl-w. Mol. Cell. 2015;59:50-61.

[19] Lang A, Grether-Beck S, Singh M, Kuck F, Jakob S, et al. MicroRNA-15b regulates mitochondrial ROS production and the senescence-associated secretory phenotype through certain 4/SIRT4. Ageing. 2016;8:484-505.

[20] Hasler, C.M. Plants as medicine: The role of phytochemicals in optimal health. In Phytochemicals and Phytopharmaceuticals, edited by F. Shahidi and C.- T. Ho, pp. 1-12. Champaign, Illinois: 2000. AOAC Press.

[21] Davies KJA. Oxidative stress, antioxidant defences, and damage removal, repair, and replacement systems. International Union of Biochemistry and Molecular Biology, Life 2000; 50:279-289.

[22] Anonymous, 2021. https://tr.wikipedia.org/wiki/Antioksidan. 1.9.2021

[23] Andreas, M.,1999. Antioxidant Status, Diet, Nutrition and Health, Boca Raton (CRC Press). 672.

[24] Halliwell, B., Gutteridge, J.M.C, Cross, C.E. Free radicals, antioxidants, and human disease. J Lab Clin Med. 1992;119: 598-620.

[25] Berköz M., Yalın S., Güler G. V., Yalçın A.Akut Lösemilerde Lipid Peroksidasyonu ve Antioksidan Enzim Aktivitesi. Erciyes Tıp Dergisi (Erciyes Medical Journal). 2008; 30(3):157-162.

[26] Valko, M., Rhodes, C.J., Monco,l J., Izakovic, M., Mazur, M. Free radicals metals and antioxidants in oxidative stress-induced cancer. Chemico-Biological Interactions. 2006; 160: 1-40.

[27] Onat, T., Emerk, K., Sözmen, E., (Ed.), 2002, Human Biochemistry, Palme Publishing, Ankara.

[28] Durak K., Bilgen Ö.F., Kaleli T., Tuncel P., Özbek R. And Turan K. Antioxidant effect of alpha-tocopherol on fracture hematoma in a rabbit. J. Int. Med. Res.1996;24: 419-424.

[29] Öğüt S. The Importance of Natural Antioxidants. Journal of Adnan Menderes University Agricultural Faculty. 2014; 11(1): 25-30.

[30] Bagchi K, Puri S. Free radicals and antioxidants in health and disease. Eastern Mediterranean Health J. 1998; 4:350-360.

[31] Diplock A T, Baum, Lucy, J A. "The effect of vitamin E on the oxidation state of selenium in rat liver" Biochem J. 1971; 123, 721-732.

[32] Bouayed J, Hoffmann L, BohnT. Total phenolics, flavonoids, anthocyanins and antioxidant activity following simulated gastrointestinal digestion and dialysis of apple varieties: Bioaccessibility and potential uptake. Food Chem. 2011; 1;128(1):14-21.

[33] Davis CD, Tsuji PA, Milner JA. Selenoproteins and Cancer Prevention. Annual Nutrition Evaluation. 2012; 32:73-95.

[34] Papas AM, Determinants of antioxidant status in humans. Lipids. 1996; 31, 77-82.

[35] Glatthaar BE, Hornig DH, Moser U. The role of ascorbic acid in carcinogenesis. Adv Exp Med Biol. 1986; 206: 357-377.

[36] Sokol D L, Zhang X, Lu P, Gewirtz A. M. Real time detection of DNA. RNA hybridization in living cells. Proc Natl Acad Sci U S A. 1998; 29:95(20):11538-43.

[37] Stefanis L, Park DS, Yan CYI, Farinelli SE, Troy CM, Shelanski ML, Greene LA. Induction of CPP-32-like activity in PC12 cells by the withdrawal of trophic support: dissociation from apoptosis. J Biol Chem.1996; 271:30663-30671.

[38] Eastwood MA. Interaction of dietary antioxidants in vivo: how fruit and vegetables prevent disease? QJM 1999;92:527-530.

[39] Neuzil, J., Gebicki, J.M., Stocker, R. Radical-induced chain oxidation of proteins and its inhibition by chain-breaking antioxidants. Biochem. J. 1993;293:601-606

[40] Şerbetçı H. Determination of Antioxidant Capacity of *Glycyrrhiza glabra* L. Plant, Master Thesis, Atatürk University, Institute of Science and Technology, Erzurum, 2007.

[41] Halliwell, B. Free radicals, antioxidants and human diseases: curiosity, cause and consequences. Lancet. 1994; 344:721-724.

[42] Grune T, Jung T, Merker K, Davıes KJA. Decreased proteolysis caused by protein aggregates, inclusion bodies, plaques, lipofuscin, ceroid, and 'aggresomes'during oxidative stress, ageing, and disease. The International Journal of Biochemistry and Cell Biology.2004; 36:2519-2530.

[43] Reische Dw., Lillard Da., Eıten Miller Rr., Akoh Cc, Mınd B., editör., Food Lipids Chemistry, Nutrition and Biotechnology, New York: Marcel Dekker Inc; 2002;335-542.

[44] Wang H., G. Co and R.L. Prior. Total antioxidant capacity of fruits. J. Agric. Food Chem. 1996;44: 701-705.

[45] Lin JK, Lin CH, Ling YC, Lin-Shian SY, Juan IM. Survey of catechins, Gallic acid and methylxanthines in green, Oolong, poor and black teas. J Agric Food Chem. 1998;46:3635-3642.

[46] Devasagayam TP, Tilak JC, Boloor KK, Sane KS, Ghaskadbi SS, Lele RD. Free radicals and antioxidants in Human Health: Current status and prospects. J Assoc Physicians India. 2004;52:794-803.

[47] Petti S, Scully C. Polyphenols, oral health and disease: A review. J Dent. 2009; 37: 413-423.

[48] Gökçe C, Tonguç MÖ. Antioxidant Nutrients İn Periodontal Health. Suleyman Demirel University Faculty of Dentistry. 2018; 39-1: 19-31.

[49] Kozlowska A, Szostak-Wegierek D. Flavonoids- -food sources and health benefits. Rocz Panstw Zakl Hig. 2014; 65:9-85.

[50] Flora SJ. Structural, chemical and biological aspects of antioxidants for strategies against metal and metalloid exposure. Oxide Med Cell Longev. 2009; 2: 191-206.

[51] avikin K, Zdunić G, Menković N, Živković J. Ćuji N, Tereščenko M, et al. Ethnobotanical study on traditional use of medicinal plants in South-Western Serbia, Zlatibor district. J. Ethnopharmacol. 2013; 146, 803-810. DOI: 10.1016/j. jeep. 2013. 02. 006.

[52] Dordević TM, Šiler-Marinković SS, Dimitrijević-Branković SI Effect of

fermentation on 354 antioxidant properties of some cereals and pseudocereals. Food Chem. 2010; 119:957-963.

[53] Ścibisz I, Mitek, M. Effect processing, storage conditions on phenolic compounds and antioxidant capacity of highbush blueberry jams. Polish Journal of Food and Nutrition Sciences. 2009;59(1), 45-52.

[54] Côté, Caillet S, Doyon G, Sylvain J F, Lacroix, M. Analyzing cranberry bioactive compounds. Critical Reviews in Food Science and Nutrition. 2010; 50, 872-888.

[55] Aggarwal B, Sundaram C, Malani C, Ichikawa NH. Curcumin: the Indian solid gold. Adv Exp Med Biol. 2007; 595: 1-75.

[56] Du, Q., Zheng, J., Xu, Y. Composition of anthocyanins in mulberry and their antioxidant activity. Journal of Food Composition and Analysis. 2008;21: 390-395.

[57] Rice-Evans, C., Miller, N.J., Paganga, G., 1996. Structure-antioxidant activity relationships of flavonoids and phenolic acids, Free Rad. Biol. Med. 20: 933-956.

[58] Kussmann M, Stover PJ. Nutrigenomics and proteomics in health and disease: Towards a systems-level understanding of gene-diet interactions. 2nd. Ed. New Jersey: John Wiley and Sons; 2017.

[59] Wu X, Beecher GR, Holden JM, et al. Concentrations of anthocyanins in common foods in the United States and estimation of normal consumption. J Agric Food Chem. 2006;54(11): 4069-4075.

[60] Smeriglio A, Barreca D, Bellocco E. Chemistry, pharmacology and health benefits of anthocyanins. Phytother Res. 2016; 30(8): 1265-1286.

[61] Bakır B, Yetkin Ay Z, Büyükbayram Hİ, Kumbul Doğuç D, Bayram D, Candan IA, Uskun E. Effect of Curcumin on Systemic T Helper 17 Cell Response; Gingival Expressions of Interleukin-17 and Retinoic Acid Receptor-Related Orphan Receptor γt; and Alveolar Bone Loss in Experimental Periodontitis. J Periodontol. 2016;87(11):e183-e191.

[62] Yang M, I Koo S, O Song W, et al. Food matrix affecting anthocyanin bioavailability. Curr Med Chem. 2011;18(2): 291-300.

[63] Afacan F.Ö., A.S. Sönmezdağ. The importance of Antocianes in Nutrition and Effects on Health Karya J Health Sci. 2020; 1: 23-28

[64] Shukitt-Hale B, Kalt W, Carey AN, et al. Plum juice, but not dried plum powder, is effective in mitigating cognitive deficits in aged rats. Nutrition. 2009; 25: 567-573.

[65] Bei R, Masuelli L, Turriziani M, et al. The impaired expression and function of signalling pathway enzymes by anthocyanins: role in cancer prevention and progression. Cur Enzyme Inhib. 2009; 5: 184-197.

[66] Chen G, Luo J. Anthocyanins: are they beneficial in treating ethanol neurotoxicity? Neurotics Res. 2010; 17(1): 91-101.

[67] Shih PH, Chan YC, Liao JW, et al. Antioxidant and cognitive promotion effects of anthocyanin-rich mulberry (Morus atropurpurea L.) On senescence-accelerated mice and prevention of Alzheimer's disease. J Nutr Biochem. 2010; 21(7): 598-605.

[68] Qin Y, Xia M, Ma J, et al. Anthocyanin supplementation improves serum LDL- and HDL-cholesterol concentrations associated

with the inhibition of cholesterol ester transfer protein in dyslipidemic subjects. Am J Clin Nutr. 2009; 90: 485-492.

[69] Langley-Evans SC. Antioxidant Potential of Green and Black Tea Determined Using the Ferric Reducing Power (FRAP) Assay. Int. J Food Sci Nutr. 2000b;51: 181-188.

[70] Dillard CJ, German JB. Phytochemicals: Nutraceuticals and human health. J Sci Food Agric. 2000;80:1744-1756.

[71] Trevisanato SI, Young-In Kim MD. Tea and Health. Nutrition Reviews. 2000; 58: 1-10.

[72] Tosun İ., Karadeniz B. The Antıoxıdant Actıvıtıes of Tea And Tea Phenolıcs. J. of Fac. of Agric., Omu, 2005,20(1):78-83.

[73] Miller R, Putnam S, Edwards M. et al. Potential health benefits of black-currant: Bioactives in fruit, Health benefits and functional foods, 1st Ed. New Jersey: John Wiley & Sons; 2013:215-250.

[74] Katsube N, Iwashita K, Tsuchida T, Yamaki K, Kobori M. Induction of apoptosis in cancer cells by Bilberry (Vaccinium myrtillus) and the anthocyanins. Journal of Agricultural and Food Chemistry.2003; 51: 68-75.

[75] Prior RL, Lazarus SA, Cao, Nuccitelli H, Hammerstone JF. Identification of Procyanidins and Anthocyanins in Blueberries and Cranberries (Vaccinium Spp.) Using High-Performance Liquid Chromatography/Mass Spectrometry. J. Agric. Food Chem. 2001;49:1270-1276.

[76] Heinonen IM, Lehtonen PJ and Hopia AI. Antioxidant activity of berry and fruit wines and liquors. J. Agric. Food Chem.1998; 46: 25-31.

[77] Sellappan, Akoh, CC, Krewer, G. Phenolic compounds and antioxidant capacity of Georgia-grown blueberries and blackberries. Journal of Agricultural and Food Chemistry, 2002;50(8), 2432-2438.

[78] Borowska, J., & Szajdek, A. Antioxidant activity of berry fruits and beverages. Polish Journal of Natural Sciences, 2003; 14.

[79] Moyer RA, Hummer K E, Finn C E, Frei B, Wrolstad, RE. Anthocyanins, phenolics, and antioxidant capacity in diverse small fruits: Vaccinium, Rubus, and Ribes. Journal of Agricultural and Food Chemistry, 2002; 50(3), 519-525.

[80] De Anchos B, González EM, Cano M P. Ellagic acid, vitamin C, and total phenolic contents and radical scavenging capacity affected by freezing and frozen storage in raspberry fruit. Journal of agricultural and food chemistry, 2000;48(10), 4565-4570.

[81] Skupień K, Oszmiański J. Influence of titanium treatment on antioxidants content and antioxidant activity of strawberries. Acta Scientiarum Polonorum Technologia Alimentaria, 2007;6(4), 83-93.

[82] Anttonen M J, Karjalainen R O. Environmental and genetic variation of phenolic compounds in red raspberry. Journal of Food Composition and Analysis.2005; 18(8), 759-769.

[83] Zheng W, Wang, SY. Oxygen radical absorbing capacity of phenolics in blueberries, cranberries, chokeberries, and lingonberries. Journal of Agricultural and Food Chemistry, 2003;51(2), 502-509.

[84] Zheng Y, Wang SY, Wang, CY, Zheng W. Changes in strawberry phenolics, anthocyanins, and antioxidant capacity in response to high oxygen

treatments. LWT-Food Science and Technology. 2007; 40(1): 49-57.

[85] Çağlar MY. Demirci M. Phenolic Compounds in Grape Berries and Their Importance in Nutrition. European Journal of Science and Technology.2017; 7:11, 18-26.

[86] Etherto PMK, Hecker KD, Bonanome, Coval SM, Binkoski AE, Hilpert KF, Griel A, Etherton, TD. Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. The American Journal of Medicine. 2002; 113: 71-85.

[87] Kaur, C. & Kapoor, H.C. Antioxidants in fruits and vegetables –the millennium's health. International Journal of Food Science & Technology. 200136;703-725.

[88] Arıduru R, Determination of antioxidant activities of some medicinal plants. Master Thesis, Sakarya University Institute of Science and Technology. 2013.

Edited by Rizwan Ahmad

The term "reactive oxygen species" (ROS) refers to a group of reactive molecules and free radicals produced by molecular oxygen. In recent decades, there has been great interest in the role of ROS in various diseases. From basic science research to clinical trials, biomedical scientists have made rapid progress toward a better understanding of ROS-metabolizing systems and their role in health and diseases. This book includes sixteen chapters that address topics such as the history of ROS, its role in autoimmunity, neurodegeneration, and aging, and recent advances in various antioxidants and their therapeutic potential.

Miroslav Blumenberg, Biochemistry Series Editor

ISSN 2632-0983
ISBN 978-1-83968-283-4
Published in London, UK

IntechOpen