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Botulinum Toxin

Edited by Nikolay Serdev

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Meet the editor

Prof. Dr. Nikolay Serdev, MD, PhD, is a renowned cosmetic surgeon. He has trained hundreds of doctors globally in minimally invasive aesthetic surgery and medical procedures as well as in his authoring techniques.
He is the creator of Scarless Serdev Suture® lifts of face and body and a pioneer in many other mini-invasive cosmetic surgery techniques: ultrasound liposculpture of the face, body and leg elongation, and beautification; T-excision and columella sliding in rhinoplasty; non-surgical body contouring; and their combination with his own suture to facial rejuvenation, beautification, and tissue volumisingvolumizing. He is also a world authority on ultrasound-assisted (VASER) body contouring and has trained doctors from around the world in its basic and advanced techniques.
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He is an editor and author of several comprehensive textbooks in the field of cosmetic and plastic surgery, including liposuction, sutures lifts on face and body areas, thread versus suture lift techniques, and rhinoplasty and has authored many chapters in aesthetic surgery and medicine books, as well as original papers in medical journals.

Contents

Preface

When botulinum toxin binds presynaptically to the cholinergic nerve terminals and decreases the release of acetylcholine, it causes a neuromuscular blocking effect, which is the basis on which it is prescribed and used as therapeutic medicine.

Botulinum toxins nowadays are used in the treatment of a wide variety of medical conditions and the list of possible new indications is rapidly expanding. They have been utilized for a variety of muscular hyperactivities, including blepharospasm, hemifacial spasm, and cervical dystonia, but also in strabismus, headaches, hypersalivation, hyperhydrosis, and other conditions that do not respond well to other medical treatments. Cosmetic use is now most important and widespread.

This book aims to review some of the therapeutic uses of botulinum toxin injections on the basis of important mechanisms of action, doses, safety, clinical uses, and side effects.

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Chapter 1

Botulinum Toxins, Diversity, Mode of Action, Epidemiology of Botulism in France

Michel R. Popoff

Additional information is available at the end of the chapter

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Abstract

Botulinum toxins (BoNTs) are the most potent toxins and are responsible for botulism, which is a neurological disease in man and animals. Botulism is characterized by flaccid paralysis and inhibition of secretions. BoNTs are produced by distinct clostridial species including *Clostridium botulinum* that consist in four physiological and genetic groups, atypical strains of *C. baratii* and *C. butyricum*. Recently, nonclostridial bacteria have been found to synthesize BoNTs. The particularity of BoNTs is to associate with nontoxic proteins to form large-size complexes that are resistant to acidic pH and protease degradation of the digestive tract. BoNTs are divided into 10 types based on neutralization by specific antisera and into more than 40 subtypes according to their sequence variations. All BoNTs retain a common core structure and mode of action, which consists in the inhibition of neurotransmitter release, notably acetylcholine. Human botulism occurs in three main forms: foodborne botulism, botulism by intestinal colonization including infant botulism, and wound botulism. In France, type B foodborne botulism is the most prevalent form, resulting from the traditional consumption of pork products such as home-made cured ham. Albeit less frequent, human botulism is still present in France including diverse types and origins.

Keywords: botulism, botulinum toxin, *Clostridium botulinum*, flaccid paralysis

1. Introduction

Botulinum toxins (BoNTs) are the most potent toxins among bacterial, animal, and plant toxins. Indeed, the lethal activity as tested in laboratory animals by determining the lethal dose 50% (LD50) is the lowest compared to that of all other toxins. Because of its extreme lethal potency,

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BoNTs are considered as the greatest threat of toxin weapon and are classified as Category A threat agent by the Centers for Disease Control and Prevention Select Agent Program [1]. In the natural conditions, BoNTs are responsible for a neurological disease in man and animals, botulism, which is characterized by flaccid paralysis and inhibition of secretions. Outbreaks of animal botulism are worldwide distributed and cause important economic losses, notably in cattle and farmed birds. Human botulism is much rarer than animal botulism, but it is a severe disease often fatal without treatment. Human botulism is the most severe food poisoning, and botulism surveillance by health and food authorities is performed in most of the countries in order to rapidly identify and withdraw contaminated foods and also to address recommendations to industrials and consumers regarding hygiene and food preservation practices. However, the paralytic effects of BoNTs are used in the treatment of numerous diseases including muscle hyperactivity such as dystonia, strabismus, limb spasticity, sphincter dysfunction, or hypersecretion (hyperhidrosis, hypersialorrhea, and drooling in neurodegenerative diseases), but also in the treatment of pain and in cosmetology. BoNTs are largely used as therapeutic drugs and are one of the drugs that have the most numerous medical indications [2, 3].

Botulism was described in the second part of the eighteenth century and at the beginning of the nineteenth century by Steinbuch (1817) and Kerner (1817–1822). Both described a particular form of foodborne poisoning due to ingestion of a "sausage poison." An increased number of fatal food poisoning cases occurred at the end of eighteenth century in the southwest German region of Wurtenberg due to a decline in hygiene of rural food productions subsequently to Napoleonic war perturbations. The paralytic disease was mainly associated to the consumption of blood sausages and was termed "sausage poisoning." This disease was also known as "Kerner's disease" and the name "botulism" was coined later in the second half of the nineteenth century from the Latin word *botulus* meaning sausage [4]. Interestingly, albeit the nature of this poisonous substance was not known, Kerner envisioned the possibility of using this poison to treat diseases associated with an overactive nervous system, including muscle hyper-contraction and hyper-secretion of body fluids. Then in 1895, Emile Pierre Marie Van Ermengem, a professor of Microbiology at the University of Ghent and who had worked in the laboratory of Robert Koch in Berlin, isolated an anaerobic-sporulating microorganism that he had named *Bacillus botulinus*, from the ham, the intestine, and spleen of one of the victims of a severe outbreak of botulism which occurred in a small Belgian village (Ellezelles). The term *Clostridium* was then used to designate anaerobic spore-forming bacteria in contrast to *Bacillus* which was reserved for aerobic or facultative anaerobic bacteria. Subsequently, the other types of botulism with the identification and characterization of BoNTs and bacterial organism producers were reported [5].

2. Botulinum toxins

2.1. Structure

BoNTs share a common structure. They are synthesized as a precursor protein (about 150 kDa), which is inactive or weakly active. The precursor that does not contain a signal peptide is

released from the bacteria by a yet unknown mechanism. The precursor is proteolytically activated in the extra-bacterial medium either by *Clostridium* proteases or by exogenous proteases such as digestive proteases in the intestinal content. The active neurotoxin consists of a light chain (L, about 50 kDa) and a heavy chain (H, about 100 kDa), which remain linked by a disulfide bridge. The structure of BoNTs shows three distinct domains: L-chain containing α-helices, and β-strands and including the catalytic zinc-binding protease motif (His-Glu-X-X-His), the N-terminal part of the H-chain forming two unusually long and twisted α-helices, and the C-terminal part of the H-chain consisting of two distinct subdomains (H_{CN} and H_{CC}) involved in the recognition of the receptor. While the three domains are arranged in a linear manner in BoNT/A and BoNT/B, both the catalytic domain and the binding domain are on the same side of the translocation domain in BoNT/E. This domain organization in BoNT/E might facilitate a rapid translocation process [6–16].

The overall sequence identity at the amino acid level between BoNTs ranges from 34 to 97%. Several domains are highly conserved which account for the common mode of action of these toxins including the central domains of L chains containing the catalytic site and the N-terminal half of the H-chains that is involved in the translocation of the L-chain into the cytosol. Thus, a similar mechanism of internalization of the intracellular active domain into target cells is shared by all the clostridial neurotoxins. In contrast, the C-terminal half of H-chain, mainly the H_{CC} subdomains, is the most divergent [17–19]. This accounts for the different receptors recognized by the clostridial neurotoxins (see subsequent text).

2.2. Botulinum complexes

BoNTs associate by non-covalent bounds with non-toxic proteins (ANTPs) produced by *C. botulinum* to form large complexes of different sizes (medium M or 12S, large L or 16S, large-large LL or 19S), also known as progenitor toxins (**Figure 1**). Botulinum complexes are synthesized in *in vitro* cultures and in naturally contaminated food or intestinal content. The complexes are stable at acidic pH, but dissociates at alkaline pH (≥pH 7) (reviewed in [20]).

All BoNT complexes contain the non-toxic non-hemagglutinin (NTNH) protein. NTNH is highly conserved. Two main classes of botulinum complexes can be distinguished based on their composition in additional proteins, the botulinum complexes containing hemagglutinins (HAs, including HA33, HA17, and HA70) (HA-BoNT complexes) and those possessing OrfX (including OrfX1, Orfx2, and OrfX3) and P47 proteins (OrfX-BoNT complexes) [17, 20–23]. The composition and structure of HA-BoNT complexes have been extensively investigated, whereas the OrfX-BoNT complexes are still poorly characterized. The stoichiometry can vary according to the strain, culture conditions (culture media, temperature, period of culture, etc.), and the method of complex preparation [20]. *C. botulinum* A produces three types of botulinum complexes M (medium), L (large), and LL (large/large) [24, 25], whereas the other *C. botulinum* types yield only M and L complexes.

The 12S or M complex results from the association of a BoNT molecule together with a NTNH at a 1:1 ratio [26]. L HA-BoNT complexes of *C. botulinum* A, B, and C consist of BoNT/NTNH/

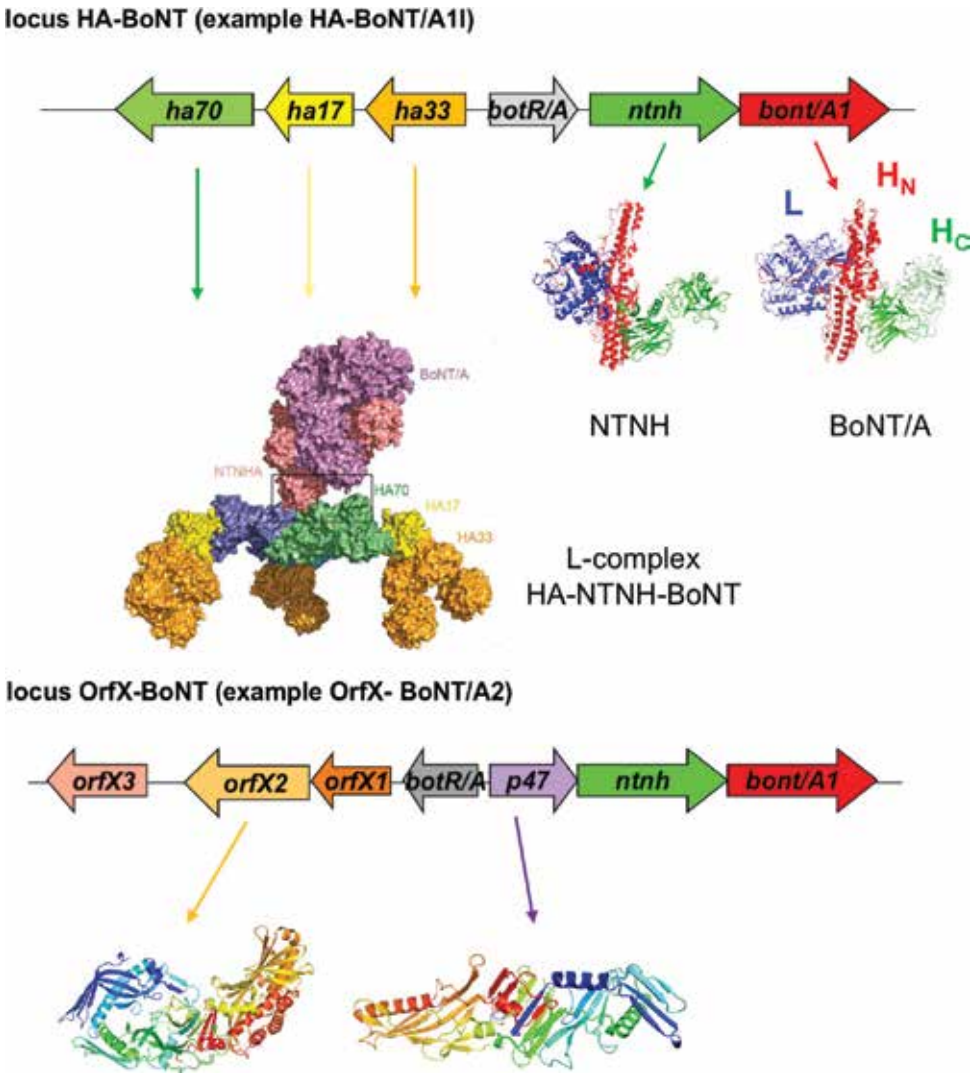

Figure 1. Genetic organization of *ha-bont* and *OrfX-bont* locus and structure of BoNT/A, NTNH, HA-NTNH-BoNT/A complex, OrfX2 and P47. L, BoNT light chain; H_N, N-terminal part of BoNT heavy chain; H_C, C-terminal part of BoNT heavy chain. The structure of OrfX-BoNT complex is not yet known.

HA70/HA17/HA33 in a molar ratio of 1:1:2:2:3 as determined by gel electrophoresis and densitometry [27]. The HA33 are likely to be at the periphery of the complex. Using stain electron microscopy and single particle averaging analysis, a stoichiometry of 1:1:3:3:6 was deduced. L HA-BoNT complexes of *C. botulinum* A or B share a similar ovoid structure with three flexible appendages, whereas the M OrfX-BoNT complex from *C. botulinum* E lacks these arms [25]. Further crystal structure analysis supports the 14 subunit complex of L HA-BoNT/A

[24]. The LL complex produced only by *C. botulinum* A is presumed to be a dimer of the L complex linked by an oligomeric HA33 consisting of four molecules and thus containing two molecules of BoNT/A [21, 22, 28]. However, a refined analysis of LL complex showed a composition of 1 BoNT/A, 1 NTNH/A, 5–6 HA17, 4–5 HA23, 3–4 HA48, and 8–9 HA34 (HA23 and HA48 resulting from HA70 nicking) [29].

The composition and organization of OrfX-BoNT complexes from *C. botulinum* A1, A2, and E is poorly characterized [30]. *C. botulinum* A2, A3, A4, A6, A7, A8, E, and F only produce M complexes devoid of hemagglutinating activity, and *C. argentinense* produces only L complex [22]. *M. botulinum* complex type A2 only contains BoNT/A2 and NTNH, although P47, Orfx2, and OrfX3 are produced in the culture supernatant, but not OrfX1 or in very low amount [31]. OrfX1 has been detected in botulinum complex type E but not type F, whereas neither OrfX2 or P47 has been evidenced in both toxinotypes [32, 33]. The structure of OrfX2 and P47 showed a similarity with TULIP family of proteins which are lipid-binding proteins [34].

NTNHs from the different *C. botulinum* types retain a high identity level (76–83.5%) and are the most conserved proteins among the botulinum complex components [17, 20]. NTNH/A, NTNH/C, and NTNH/D contain a cleavage site within their N-terminus, yielding 15 kDa N-terminal and 115 kDa C-terminal fragments. NTNH/A is split into 13 and 106 kDa fragments by cleavage between Pro144/Phe145 [35]. NTNH/C and NTNH/D are cleaved at Lys127 by a trypsin-like protease with 7–13 amino acids removed from the N-terminus of the 115 kDa fragment that subsequently results in three proteins starting at Leu135, Val139, or Ser141 [36]. NTNH is only cleaved in the 12S (M) complexes from *C. botulinum* types A, C, and D, but not in the L (16S) or LL (19S) complexes. The cleaved NTNH molecules constituted a nicked structure since the two fragments still remain together after NTNH purification [36]. In contrast, NTNH/E and NTNH/F show an identical deletion of 33 residues in the corresponding region of NTNH/A, NTNH/C, and NTNH/D encompassing the cutting site, and NTNH/G possesses a slightly different sequence in this region. It is presumed that the processing and additional sequence of NTNH in *C. botulinum* A, C, and D are responsible for forming 12S-, 16S-, and 19S-sized complexes. The inability of *C. botulinum* E and F to form L complexes may result from the absence of hemagglutinin (HA) or other related proteins that bind to NTNH and from the absence of a putative binding site in NTNH/E and NTNH/F [17, 22].

BoNT and NTNH share a weak amino acid sequence identity (~20%), but both proteins retain a common structure (**Figure 1**). NTNH associates with BoNT by non-covalent bonds in a pH-dependent manner to form an interlocked compact M complex, which is resistant to acidic pH and protease degradation, whereas each protein separately is sensitive to proteolysis [23, 25, 26, 37]. Thereby, NTNH is a non-toxic protein which acts as a chaperone protein to protect BoNT. NTNH does not contain the catalytic HExxH motif, but another zinc-binding motif, KCLIK, at the same position. Indeed, NTNH binds one zinc atom per each molecule but exhibits no proteolytic activity [38]. This strongly supports that all NTNH and BoNT variants derive from a common ancestor gene by duplication and subsequent independent reshuffling.

HA33-35 is the most abundant hemagglutinin component of the HA-BoNT complexes. Type A HA35 binds to oligosaccharides containing galactose-β1-4glucose-*N*-acetyl-D-neuraminic acid (Galβ1-4GlcNAc) [39]. Thereby, hemagglutination induced by L and LL type A botulinum complexes is mainly mediated through HA35 binding to erythrocyte membrane glycolipids and glycoproteins containing Galβ1-4GlcNAc [39, 40]. Similarly, HA33 from types C and D botulinum complexes binds to paragloboside on Galβ1-4GlcNAc and also sialylglycolipids (GM3), as well as sialoglycoproteins (sialosylparagloboside) on the *N*-acetyl-D-neuraminic acid-α2-3-galactose-β1 motif [41]. The importance of HA33-35 in hemagglutination is also supported by monoclonal antibody studies. Type C-specific monoclonal antibodies against HA33 inhibit hemagglutination, contrary to those against HA50 and HA17 [42]. However, type C HA70 and its derivative HA50 recognize sialosylparagloboside and GM3 at the *N*-acetyl-D-neuraminic acid-α2-3-galactose-β1motif in erythrocyte membranes, like the corresponding L botulinum complex. Thus, HA50 could also be involved in hemagglutination [41]. HA35 purified from *C. botulinum* A is predominantly a dimeric, β-sheet protein in aqueous solutions. In *C. botulinum* A, five N-terminal amino acids are removed from HA35, but similar posttranslational modification has not been observed in HA33 from *C. botulinum C*. The significance of HA35 processing on its biological activity is not known [43]. It was first discovered that the 31 C-terminal amino acids, which contain a predicted carbohydrate recognition site, play an essential role in hemagglutination [44]. The structure of type C HA33 shows two β-trefoil domains consisting of a six-stranded, antiparallel β-barrel capped on one side by three β-hairpins. Related β-trefoil structures bind to oligosaccharides and are found in other proteins, including various lectins like the ricin B-chain, cytokines, trypsin inhibitor, xylanase, as well as the C-terminal part of BoNTs. Type A and B HA35 retain a similar structure related to the carbohydrate-binding site of ricin, a plant toxin. It is worth noting that Asp263 and Asn285 of HA35, which are conserved in the lactose-binding site for ricin B chain, are critical for carbohydrate binding [45–47]. HA17 also adopts a β-trefoil fold, whereas HA70 forms a three-bladed propeller-like trimer with a pore located at the center of the trimer [48, 49].

More recently, a novel function has been attributed to HA complexes consisting in the disruption of intercellular junctions between intestinal epithelial cells. HAs recognize E-cadherin, which plays a crucial role in basolateral junction. The interaction of HAs with E-cadherin is species and isoform specific. Thereby, HAs directly bind to the extracellular domain of (epithelial) E-cadherin, but not of (neural) N-cadherin, nor (vascular endothelial) VE-cadherin. Type B HAs specifically bind to human, bovine, and mouse E-cadherin but not to that of rat and chicken [50]. This is consistent with the fact that botulism type B is common in humans and is rarely observed in chickens. Type A BoNT complexes also recognize human E-cadherin, whereas type C BoNT complexes do not [50]. The combination of HAs (HA33, HA17, and HA50/70) organized in complex is required for the optimum binding to E-cadherin, whereas individual HAs do not interact with E-cadherin. HAs assemble in a threefold symmetric hetero-dodecameric structure, and the whole HA complex exhibits the highest affinity to E-cadherin. The minimal HA complex interacting with E-cadherin consists of domain 3 of HA70 (Pro-378-Asn-626), one

molecule of HA17, and two HA33 molecules [51]. HAs bind to the distal extracellular domain (EC1) of E-cadherin near the cadherin trans-dimer interface [50]. Thus, the HA-binding sites to carbohydrates and E-cadherin are functionally and structurally distinct [52].

The structures of OrfX2 and P47 are unrelated to that of HAs and show that they belong to the tubular lipid-binding (TULIP) protein superfamily. Thereby, OrfX1 and OrfX2 have been found to bind to phosphatidylinositol [34]. In contrast to HAs, OrfX proteins and OrfX complexes have not been reported to interact with E-cadherin or to alter the intestinal epithelial barrier. This raises the question whether OrfX complexes are involved in BoNT passage through epithelial barriers. In *C. botulinum* strains type E, F, and some type A, BoNTs form complexes lacking HAs and are responsible for foodborne botulism, which is as severe as the classical type A and B botulism.

2.3. Botulinum toxin gene organization

The BoNT and ANTP genes are clustered in close vicinity in a DNA fragment which is called the botulinum locus. BoNT and ANTP genes are organized in two operons. The operon localized in the 3′ part of the botulinum locus contains *ntnh-bont* which is highly conserved in all *C. botulinum* strains. In *C. botulinum* types E and F and certain *C. botulinum* A strains, this operon contains an additional gene called *p47* encoding a 47-kDa protein (**Figure 1**). The second operon consists of the *ha* or *orfX* genes and is localized upstream of the *ntnh-bont* operon. The *ha* or *orfX* operon is transcribed in opposite orientation to that of the *ntnh-bont* operon and shows more strain variation. In *C. botulinum* B, C, D, and some A strains, the *ha* operon consists of three genes (*ha70*, *ha17*, and *ha33*). The *ha* genes of *C. botulinum* G only comprise *ha*17 and *ha*70. The *ha* genes are missing in the non-hemagglutinating toxinotypes A1, A2, A3, A4, E, and F and an *orfX* operon (*orfX1*, *orfX2*, *orfX3*) instead of *has* lies upstream of the *ntnh-bont* operon [53–55] (**Figure 1**). It is worth noting that a same *bont* gene can be inserted into a HA or a OrfX locus. However, *bont*/*A1* is the only gene which has been found in either of the two types of botulinum locus.

The *bot*R gene encoding for an alternative sigma factor is a positive regulator of the *ntnh-bont* and *ha* operons. *botR* is localized differently according to the *C. botulinum* strains, either between the *ntnh-bont* and *ha* operons or upstream of the *ha* operon. This gene is missing in *C. botulinum* E and toxigenic *C. butyricum*.

Most of *C. botulinum* strains produce only one type of BoNT, and the botulinum locus is present in a single copy on the genome. However, some rare strains synthesize two different BoNTs: BoNT/A-BoNT/B, BoNT/A-BoNT/F, and BoNT/B-BoNT/F producing strains have been isolated. The two neurotoxins are usually produced in different proportions. Thus, in Ba and Bf strains, BoNT/B is produced 10 times more than BoNT/A and BoNT/F. Some clostridial strains contain silent neurotoxin genes. Several *C. botulinum* A strains isolated from foodborne and infant botulism contain a silent *bont*B gene. These strains are noted A(b). These strains contain two distinct botulinum loci. One *C. botulinum* strain has been found to harbor three botulinum loci containing *bontA2, bontF4,* and *bontF5* [56].

The botulinum loci are distributed on different genetic elements, including chromosome, plasmid, or phages depending on the species and strain of Clostridia. In *C. argentinense*, *C. botulinum* type B, mainly in subtype B1, bivalent, and non-proteolytic strains, and in some *C. botulinum* A strains, the botulinum loci are located on large plasmid. For example, in the bivalent strain Ba657, the two botulinum loci, locus A and locus B, are harbored by the same plasmid (pCLJ) separated by approximately 97 kbp. Similarly, the neurotoxin genes, *bontB* and *bont/f*, from one Bf strain are located on a same plasmid (pBf), which is very much related to pCLJ. In *C. botulinum* type E and neurotoxigenic *C. butyricum* strains, the *bont/E* loci are mainly on the chromosome. However, in three *C. botulinum* E strains from 36, *bont/E1* is located on a large plasmid. In *C. botulinum* C and D, it has been clearly evidenced that BoNT is encoded by bacteriophages (reviewed in [57]).

The location of botulinum locus within chromosome or plasmid seems to occur not at random but at specific sites. Indeed, in strains from group I or II, whose genome sequencing is available, five specific sites of botulinum locus integration have been identified. *orfX-bont/A2*, *orfX-bont/A1*, and *orfXbont/F* loci are located in the *ars* operon, which contains three to five genes involved in arsenic reduction. *orfX-bont/A1* and *orfX-bont/F*loci share a similar integration site at the 5′ end of the *ars* operon, whereas *orfx-bont/A2* locus is inserted between two copies of *arsC* gene. *ha-bont/A1* and *ha-bont/B* loci, which contain a recombinant *ntnh* gene type A and type B strains, are found in the *oppA/brnQ* operon, encoding for extracellular solute-binding protein and branched chain amino acid transport proteins, respectively. This operon is lacking in non-proteolytic *C. botulinum* type B, *C. botulinum* type E, and *C. butyricum* type E strains. The third integration site is the *rarA* gene in group II and V strains, which contains the *orfX-bont/E* locus in *C. botulinum* type E and *C. butyricum* type E strains. *rarA* encodes a resolvase protein involved in recombination or insertion events of transposons. Interestingly, the botulinum E locus is inserted in the same codon [102] of *rarA* gene in both *C. botulinum* type E and *C. butyricum* type E strains, and the inserted botulinum locus contains an additional intact *rarA* gene [58]. The trivalent strain A2f4f5 contains the *orfX-bont/A2* and *orfX-bont/F4* loci located in the chromosome at the *arsC* and *pulE* (type II secretion system protein E) genes, respectively [56]. In *C. botulinum* F, the *orfX-bont/F6* locus has been found in a new chromosomal integration site *topE* [59].

Two specific sites of botulinum locus location have been identified on plasmids from group I strains, one contains *orfX-bont/A3*, *orfXbontT/A4* from Ba strain, or *orfX-bontF* from Bf strain, and the second harbors the *ha-bont/B* locus from *C. botulinum* B1 strain or bivalent Ba4 or Bf strains. The *ha-bont/B4* locus in nonproteolytic strains is located on a plasmid different from those of group I strains. However, the downstream flanking region of the HA-npB locus contains an IS element, a transposon-associated resolvase, and a site-specific recombinase [58]. It is worth noting that *C. botulinum* plasmids harboring *bont* genes such as pCLJ, pCLL, and pCDC-A3 (related to pCLK) are transferable by conjugation into a group I *C. botulinum* strain [60].

The toxin gene location on the various genetic elements chromosome including mobile genetic elements (plasmid, phage) supports horizontal *bont* gene transfer between *Clostridium* strains and also between clostridia and non-clostridia strains. In addition, insertion sequences or transposases genes have been identified in the flanking regions of most of botulinum loci. These genetic elements are associated to gene mobility and contribute to the extreme plasticity of these BoNT-producing bacteria. It is worth noting that most of the insertion sequences are partially modified, suggesting a very ancient process of gene mobility and subsequent DNA

rearrangement or modification (review in [61–63]. Indeed, the BoNT-producing clostridia strains are heterogeneous and do not form a unique bacterial species. The *C. botulinum* species has been designed on the basis of only one phenotype, the production of a paralytic toxin. However, it appeared that they show variable physiological and biochemical properties and they have been divided into four physiological groups (I–IV). Moreover, it was shown that atypical strains of other *Clostridium* species than *C. botulinum* such as *C. baratii* and *C. butyricum* were able to synthesize a BoNT related to those produced by *C. botulinum*. Genetic analysis including whole genome sequencing confirmed the distinction of the multiple groups and species of BoNT-producing bacteria [64–66]. More recently, *bont* genes have been found in the genome of non-clostridial species (see subsequently and in **Table 1**). Clostridia and other bacteria, which contain *bont* genes, are from the environment, raising intriguing question which

Botulinum toxin type	**BoNT/A**	**BoNT/B**		**BoNT/E**	**BoNT/F**	
Subtypes	A1, A2, A3, A4, A5, A6, A7, A8	B1, B2, B3, B5, B6, B7, B8	B4	E1, E2, E3, E6, E7, E8, E9, E10, E11, E12	F6	F2, F2, F3, F4, F5, F8
Enzymatic substrate (cleavage site)	SNAP25 (QR)	VAMP1, 2, 3 (QF)		SNAP25 (RI)	VAMP1, 2, 3 (QK) F5: VAMP2 (LE)	
Neurotoxin-producing bacteria	*C. botulinum* group I		*C. botulinum* group II		*C. botulinum* group I	
Main physiological properties	Proteolytic Lipase Temperature growth: minimum 10–12°C, optimum 30–40°C Highly heat-resistant spores		Non-proteolytic Lipase Growth at low temperature: minimum 2.5–3°C, optimum 25–30°C Moderate heat-resistant spores		Idem group I	
Botulism	Human, occasionally animal					

Botulinum toxin type	**BuNT/E**	**BaNT/F**	**BoNT/C**	**BoNT/D**	**BoNT/G**	**BoNT/H**
Subtypes	E4, E5	F7	C/D, D/C		G	H or F/A or H/A
Enzymatic substrate (cleavage site)	SNAP25 (RI)	VAMP2 (QK)	SNAP25 (RA) Syntaxin (KA)	VAMP1, 2, 3 (KL)	VAMP1, 2, 3 (AA)	VAMP1, 2, 3 (LE)
Neurotoxin-producing bacteria	*C. butyricum*	*C. baratii*	*C. botulinum* group III		*C. argentinense* group IV	*C. botulinum* group I
Main physiological properties	Non-proteolytic Glucidolytic	Lecithinase	Non-proteolytic Lipase Temperature growth 37–40°C		No protease No lipase	Group I
Botulism	Human, animal not reported		Animal, very rare in human		No natural case reported	Human

Botulinum toxin type	BoNT/X	BoNT/I or BoNT/Wo	BoNT/J or eBoNT/J or BoNT/En	Cp1 toxin (BoNT homolog)	BoNT/Ba BoNT/Bf BoNT/Ab BoNT/Af BoNT/A(B) BoNT/A2F4F5
Subtypes	Bivalent BoNT/B2-BoNT/X				
Enzymatic substrate (cleavage site)	VAMP1, 2, 3, 4, 5 Ypkt6 (RA)	VAMP2 (WW)	VAMP2 (DL) SNAP25, 23 (KD) Syntaxin (MD)	?	
Neurotoxin-producing bacteria	*C. botulinum* strain 111 group I	*Weissella oryzae*	*Enterococcus faecium*	*Chryseobacterium piperi*	Bivalent and trivalent *C. botulinum* strains Group I
Main physiological properties	Group I	Gram-positive bacillus Nonspore-forming Facultative anaerobic	Gram-positive cocci	Gram-negative bacillus Strictly aerobic Non-spore forming	Group I
Botulism	Infant botulism Japan	No natural botulism case reported			Human botulism

Table 1. Botulinum toxin (BoNT) types, subtypes, and their main properties including enzymatic substrates and cleavage sites, as well as the neurotoxin-producing microorganisms with their main physiological properties and involvement in naturally acquired botulism.

are the molecular mechanism and selection pressure of neurotoxin gene transfer and which are the advantages conferred by genes encoding paralytic toxins for higher organisms. It is worth noting that whether *bont* genes can be mobilized in diverse bacteria, their transfer is mainly restricted to *Clostridium* species.

2.4. Botulinum toxin diversity

BoNTs form a family of diverse proteins which share the common property to induce a flaccid paralysis. Historically, it was found that these toxins can be antigenically distinguished. On the basis of neutralization of the biological effects on small rodents with specific antisera, seven BoNT types (A–G) were identified. Each type-specific antitoxin only neutralizes the corresponding BoNT type. The differences in amino acid sequences range from 37.2 to 69.6% [19]. In 2013, a novel eighth BoNT type called H (or F/A or H/A) has been described from a bivalent *C. botulinum* strain isolated from an infant botulism case and producing both BoNT/B2 and

BoNT/H [67, 68]. It was claimed that this novel BoNT type was not neutralized by the already known anti-BoNT sera justifying its assignment to a novel type. More recently, genome analysis showed the presence of a related *bont* sequence in an OrfX locus in *C. botulinum* type B strain 111 which also produces BoNT/B2. BoNT/X retains a low sequence identity with the other BoNT types, and it is not recognized by the antibodies against the previous BoNT types [69]. Moreover, *bont*-related sequences have been identified in non-clostridial bacteria including Gram-positive/Gram-negative, spore-forming/non-spore-forming, anaerobic/aerobic bacteria such as *Weissella oryzae* (BoNT/Wo or BoNT/I) from fermented rice [70], an *Enterococcus faecalis* strain (BoNT/J, or BoNT/En, or eBoNT/J) isolated from a cow [71, 72], and *Chryseobacterium piperi* (Cp1) from sediment [73] (**Table 1**). This suggests a complex and long evolution of *bont* genes, the ancestral source of which still remains mysterious [63, 74, 75].

An increased sequencing of *bont* genes and/or whole genome of individual strains shows that each BoNT type contains variable isoforms based on sequence variations. Therefore, BoNT types are divided into subtypes which were initially defined as displaying at least 2.6% amino acid sequence difference [76]. However, some BoNT subtypes, notably from types B and E, only exhibit 0.9–2.1% amino acid sequence difference, but they were assigned to distinct subtypes according to phylogenetic clade analysis. Among more than 500 BoNT sequences, 41 subtypes have been identified [19] (**Table 1**).

Amino acid sequence variations might impact BoNT biological functions including receptor recognition, the efficiency of entry into cells and persistence, recognition by monoclonal antibodies, and enzymatic activity. For example, BoNT/A2 has been shown to enter more efficiently into neuronal cells than BoNT/A1 and to have a higher affinity for its receptor [77, 78]. BoNT/A2 induces a faster paralysis than BoNT/A1/A4/A5, and BoNT/A3 has a shorter duration of effect [79]. In addition, BoNT/A2 retains a lower immunogenicity [80]. Thus, BoNT/A2 would be a more efficient therapeutic agent than BoNT/A1 [81–83]. BoNT/A8 binds less efficiently to gangliosides embedded into a membrane and has a lower enzymatic activity than BoNT/A1 [84]. BoNT/B1 binds to synaptotagmin 1 and 2 receptors, whereas BoNT/B2 only recognizes synaptotagmin 2 [85]. In contrast to the BoNT/F subtypes which cleave VAMP1 and VAMP2 at QK site, BoNT/F5 uses a distinct cleavage site (LE) [86] (**Table 1**). Monoclonal antibodies against BoNT/B differently recognize the subtypes BoNT/B1 to BoNT/B5 [87]. Similarly, monoclonal antibodies against BoNT/A recognize and/or neutralize the distinct BoNT/A subtypes with variable efficiently [88, 89].

3. Mode of action

BoNTs enter by oral route (foodborne botulism) or are produced directly in the intestine (infant or intestinal botulism) subsequently to a *C. botulinum* intestinal colonization. BoNTs are able to transcytose across the intestinal mucosa (review in [90] or can pass through the paracellular way with the help of HA complexes (review in [91]). After diffusion into the extracellular fluid and blood stream circulation, BoNTs target motoneuron endings.

Each type of BoNT and TeNT recognizes specific receptors on demyelinated terminal nerve endings, mainly through the H_{CC} subdomain. BoNT/A/C/E/F exploit the three isoforms of

the vesicle protein SV2 as specific receptors, while BoNT/B and /G bind to synaptotagmin I or II [92–98]. BoNT/C and BoNT/D interact with gangliosides (GD_{1b}, GT_{1b}) and phosphatidylethanolamine, respectively, by their H_{CC} subdomain [99]. Gangliosides (GD_{1b}, $GT_{1b,}$ and GD_2) and SV2A/B/C also mediate the entry of BoNT/D into neurons, but by a different mechanism than that used by BoNT/A and BoNT/E [100, 101]. The role of H_{CN} subdomain, which may interact with phosphatidylinositol phosphates [102], is still unclear. The co-presence of the *ad hoc* ganglioside(s) and protein receptors likely facilitates the identification of cell subset targeted by BoNTs at very low concentrations encountered in the physiological medium during the disease. At higher concentrations, binding to the protein receptor is likely sufficient for mediating toxin binding. Indeed, the number of cell types affected by these toxins expands with increasing toxin concentrations. Therefore, BoNTs can target numerous neurons but not all, as well as non-neuronal cells at high concentrations, inhibiting the release of various compounds.

Neurotoxin bound to its receptor is internalized by receptor-mediated endocytosis. Acidification of the vesicle lumen triggers a conformational change of the neurotoxin and subsequent translocation of the L chain into the cytosol. In addition, the disulfide bond between the two chains has a crucial role in the translocation process [103–106]. Then, the L chain refolds in the neutral pH of the cytosol. Cytosolic translocation factors such as β-COPI are possibly involved in this mechanism, as it has been found for diphtheria toxin [107–110].

L chains of all clostridial neurotoxins are zinc-metalloproteases that cleave one of the three members of the SNARE proteins. BoNT/B, D, F, and G attack synaptobrevin (or VAMP), BoNT/A and E cleaves SNAP25, and BoNT/C1 cut both SNAP25 and syntaxin. The cleavage sites are different for each neurotoxin. The cleavage of SNARE proteins occurs only when disassembled. Since VAMP, SNAP25, and syntaxin play a major role in the regulated fusion of synaptic vesicles with the plasma membrane at the release sites, their cleavage induces a blockade of the neurotransmitter exocytosis.

SNAP25 cleavage by BoNT/A or BoNT/E alters SNAP25 and synaptotagmin interaction, thus strongly reducing the responsiveness to Ca^{++} of exocytotic machinery [111–114]. Indeed, the removal of the nine C-terminal amino acids of SNAP-25 by BoNT/A deeply disrupts the coupling between Ca^{2+} sensing and the final step in exocytosis [112]. Truncated SNAP-25 can behave as a dominant-negative mutant upon the exocytotic process, suggesting that after BoNT/A treatment, the block of release is due to both functional elimination of SNAP-25 and accumulation of the cleavage product which competitively inhibits exocytosis [115–117]. In contrast, the blockade of exocytosis by BoNT/E is only due to the elimination of functional SNAP-25 and not to the production of competitive antagonists of SNARE complex formation. Indeed, the inhibition of exocytosis by BoNT/E can be rescued by supplementing the C-terminal portion of SNAP-25 removed by the toxin [118–120]. Truncation of SNAP-25 by BoNT/E destabilizes the four-helix bundle of the SNARE complex [118, 119], and SNAP-25 truncated by BoNT/E is not retained by syntaxin [121].

VAMP cleavage abolishes the interaction of VAMP with the adaptor protein AP3 and affects synaptic vesicle recycling *via* early endosomes [122]. The blockade of neuroexocytosis likely results from a disturbance of synaptophysin-1/VAMP2 interaction and of coupling between

detecting Ca^{2+} and synaptic vesicle triggering [112]. Since the synaptic vesicles docked with unproductive complexes cannot fuse or undock, they stay at the fusion sites (with slightly increased numbers), irreversibly plugging the fusion sites that would normally accommodate intact vesicles. This progressively reduces the number of active release sites to which exocytosis can occur. When VAMP is cleaved by BoNT/B or /G, the VAMP portion (~20 amino acids) remaining in the synaptic vesicle membrane does not contain interaction sites for the other SNAREs. Therefore, the synaptic vesicle membrane is no longer linked to a SNARE complex, and fusion with the plasma membrane cannot occur. When VAMP is cleaved by BoNT/D or /F, the C-terminal fragment remaining in the vesicle membrane is long enough to anchor the synaptic vesicle to the SNARE complex, but fusion cannot occur because the SNARE complex cannot transit into the thermally stable four-helix bundle.

BoNT/C cleaves both syntaxin-1 and SNAP-25, but *in vitro* cleavage of SNAP-25 by BoNT/C occurs with a low efficiency (~1000-fold difference) versus cleavage by BoNT/A or /E [123, 124]. This raises the following question: which of the two targets is involved in BoNT/C neuroexocytosis blockade?

Although the physiological properties induced by the cleavage of either VAMP, SNAP25, or syntaxin are not equivalent at the neuromuscular junctions, all the clostridial neurotoxins cause a blockade of the regulated neurotransmission, which varies in intensity and duration according to each neurotoxin type.

4. Epidemiology of botulism in France

4.1. Main clinical forms of human botulism

Several clinical forms of botulism are distinguished according to the mode of acquisition of BoNT and/or neurotoxigenic bacteria. Foodborne botulism occurs after the consumption of food contaminated by *C. botulinum* in which sufficient amount of toxin has been produced. Foods stored for a sufficient period such as home-made canned foods, home-fermented products, or commercial minimally heated and chilled foods are at risk of botulism. Ingestion of preformed BoNT in food is responsible for botulism by intoxication. Foodborne botulism is the main form in adults.

Infant botulism results from the ingestion of *C. botulinum* spores that germinate, multiply, and produce BNT in the infants intestinal content. A low contaminating dose of 10–100 *C. botulinum* spores is sufficient to induce intestinal colonization and production of BoNT in the intestinal tract, since the intestinal microbiota, which has an inhibitory effect on the growth of *C. botulinum* in the digestive tract, might be not sufficiently developed or non-functional in babies under 1 year.

Botulism by intestinal colonization occasionally occurs in adults. Predisposing factors consist in factors that perturb or modify the microbiota composition such as antibiotherapy, intestinal surgery 1 or 2 weeks prior consumption of a food contaminated by *C. botulinum* spores, chronic inflammation, and necrotic lesions of the intestinal mucosa, which might support the intestinal growth of *C. botulinum*.

Wound botulism is caused by *C. botulinum* growth and toxin production in a contaminated wound or a lesion-like tetanus. Wound botulism is much rarer than tetanus. Drug users by injection who handle contaminated materials or drugs are notably at risk of wound botulism.

Inhalational botulism is very rare. A few cases have been reported in laboratory workers preparing concentrated BoNT by continuous centrifugation and in two patients who inhaled cocaine (review in [125]). BoNT dissemination by aerosol has been considered as a possible bioterrorist attack.

Iatrogenic botulism is a recent novel form of botulism which develops subsequently to toxin overdoses for a therapeutic or a cosmetic purpose or to a hematological dissemination of toxin at a therapeutic dose.

4.2. Botulism in France

4.2.1. Foodborne botulism

The first case of human botulism was reported in 1875. The disease was very rare until the second war, since the consumption of canned foods was not traditional in France. This not excludes that the disease was underestimated or misdiagnosed. Only 24 cases were recorded from 1875 to 1936 and eight from 1936 to 1940 [126, 127]. In contrast, in the USA where the first industrial canned foods treated by heating were developed, large outbreaks of botulism occurred from 1899 to 1954, 514 outbreaks, 1350 cases including 861 deaths [127]. However, the incidence of botulism was very high in France during the Second World War. About 500 outbreaks and 1000 cases were estimated between 1940 and 1944 [126]. Food deprivation and poor quality of home-made food preservation were the main factors responsible for this high incidence. Type B botulism predominated, and most of the incriminated foods (93%) were from pork meat, notably cured ham [126].

The incidence of botulism decreased after the Second World War (**Figure 2**). Albeit no systematic recording of botulism cases was performed during this period, only a few outbreaks were identified, mainly in the Anaerobe Laboratory of Pasteur Institute. During the period 1956–1970, a 22.4 annual mean of botulism cases was observed based mainly on the detection of BoNT and/or *C. botulinum* in the incriminated food. Since 1971, the diagnosis of human botulism was improved by the detection of BoNT in patient's serum [128]. Thus, the incidence of botulism increased to an annual mean of 76 cases per year within the 1971–1977 periods. This corresponds to a better survey of human botulism, but possibly also to the introduction of novel foods or modes of food preservation at risk of botulism, such as minimally heated and chilled foods or vacuum-packed chilled foods. Type B was the most frequent type of botulism (96.9% of outbreaks), and home-made or small-scale preparation of ham was the main source of botulism (63.7% of the outbreaks). However, commercial products or restaurant meals were incriminated or suspected in 30 (7.2%) outbreaks and were responsible for six deaths [129].

From 1986, human botulism is subjected to mandatory declaration to health authorities and since 1998 botulism declarations are coordinated by the national organism of disease survey InVS (Institut national de Veille Sanitaire called Sante Publique France since 2016). Since 1980, human botulism decreased, but every year, 10–40 cases are recorded in France. Home-made

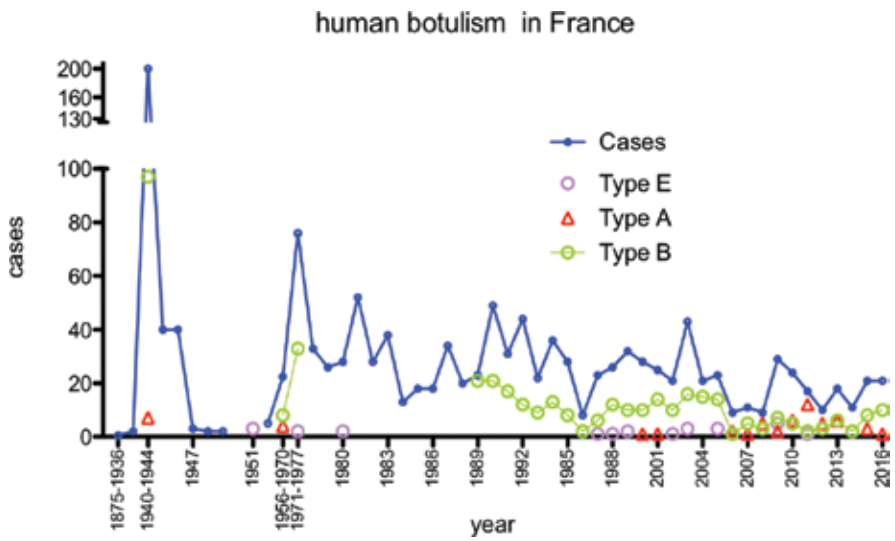

Figure 2. Incidence of human botulism in France, 1875–2016. The numbers indicated in the period ranges 1875–1936, 1940–1944, 1956–1970, and 1971–1977 are the annual mean values. Total cases (blue), type B botulism (green), type A botulism (red), type E botulism (purple), according to [127, 129–135, 147–151]. The two outbreaks of *C. baratii* type F botulism in 2014 and 2015 are not reported in the figure.

preserved foods are less used but remain traditional in certain areas of France. Type B is predominant, and cured ham and pork meat preparations are the main origin of human botulism [130–135]. Pork is often a healthy carrier of *C. botulinum* type B and rarely develops botulism symptoms [136, 137]. Insufficient or inadequate sanitary measures in the preparation of pork meat and absence or insufficient heat treatment are the main risk factors. However, more diverse types and sources of botulism occurred since 2000 (**Figure 2**). Botulism type A, which was extremely rare in France, was more frequent since 1997 notably from canned vegetables [132]. Severe outbreaks of botulism type A occurred in 2008, one from commercial "enchiladas" containing chicken meat, vegetables, and cereal cake, and another one from home-made pumpkin jam [134]. During the period 2010–2012, botulism type A was predominant (23 cases out of 51) and resulted from diverse origins: home-made canned beans, commercial tapenades (olives, dried tomatoes), commercial pasta, and imported home-made eggplant preparation [135]. Only one outbreak of botulism type A (from home-made pheasant pie) was recorded within 2013–2016 [133].

Botulism type E is extremely rare in France. An outbreak of botulism type E occurred in 2009 after the consumption of smoked and vacuum-packed fish which was bought a few days ago in Finland. The fish was from Canada and was processed in Finland [134]. In 2010, an unusual case resulted from a ham contaminated with *C. botulinum* B and a novel *C. botulinum* E subtype (E12) [135, 138]. It was hypothesized that marine salt used for the ham preparation could be the origin of the contamination.

Two atypical outbreaks of botulism type F occurred in 2014 and 2015. Both were *Clostridium baratii* F7 botulism. The first outbreak included two patients, one of which was totally paralyzed and showed a very high level of BoNT/F in the serum (400 mouse lethal doses/ml),

but she recovered after 46 days in intensive care unit. The origin of this outbreak was not determined [139]. The second outbreak concerned three patients who have had their meal at the same restaurant on the same day. A Bolognese sauce prepared 2 days in advance with industrial ground meat was the common food. A sample of the ground meat in the refrigerator of the restaurant was contaminated with *C. baratii* F7 [140, 141].

4.2.2. Infant botulism

Infant botulism is a rare form of botulism in France. Only 15 cases were identified from 2004 to 2016. They resulted from group I *C. botulinum* type A or B and from different subtypes: A1(B), A2, Bf, B2, and B5. All food samples investigated for the origin of contamination were negative. In two outbreaks, an environmental contamination was strongly suspected. In one of them, the baby's home was close to a reconstruction work. *C. botulinum* B was identified in stool sample of the baby and soil samples of the reconstruction work [133]. Another 2-month-old baby developed botulism with several relapses over a period of 4 months. *C. botulinum* A2 was isolated from stool samples all along the course of the disease. The particularity of this strain was its high resistance to penicillins and to metronidazole [142]. It was the first report of an antibiotic-resistant clinical *C. botulinum* strain. The baby's home was at proximity of a thermal power station that intermittently released sprays of vapor and smoke/dust and that was suspected to be the origin of the contamination.

4.2.3. Wound botulism and inhalation botulism

Only one case of wound botulism was identified from 1995 to 2017. In 2008, a patient had an open fracture of the leg abroad and was hospitalized again when back to France for persistent suppuration of the wound. He developed a type B botulism during the course of the second hospitalization [134]. Wound botulism in injection drug users was reported in several European countries and North America, but no such case was reported in France [143, 144]. However, in 2007, two patients who inhaled cocaine developed a botulism type B [145].

4.2.4. Botulism diversity in France

Albeit botulism is a rare disease, human botulism is identified every year in France. Foodborne botulism is the main form of botulism in France. Historically, home-made cured ham or pork products were the main source of type B botulism. During the recent period, home-made preserved foods including ham are no longer commonly used, but human botulism is still present albeit to a lower extent than in the past. Thereby, the origin of botulism is more diverse including imported products, commercial minimally heated foods, or meals at a restaurant. The diversity of BoNT types and subtypes as well as of the BoNT-producing clostridia reflects the diverse origins of human botulism in France [146].

5. Conclusion

BoNTs form a wide diverse family of toxins which target specific neurons, leading to the inhibition of release of neurotransmitters, notably acetylcholine. At least 10 BoNT types and more

than 40 subtypes have been identified. All BoNTs retain a common core structure and mode of action which consists in the inhibition of neurotransmitter release, notably acetylcholine, leading to flaccid paralysis. However, they use distinct pathways and distinct intracellular targets to drive the blockade of neurotransmission. Indeed, the distinct BoNT types recognize different neuronal receptors such as different sets of gangliosides and different membrane proteins (SV2 isoforms, synaptotagmin) and target either one of the three SNARE proteins at distinct cleavage sites. In addition, BoNTs are produced by diverse bacterial species, mainly from the *Clostridium* genus which are environmental bacteria. This raises the questions about the evolution and selection pressure involved in the emergence of so diverse bacterial proteins with unique function on the neurological system of higher eukaryotic organisms. BoNTs are responsible for severe neurological disease in man and animals which are still present in some countries such as in France. However, they also constitute valuable therapeutic tools for the treatment of diverse neurological dysfunctions. The increased number of medical indications of BoNTs contrasts with the high poisonous activity of these toxins. The wide BoNT diversity offers a panel of natural variants which can be adapted to specific applications.

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Chapter 2

Botulinum Toxin Adverse Events

Raffaela Pero, Sonia Laneri and Giovanna Fico

Additional information is available at the end of the chapter

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Abstract

Botulinum toxin acts at the neuromuscular junction (motor plaque) blocking the release and effects of acetylcholine (ACh), a neurotransmitter of both the central nervous system (CNS) and the peripheral nervous system (SNP). By inhibiting the release of acetylcholine, botulinum toxin interferes with the nervous impulse and causes a characteristic flaccid paralysis of the muscles. This effect is used to decrease wrinkles of the facial skin and chin providing a smooth appearance and for the treatment of a variety of human syndromes characterized by hyperfunction of selected nerve terminals. Side effects of this treatment are rare, but are essentially related to the active ingredient of the drug or to medical malpractice. These adverse events and their possible therapy are discussed in this chapter.

Keywords: botulinum toxin, adverse events, therapy, esthetic, motor endplate

1. Introduction

Botulinum toxin is a neurotoxic protein produced by the anaerobic bacterium *Clostridium botulinum*. There are seven types of distinct botulinum toxin and are indicated with the alphabet letters: A, B, C, D, E, F, and G [1].

Recently, a novel botulinum neurotoxin (BoNT/X) has been identified [2] and the first botulinum-like toxin outside the *Clostridia* family has been described [3].

The currently used in esthetic medicine is botulinum toxin type A (BoNT-A). It is used for wrinkles of expression and for those dynamic wrinkles linked to the hypertonia of mimic muscles [4]. Botulinum toxin acts at the level of the neuromuscular junction (motor endplate) blocking the release and effects of acetylcholine, an ester of acetic acid and choline, responsible for neurotransmission both at the central nervous system (CNS) level and at the

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peripheral nervous system (SNP) level. The enzyme acetylcholineesterase, present in the presynaptic nerve endings, continuously hydrolyses the acetylcholine which is then immediately resynthesized and stored through an active transport mechanism by means of a specific carrier protein, within synaptic cholinergic vesicles of storage. Within these cytosolic vesicles, acetylcholine is transported to the presynaptic region of the neuron (synaptic button) where it waits for the ionic signal (calcium ions) to release its role as a neurotransmitter [1].

Acetylcholine is normally released into the synaptic space trough a potential action that, by following the axon of the neuron at the last termination level of the final arborization of the axon, determines the opening of voltage-dependent ion channels: the channels of calcium. The calcium ions, present in the synaptic space, penetrate inside the synaptic button and start the realizing process of ACh into the synaptic space where it acts on specific receptors (ACh receptors). ACh receptors are located on the postsynaptic cell membrane of the muscle fibrocell, which are of two types: nicotinic and muscarinic. Interacting with ACh receptors, the neurotransmitter achieves its effects by determining, at the postsynaptic level, the opening of sodium-potassium ion channels through which the sodium ions penetrate into the muscle fibrocell which, thus, initiate muscle contraction. Immediately afterward, ACh is hydrolyzed by acetylcholinesterase. By inhibiting the release of acetylcholine, botulinum toxin interferes with the nervous impulse and causes a flaccid paralysis of the muscles. Botulinum toxin is in fact a real muscle relaxant [4].

Botulin toxin is a double-chain polypeptide consisting of a heavy chain and a light chain. The former has a molecular weight of 100 KDa while the latter has a molecular weight of 50 KDa. The heavy chain is linked to the light chain via sulfide bridges. The two chains perform different functions. The heavy chain binds to a receptor on the cell membrane of the synaptic button, the SV2 receptor, and begins the endocytosis phenomenon through which the botulinum toxin enters into the synaptic button. The heavy chain works like a sort of light chain conveyor [5].

Once penetrated into the synaptic button, the botulinum toxin releases the light chain that can perform its protease function capable of hydrolyzing the proteins of the SNARE complex (SNAP-25, syntaxin, synaptobrevin) of the neuromuscular junction preventing the release of ACh from synaptic vesicles [6].

The proteins of the SNARE complex play a crucial role in the release of ACh, because they favor the fusion between the membrane of the synaptic vesicles in which the acetylcholine and the membrane of the synaptic button are stored. The protein that is hydrolyzed is SNAP-25, and in this way, the fusion between the synaptic vesicle membrane in which the acetylcholine and the synaptic membrane are crammed is made impossible, and it is for this reason that the acetylcholine cannot be released into the synaptic space of the motor plate and the characteristic flaccid paralysis of the treated muscles is determined [7].

In 1980, botulin toxin was first described and used by ophthalmologists in humans for the treatment of strabismus [8], while its esthetic use was first reported in 1992 by Carruthers and Carruthers [9].

1.1. Commercial products of botulinum toxin

The most widespread toxin in the world has the trade name of Botox. Botox for esthetic use are called:

- Vistabel® 50 U (corresponding to Botox® used in pathology); the storage of the solution requires a temperature between 2 and 8°C, because the toxin is thermolabile. According to the technical sheet, it is maintained for up to 4 hours. According to some scientific works, in 12 hours, the effect is reduced to 50%. According to other works, the effect remains intact for 6 weeks. When inserting the needle into the bottle, the syringe must be sucked: this is a sort of test to verify that the product is actually under vacuum and has therefore been stored correctly;
- Azzalure® 125 U (corresponding to the Dysport® used in pathology); and
- Bocouture® 50 U (corresponding to Xeomin® used in pathology) is a bare toxin (not a complex protein like the previous ones). Units are not equivalent. The conversion rate is 2.5 (1 U Vistabel or Bocouture = 2.5 U Azzalure). Bocouture not requires the cold chain; it is stored at room temperature (0–25°) for 3 years and presents less risk of allergies as albumin is absent in the commercial preparation [10].

1.2. Therapeutic uses of BTX

In the last 20 years, the therapeutic spectrum of botulinum toxin has greatly increased. BoNT-A has been used for a wide range of established and emerging applications grouped into the following categories:

- neurological,
- otolaryngological,
- ophthalmological,
- urological disorders,
- esthetic,
- gastrointestinal/proctological disorders,
- pain, and
- symptomatic treatment of Parkinson's disease (PD) [11–13].

1.3. Esthetic uses of BoNT-A

In 2002, AIFA authorized the esthetic use of BoNT-A with the following indication: "Temporary improvement of vertical wrinkles, moderate to severe, between eyebrows to wrinkling, in adults aged <65 years, when the severity of such wrinkles has an important psychological impact on the patient." Although this is the only indication for esthetic use approved by the regulatory authority, many physicians use the toxin in off-label mode at injection sites other than those approved, in particular for periocular and frontal wrinkles [14]. Actually, botulinum toxin is approved by the US Food and Drug Administration (FDA) for esthetic use in the treatment of:

- axillary hyperhidrosis,
- glabellar lines, and
- lateral canthal lines.

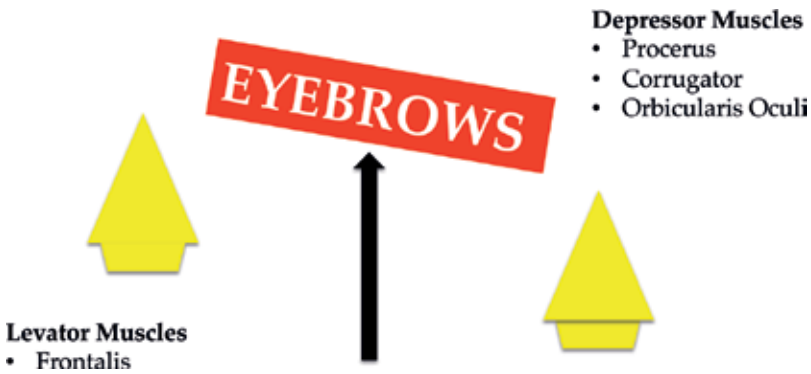

Figure 1. Fronto-orbital balance of the eyebrows: levator muscles and depressor muscles. The fronto-orbital balance clarifies botulinum toxin action: relaxing of the frontalis muscle determines a strength increase of depressor muscles, with possible ptosis. Instead, relaxing of the depressor muscles causes a strength increase of the frontalis.

The dynamic rhytides of the upper third of the face are the best indication of botulinum toxin [15, 16].

These dynamic wrinkles depend on both the muscle factor and the photoaging. If the muscle factor (young subject) predominates and if the skin is fine, you can hope for a good result; if photoaging is predominant (older subject) and if the skin is thick, the result is less good. Despite the apparent ease of injections, the correction of these glabellar wrinkles in particular requires a good understanding of the anatomy and function of the fur muscles of the region. It is necessary to respect the depression/elevator balance, which is not the same for each face, and the type of frowning to choose the appropriate doses and to respect the recommended injection points (**Figure 1**) [17].

2. Adverse events

Side effects are essentially related to active ingredient of the drug and are referred to both therapeutic and esthetic use.

2.1. Effects related to the drug

Regarding the side effects related to the drug, those most frequently reported are:

- injection of high doses of this drug (more than 200 units in every injection); and
- booster within less than 1 month is dangerous [18].

Side effects of this treatment are rare, but can include bruising, headache, allergic reactions due to allergy to human albumin or sodium chloride present as an excipient in the drug, facial and palpebral edema, injection site pain, eye pain, erythema, psoriasis, skin infections, vertigo, nausea, fever, blepharitis, xerostomia, respiratory virosis, itching, asthenia, muscle weakness, psychiatric disorders, and pneumonia ab ingestis ineffectiveness of the drug (the formation of antibodies against botulinum toxin neutralizes the effect of the toxin itself).

In many cases, side effects can be minimized by lower injection doses [19].

2.2. Side effects of esthetic use

In esthetic, the dose of use is between 6 and 400 units; the maximum dose is between 400 and 600 U; the LD50 or toxic dose is between 2500 and 3000 U.

The most common reported side effects are mild and transient, and include injection site discomfort, erythema, bruising, temporary headaches, and rarely, prolonged migraine headaches [20].

A recent study on the safety of botulinum toxin described that the treatment-related adverse events were:

- eylid ptosis,
- brow ptosis,
- eye sensory disorders in the upper face,
- lip asymmetries, and
- imbalances in the lower face [18].

Eyelid ptosis is due to the interference with the function of the upper eyelid levator muscle. It can mainly occur when there is an excessive diffusion of the toxin to the frontalis muscle. It is therefore necessary to avoid high dosage, to inject slowly and firmly to press the eyeball with a free finger to prevent any possible diffusion of the drug into the orbital area. Eyelid ptosis appears after the 2nd day and can last from 1 to 2 months. Therapy is based on the administration of an eyedrop (Iopidine®) based on apraclonidine (α-adrenergic) which causes, in addition to mydriasis, the contraction stimulation of the Muller muscle of the upper eyelid, resulting in elevation of the lash margin (**Figure 2**) [21].

Eyelid ptosis is connected with the unwanted diffusion of the product toward the eyelid lift if the corrugator has been injected too low and too far outside. This complication is always feared, even if exceptional for an experienced operator, and hardly dissipates before 4–6 weeks. A possible asymmetry of the eyebrows can be corrected secondarily if it is an excessive and/or asymmetrical lift, while the lowering is more difficult to modify. The frontal muscle should not be injected too low, especially in men who already have eyebrows and a fairly low forehead [22, 23].

Lateral brow ptosis is due to chemodenervation of the frontal leaflet and therefore the orbicularis muscle of the eyes (pars superior) pulls down the lateral third of the eyebrow.

Medial brow ptosis can occur after excess of dosage or injections too low in the frontal muscle. Prevention consists of the injections at least 2 cm above the orbital rim.

Lateral brow elevation (mephisto sign) is caused by a compensatory contraction of the lateral portion of the frontal muscle. The remedy consists in the botulinum toxin treatment of the external portion of the frontalis muscle [24, 25].

A full blockage of the frontal mimic muscles can be avoided with intradermal injections to obtain a better distribution of botulinum toxin and with lower concentration in the underlying muscular tissue [26].

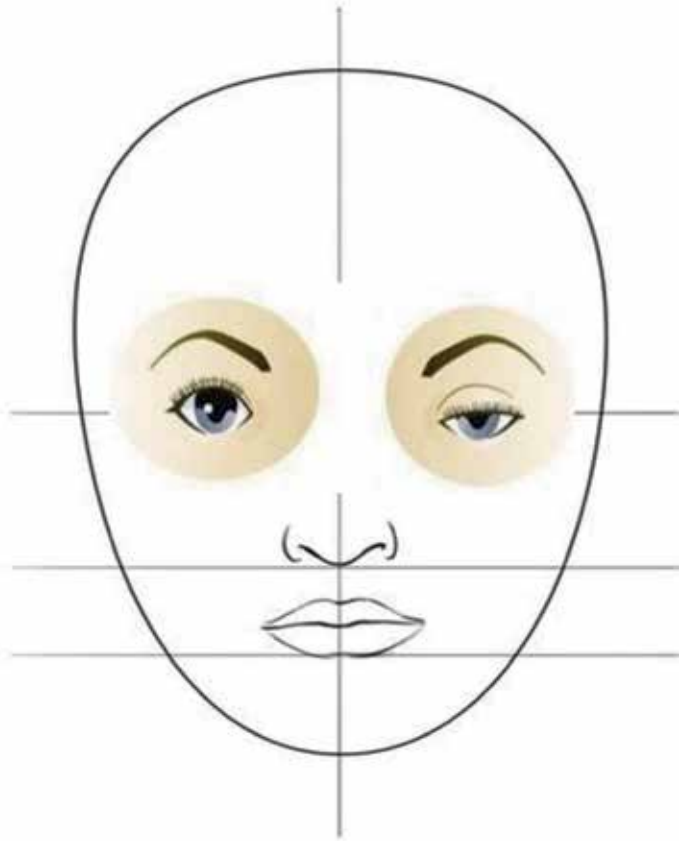

Figure 2. Schematic representation of eyelid ptosis complication of BoNT-A administration: uilateral eyelid ptosis.

A scleral show, greater evidence of sclera, can be verified after a functional deficit of the eye's orbicularis (pars inferior) following interference with the function of this muscle.

Ectropion, anomalous reversal toward the outside of the lower eyelid, is due to functional deficit of the orbicularis muscle of the eye (pars inferior) for chemodenervation of the orbicularis muscle.

A strabismus, deviation of the visual axes, is caused by the malfunction of the extrinsic oculomotor muscles (lateral rectus) with consequent inability of binocular representation at the retinal level.

Diplopia is caused by the involvement of the lateral rectus muscle through the diffusion of the toxin inside of the secondary orbitary cavity with inoculation too deep and close to the margin orbital. Temporary monolateral ocular bandage may be useful (**Figure 3**) [27–29].

Smile asymmetry is due to the toxin diffusion into the nearby zygomaticus major muscle and asymmetry of mouth mobility is caused by the blockage of the zygomatic muscle with ptosis of the lip (**Figure 4**).

Difficulty in whistling occurs after a functional deficit of the orbicular muscle of the mouth. Incidence may be reduced using diluted doses of botulinum toxin [30, 31].

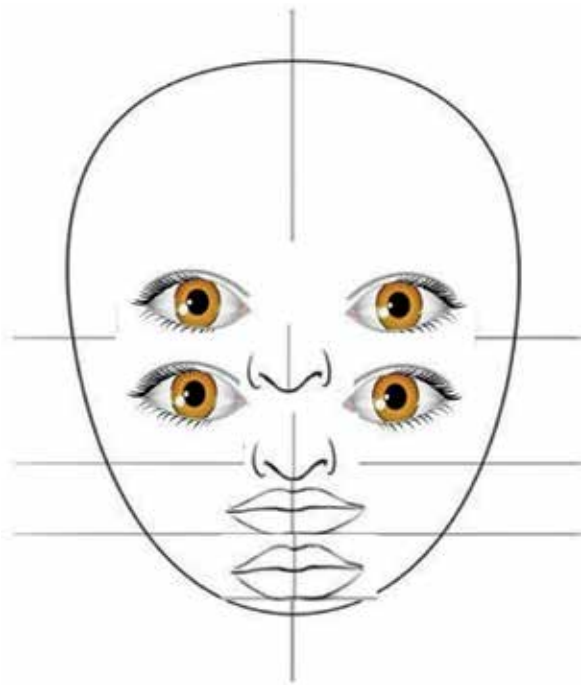

Figure 3. Schematic representation of diplopia complication of BoNT-A.

Botulinum toxin is often interesting to mitigate the fold of the marionette, which gives the face a sad and aged appearance, injecting the depressor of the corner of the mouth, which lowers the labial commissures. The injection must be low to prevent the lips from spreading to the orbicularis [32].

At the neck, the attraction through the posterior platysmal cords of the area in which the falling cheeks are delineated can be attenuated by the Nefertiti lift, injecting two or three small doses along the posterior platysmal chord and the mandibular edge. The anterior and posterior platysmal chords can be mitigated by small doses of botulinum toxin, injected every 2 cm, pinching and attracting the rope forward [33, 34].

All of these events resolved spontaneously maybe dose-dependent and were attributed to local diffusion of BoNT into adjacent areas [35].

Serious adverse events related to the cosmetic use of botulinum toxin include thyroid eye disease in a patient with Graves hyperthyroidism, sarcoidal granuloma, pseudoaneurysm of the frontal branch of the superior temporal artery, and respiratory damage [36–39].

2.3. Side effects of therapeutic use

Recent studies demonstrate that BoNT trafficking is not restricted to the neuromuscular junction, but also involves internalization of the toxin by spinal cord motor neurons and fast axonal retrograde transportation. Toxin's effect is sometimes observed beyond the site of local injection. Major adverse events can include:

- death,
- anaphylaxis,
- dysphagia,
- respiratory insufficiency, and
- muscle weakness.

These systemic events are rare and observed only at high dosages or in patients with underlying medical conditions predisposing to the complications [40–44].

Bahtia et al. reported on three patients in whom treatment of their dystonia with therapeutic doses of botulinum toxin resulted in clinical muscle weakness distant from the site of injections. It may be speculated that repeated injections at intervals of 10–12 weeks as in their patients may have an impact on toxin binding and diffusion. In fact, according to authors, the cause is most likely presynaptic inhibition due to systemic spread of the toxin [45]. Even in the case of repeated blepharospasm treatments with BoNT-A, an induction of acute myasthenic crisis has been demonstrated [46].

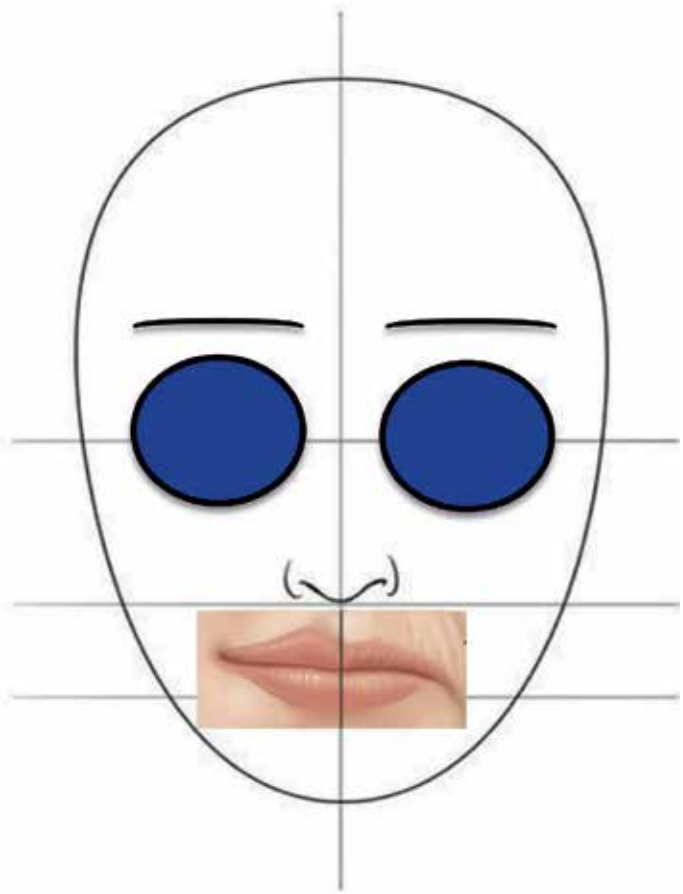

Figure 4. Schematic representation of asymmetry of mouth mobility of BoNT-A administration.

Systemic adverse events have been reported at the time of botulinum toxin A injection (6% injection episodes) and at follow-up (22% injection episodes) in children with cerebral palsy (CP), and children in Gross Motor Function Classification System (GMFCS) levels IV and V have increased rates of systemic adverse events [47].

Tugnoli et al. describes a first case of generalized muscular weakness associated with signs of systemic cholinergic autonomic impairment who was treated with 1400 U of BoNT-A for axillary and palmar hyperhidrosis. The authors assert that this case is consistent with a mild but diffuse Botulism-like syndrome, probably related to the high BoNT-A doses uses and to numerous intradermal injections and the slight build of their patient [48].

All these data demonstrate the possible risk of unwanted adverse effects due to spreading of the toxin [42].

2.4. Diffusion and migration of BoNT

In the diffusion phenomena, the concentration gradient and the BoNT molecular size determine the movement of the toxin beyond the immediate injection site through Brownian motion even if these muscles are separated by fasciae. In migration instead, a haematic and neuroaxonal transport of BoNT occurs, which is distant from the muscle and is related to systemic side effects that may be fatal if left untreated [49, 50].

Experimental studies in rodents have shown that botulinum toxin receptors exist in the central nervous system and a small amount of botulinum toxin crosses the blood-brain barrier. This raises the possibility that botulinum toxin is transported retrogradely, similar to tetanus toxin, and may cause centrally mediated side effects [51].

Botulinum toxin type-A can induce autonomic effects such as biliary colic, impairment of gastrointestinal and cardiovascular autonomic pathways, and inhibition of autonomic cholinergic pathways in the bladder. Cholinergic receptors in the pharyngeal and laryngeal sphincters are likely to be inhibited by systemic spread of BoNT and may be the main reason for dysphagia/dysphonia [52–54].

One of the suggested mechanisms for transport of the toxin from one part of the body (neck) to a remote location (toes) is the vascular spread via absorption through the capillary system and the retrograde axonal spread of the toxin. The injection of proximal upper extremity muscles with BoNT-A can determine diffusion of the toxin into the surrounding muscles resulting in dysphagia. These data suggest a systemic spread even when toxin is injected in sites anatomically adjacent to the locus of the side effect. Retrograde axoplasmic spread of the toxin is the second possible mechanism for the observed distant adverse events.

Recent studies show retrograde transport of enzymatically active toxin molecules via microtubules in the axon to both sensory and motor regions in the spinal cord after intramuscular and intraneural injections of BoNT-A. In fact, antinociceptive effect of BoNT-A may occur through retrograde spread of BoNT-A from the sensory nerves in the periphery to the central nervous system. Moreover, distant effects also may be caused by intrafusal uptake of the toxin in the muscles spindles as well as neuroplastic changes post-BoNT-A injections. Diffusion of BoNT is affected by a variety of factors; however, dose, concentration, and volume probably are the greatest contributors that increase the risk of diffusion. In general, the BoNT reduction in amplitude increased with increasing doses and with increasing concentration [55–57].

To limit diffusion is target muscle localization using EMG and endoscopic or imaging guidance [58].

2.5. Nonresponsiveness to treatment with BoNT

Nonresponsiveness to BoNT could be as a result of possible factors that include misdiagnosis, insufficient dose, problems with toxin storage and preparation, and administration. Another possible reason for lack of clinical effect is immunoresistance to BoNT, which refers to ineffectiveness of the toxin as a result of development of neutralizing antibodies against the toxin [59].

The formation of neutralizing antibodies to BoNT is increased by a short time period between injections, the administration of booster injections, and the use of high BoNT doses. To prevent antibody formation against BoNT, the practitioner can use a newer BoNT formulation with the lowest protein content [60].

3. Contraindications and interactions with some medications

BoNT is contraindicated in patients with known peripheral motor neuropathies or neuromuscular disorders, such as Eaton-Lambert syndrome, multiple sclerosis, and myasthenia gravis, because further chemodenervation may exacerbate muscle weakness. The cause is to be found in a reduced release of acetylcholine in the neuromuscular endplate, due to the effect of autoantibodies against the presynaptic channels of calcium [61].

The treatment can be performed in the 18–65 age range. Other contraindications are represented by:

- allergy to human albumin and/or sodium chloride,
- skin infections,
- presence of scleral show,
- senile ectropion,
- pregnancy,
- lactation,
- dysphagia, and
- psychiatric disorders.

Aminoglycoside antibiotics that can enhance the effect of botulinum toxin are netilmicin, tobramycin, gentamicin, neomycin, amikacin, kanamycin, and streptomycin. Other drugs that also interfere with neuromuscular transmission are muscle relaxants such as D-tubocurarine, baclofen, thiocolchicoside, tizanidine, diazepam, dantrolene, and pridinol [62, 63].

4. Rehabilitation of the motor endplate

The rehabilitation of the motor endplate can be very useful in case of side effects following treatment with botulinum toxin.

Radioiodinated botulinum toxin A (125I-BoNT/A-complex, 67 or 344 U free-125I-BoNT/A) was injected into the gastrocnemius muscle of rats and measured in various tissues at different time points. These "in vivo" studies allowed to establish that after 24 hours, the toxin is no longer present in the infiltrated muscle.

Thus, the side effects reported seem to be related to the damage caused by toxin caused and not to the presence of it in the muscles. These effects can be visible after 10–12 days [64].

For this reason, it is useless to administer the antitoxin which exerts its action by binding to the toxin still in circulation, complexing it and making it inactive. Furthermore, the healing capacity depends on the regeneration of the affected synaptic terminations.

Because the light chain of botulinum toxin causes proteolysis of the SNAP 25 protein, reducing its endocellular pool, one must reestablish its own physiological endocellular pool.

In practice, it is necessary to stimulate the biosynthesis of the SNAP25 protein to favor the structural and functional recovery of the motor endplate.

The aim of the therapy is to stimulate the biosynthesis of the SNAP 25 protein, consisting of about 200 amino acids. So, we can correct side effects such as ectropion, diplopia, palpebral ptosis, strabismus, scleral show, and asymmetries of smile and mouth mobility.

To improve the biosynthesis of the SNAP 25 protein, it is necessary to take:

a. A proteic diet (meat, fish);

b. Amino acids such as arginine and cysteine as they belong to the molecular composition of the SNAP-25 protein. Then, we supplement other amino acids: arginine, bioargin, and cysteine;

c. L-acetylcarnitine which is an agonist of the mitochondrial growth function and reparative agents (NGF), expounds an antioxidant activity in the neurons of the central and peripheral nervous system. L-acetylcarnitine is structurally similar to acetylcholine and plays an indispensable role for proper cellular energy, metabolism, and neurotransmission;

d. Alpha-lipoic acid (also called thioctic acid), a fat-soluble vitamin that participates in various antioxidant mechanisms such as the regeneration of reduced glutathione (GSH) and ascorbic acid; and

e. L-carnosine, a dipeptide composed of β-alanine and L-histidine; it has the ability to promote protein regeneration even in difficult situations such as in the late stage of the life cycle. It has antioxidant properties.

This therapy is able to guarantee fast responses (7–10 days) and in 80% of cases [65–68].

5. Conclusions

The use of BoNts continues to steadily expand and multiply. New indications of clinical use of BoNTs are continuously emerging in medical therapy and further applications will be developed in the future. Adverse events occur more frequently after the clinical use of the toxin, but may also disclose after its esthetic use. The safe utilization of BoNTs requires knowledge of its indications and pharmacology, anatomy of the treated muscles to avoid serious complications.

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Chapter 3

Experimental Comparative Effects of Botulinum Toxin A between Subtypes A1 and A2 in Movement Disorders in Rats

Tomoko Kohda, Yasushi Torii and Hidemitsu Nakajima

Additional information is available at the end of the chapter

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Abstract

In the present review, we describe here experimental comparative and beneficial effects of botulinum neurotoxin A (ANTX) between subtypes A1 and A2 in the pathology of movement disorders, particularly rat Parkinson's disease model. We and other laboratories have shown the beneficial effects, and this novel strategy for intractable brain disorders might confer potent and safety therapy in bedside. First, we show the characteristics of ANTXs in the genetic aspects of these subtypes, and our intriguing findings of immunological profiles in the subtypes between A1NTX and A2NTX. Then, we state the distinct diffusion in the body between A1NTX and A2NTX. Importantly, we describe that the intra-brain treatment of small animals with A2NTX subtype results in improvements of pathologies more effectively and provides greater safety than those of A1NTX in a rat 6-OHDA Parkinson's disease (PD) model. Finally, we represent that the different efficacies between ANTXs are likely due to each localization in the brain; A2NTX is strictly limited in the injected regions, while A1NTX diffused other brain regions. Thus, therapeutic avenue using A2NTX in incurable PD including other movement disorders could be a druggable target in the future.

Keywords: botulinum neurotoxin type A, pharmacokinetics/pharmacodynamics, Parkinson's disease, therapeutics, safety, experimental, rats

1. Introduction

Clostridium botulinum produces highly potent neurotoxin, which causes a persistent paralysis of peripheral nerve terminals. The toxin is classified into seven serotypes (A–G). Type A,

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B, E, and F toxins are responsible for human botulism, whereas type C and D toxins cause botulism in other animals. The toxins are large complexes, known as progenitor toxins, which differ in terms of molecular size. The progenitor toxins are containing a neurotoxin (NTX) and several nontoxic components. The nontoxic proteins compose a nontoxic non-hemagglutinin component (NTNHA) and several hemagglutinin (HA) component proteins. In botulinum complex, the proteins are not covalently linked, but their association occurs in culture. The sizes of complex toxins differ, from 900 kDa (LL toxin for type A) to 500 kDa (L toxin for types A, B, C, D, and G), down to 300 kDa (M toxin for types A, B, C, D, E, and F). The LL toxin is a dimer of L toxin, which consists of NTX, NTNHA, and HAs. The M toxin consists of NTX and NTNHA. The complex toxin is also stable at acidic pH but dissociates at alkaline pH (pH $\geqq$ 7).

NTXs are released from *C. botulinum* as single polypeptides with molecular mass about 150 kDa, which are proteolytically activated and composed of light chain (50 kDa) and heavy chain (100 kDa) by disulfide bond. The light chain (LC) acts as a zinc-dependent endopeptidase. The heavy chain is divided into two different functional domains: the amino-terminal (H_N) domain and the carboxyl-terminal (H_C) domain. The H_C acts as the receptor-binding domain, and H_N acts as pH-dependent translocation domain of LC from endosome to cytosol. Neuronal endocytosis is driven by the formation of protein complex between the vesicle N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE), VAMP2, and the plasma membrane SNAREs, synaptosomal-associated protein of 25 kDa (SNAP-25) and syntaxin [1]. Type C toxin cleaves both SNAP25 and syntaxin; type B, D, F, and G toxins only target VAMP; and type A and E toxins cleave SNAP25. NTX inhibits endocytosis by the cleavage of one of the three proteins. Due to high efficiency and longevity, the toxins are the most widely used therapeutic proteins.

Due to their high efficacy, tolerance, longevity, and safety property, botulinum toxins are the most widely used therapeutic proteins. Most of the toxins have several subtypes based on amino acid sequence variability. The type A toxins have been subclassified into 10 subtypes (A1–A10) [2]. Especially, the toxin products used as treatment for neurologic disorders are LL toxin and NTX, produced by botulinum toxin subtype A1. The other subtype of toxins is not used clinically, however has been conducted in researches. We have been studying the biological characteristic and pharmacology of A2 toxin.

Several species of botulinum neurotoxin are known to act on cholinergic terminals of the peripheral neuromuscular junction and the central nervous system (CNS) [3–5]. NTXs cause robust inhibition of the voluntary nervous circuits by blocking the release of acetylcholine (ACh) [6]. The therapeutic application of A1NTX for neurological disorders such as bradykinesia, urinary dysfunction, hemifacial spasm, and cervical dystonia is well established [7]. The type A organisms have been classified into 10 subtypes (A1 to A10) based on the amino acid sequence variability of NTX [2]. All 10 subtypes bind to presynaptic protein SNAP-25 with similar affinity, but A1NTX and A2NTX cleave SNAP-25 more efficiently than that of other subtypes [4, 8, 9].

Recent studies investigated the direct administration of ANTX to the CNS as a therapeutic strategy for the treatment of neurological disorders [3–5]. Parkinson's disease (PD) is characterized by imbalanced cholinergic hyperactivity in the striatum of affected individuals [10, 11]. Interruption of ACh release in the striatum by direct injection of BoNT/A has been reported in the rat unilateral 6-hydroxydopamine (6-OHDA) model of PD [12].

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This paper will review the recent advance in the genetic, immunological, diffusion in the body and experimental animal model of PD in botulinum toxin A.

2. Genetic diversity between A1NTX and A2NTX

2.1. Genetic diversity of gene clusters encoding ANTX complexes

The neurotoxin and nontoxic protein genes are defined as the NTX gene cluster. There are two types of nontoxic components of gene organization (HA and Orfx clusters), and *C. botulinum* type A strains were classified according to their harboring of these clusters. The NTX genes are encoded by mobile genetic elements that enable horizontal transfer among different isolates, which is thought to contribute to evolution of the NTX gene loci and thereby to the large number of distinct NTXs that are currently known [13]. Further type A strains have been classified as boNT and HA gene cluster typing to be applied for molecular characterization of type A strain. Genes encoding components of the A1NTX and A2NTX complex are arranged clusters. Type A strains possess HA cluster genes and A1NTX to NTX gene cluster typing 1, Orfx cluster genes and A2NTX to NTX gene cluster typing 2, and HA cluster genes, Orfx cluster genes, and A1NTX with unexpressed or expressed BNTX to NTX gene cluster typing 3 [14]. Umeda et al. have reported that *C. botulinum* type A isolates genotypes by combining the results of NTX subtype (subtype A1 or A2) gene detection with ha33 and/or p47 gene detection by multiplex PCR. Ten isolates associated with infant botulism in Japan were divided into NTX gene cluster typings 2 and 3 by origin (honey feeding or not) and period (1986–1987 and 1999–2007). And, four isolates associated with food-borne botulism in Japan were divided into NTX gene cluster typings 1 and 3. The multiplex PCR method is easily capable of classification of NTX gene cluster typing [15]. Further, genetic characterization was performed in ten botulism cases in Japan between 2006 and 2011. Except two type B isolates, eight type A isolates are NTX gene cluster typings 1 and 3 which are associated with HA cluster genes [16]. NTX gene cluster typing 2 is predominant in Europe, while NTX gene cluster typings 1 and 3 are predominant in the USA [14, 17, 18]. As *C. botulinum* type A is rarely found in Japanese soil, there is a possibility that imported foods are related to botulism cases.

2.2. Immunological differences between A1NTX and A2NTX

The difference in amino acid sequence between subtype A1 and A2 toxins' light chain is 5%, while the difference in heavy chain is 13%. The similarity of heavy chain is lower than light chain. These differences appear to indicate that characteristic antigenicity in the heavy chain is more conserved than that in the light chain [19]. Differences in antigenicity among subtypes were evaluated using monoclonal and polyclonal antibodies [20–23]. Among eight and seven monoclonal antibodies against A1NTX and A2NTX, respectively, each of which recognized different epitopes, each three specifically reacted with A1NTX and A2NTX. Neutralizing single monoclonal antibodies against A1NTX and A2NTX that recognized LC, H_N, or H_C have been reported, respectively (**Tables 1** and **2**). Each neutralizing antibody mostly neutralized only toxins of their own subtypes. It is suggested that the epitopes of neutralizing are present in every domain of both subtypes. The 3B10 and 5G2 that are reacting with LC and H_N, respectively, specifically

recognized and neutralized A2NTX. These monoclonal antibodies recognizing epitopes are considered to function as A2NTX properties. In type B, differences in biological activities among the subtypes B1, B2, and B6NTX appeared to be attributable not only to the function in H_C but also to the function in H_N [24]. For binding of monoclonal antibodies to NTX, KD values of 1F11 for A1NTX were 500 hold higher than that for A2NTX and only neutralized A1NTX. However, the KD values of 5C7 for A2NTX were 16 hold higher than that for A1NTX did not neutralize both NTXs. The neutralization of monoclonal antibody did not correspond to its affinity. And, OD values obtained by ELISA did not necessarily correlate with KD values (**Table 3**).

Type A antitoxin in standard and therapeutic preparation is a polyclonal antibody purified from immunized sera with A1NTX; however, there was no report on the reactivity of the standard type

mAb[1]	ELISA (OD_{450})[2]			Blotting	Neutralization[3]	
	A1NTX	A2NTX	Toxoid/A1NTX		A1NTX	A2NTX
1D4	0.879	0.795	0.787	L	-	-
1E2	0.741	0.820	0.725	L	++	+
1B12	0.748	0.372	0.505	H_N	++	-
1F11	0.953	0.404	0.853	H_N	++	-
10H3	0.725	0.090	0.453	H_N	-	-
4E4	0.967	0.005	0.002	Hc	++	-
6D9	0.565	0.235	0.151	Hc	-	-
9A3	0.643	0.040	0.051	Hc	++	-

[1]: MAbs were raised against A1NTX.

[2]: Values were obtained with each mAb at 1 μg/ml.

[3]: Mix mAb (10 μg/ml) 300 μl with A1NTX (40 LD_{50}/ml) 300 μl in GPB, and incubate at room temperature for 30 min. Inject intraperitoneally 0.5 ml of the mixture to a mouse and monitor the mouse for 4 days. (-): time to death≤24 h; (+): 24 h< time to death≤96 h; (++): time to death>96 h

Table 1. Properties of mAbs raised against A1NTX.

mAb[1]	ELISA (OD_{450})[2]			Blotting	Neutralization[3]	
	A1NTX	A2NTX	Toxoid/A2NTX		A1NTX	A2NTX
3B10	0.063	0.824	0.305	L	-	++
4G12	0.745	0.805	0.432	L	-	+
9B3	0.614	0.894	0.466	L	-	-
2A12	1.185	0.969	0.404	H_N	-	-
5G2	0.025	0.539	0.222	H_N	-	++
5C7	0.601	0.591	0.085	Hc	-	-
6A5	0.015	0.865	0.338	Hc	-	+

[1]: MAbs were raised against A2NTX.

[2]: Values were obtained with each mAb at 1 μg/ml.

[3]: Mix mAb (10 μg/ml) 300 μl with A2NTX (40 LD_{50}/ml) 300 μl in GPB, and incubate at room temperature for 30 min. Inject intraperitoneally 0.5 ml of the mixture to a mouse and monitor the mouse for 4 days. (-): time to death≤24 h; (+): 24 h< time to death≤96 h; (++): time to death>96 h

Table 2. Properties of mAbs raised against A2NTX.

mAb[2]	against NTX	K_D (M)[3] (A1NTX)	K_D (M)[4] (A2NTX)
1D4	A1	3.8×10^{-9}	3.3×10^{-9}
1E2	A1	9.2×10^{-10}	8.5×10^{-10}
1B12	A1	2.5×10^{-9}	2.7×10^{-10}
1F11	A1	6.2×10^{-10}	1.3×10^{-8}
10H3	A1	1.3×10^{-9}	ND[5]
4E4	A1	4.2×10^{-10}	ND
6D9	A1	2.8×10^{-9}	3.2×10^{-9}
9A3	A1	8.1×10^{-11}	ND
3B10	A2	ND	8.9×10^{-10}
4G12	A2	7.7×10^{-9}	3.7×10^{-8}
9B3	A2	7.7×10^{-9}	1.5×10^{-8}
2A12	A2	ND	ND
5G2	A2	ND	8.7×10^{-8}
5C7	A2	1.6×10^{-8}	2.7×10^{-9}
6A5	A2	ND	2.7×10^{-9}

[1]: Association (k_a) and dissociation (k_d) rate constants were determined by surface plasmon resonance in biacore, and K_D was calculated as k_d/k_a.

[2]: MAbs were raised against A1NTX.

[3]: K_D for mAbs binding to A1NTX.

[4]: K_D for mAbs binding to A2NTX.

[5]: Not determinable.

Table 3. Equilibrium dissociation constants (***KD***)[1] of A1NTX and A2NTX with mAbs against A1NTX and A2NTX.

A antitoxin with other subtype toxins. The A1 antitoxin had equivalent potency both the A1NTX and A2NTX; however, neutralization titer of A2 antitoxin was 4–9 hold higher against A2NTX than against A1NTX. It seems that the difference between the antibody titers against the test NTX was due to the standard antitoxin having different reactivities with the NTXs. The binding analysis comparing these antitoxins and NTXs by SPR showed that the A1 antitoxin had a higher binding affinity and slower dissociation speed with the A1NTX than with the A2NTX. The A2 antitoxin showed a higher binding affinity than with the A1NTX [22]. Although these NTXs show a low level of sequence difference, they have marked a difference in antigenicity, and antitoxin preparation should be used for each subtype's diagnosis and therapy of botulism.

3. Diffusion into the body of botulinum toxins A1 and A2

Botulinum toxins type A have been researched and developed for use as important therapeutic agents for neurological disorders such as blepharospasm, hemifacial spasm, various dystonias, and overactive bladder [7, 25]. Botulinum toxin type A products, which are used as treatment for neurologic disorder, are produced from LL toxin or NTX derived from subtype A1 organisms [26]. The toxins show high-level efficacy at very low doses, but their adverse effects are

becoming an issue. In the treatment for torticollis, cervical dystonia, and cosmetic cases, patients showed dysphagia or respiratory compromise [27–29]. In clinical studies of treatment for spasm, patients who received high-dose toxin showed weakness around the site of administration as well as symptoms of botulism [30–32]. The A1 toxins spread to distant regions is considered to be due to transport via the body fluid or nerves [33–35]. In addition, A1 toxin was reported to transport via a retrograde axonal route in visual nerve and facial motoneurons in rats [3].

The first report of the diffusion of A2 toxin in the body was grip strength study in mice to compare with A1 toxin [36]. This study was evaluated by measurement of contralateral grip strength as indicator of toxins' diffusion. The toxins used were A1 L + LL toxin, onabotulinumtoxinA (A1LL toxin), and A2M toxin and were injected into one side of the gastrocnemius muscle, and grip strength of the contralateral hind leg was measured. The study evaluated that the doses causing a 20% reduction in the grip strength before injection were calculated and these values were termed the 20% toxic doses (TD_{20}). The TD_{20} of A1L + LL toxin, A1LL toxin, and A2M toxin were 17.0, 16.2, and 37.3 U/kg, respectively. The grip strength test was conducted for change in toxins' forms, measurement sites, and animal species [37]. The grip strength test using rats' forelegs was conducted using A1 neurotoxin (A1NTX), A1LL toxin, and A2NTX (**Figure 1**). The study evaluated that 50% toxic doses (TD_{50}), which caused a 50%

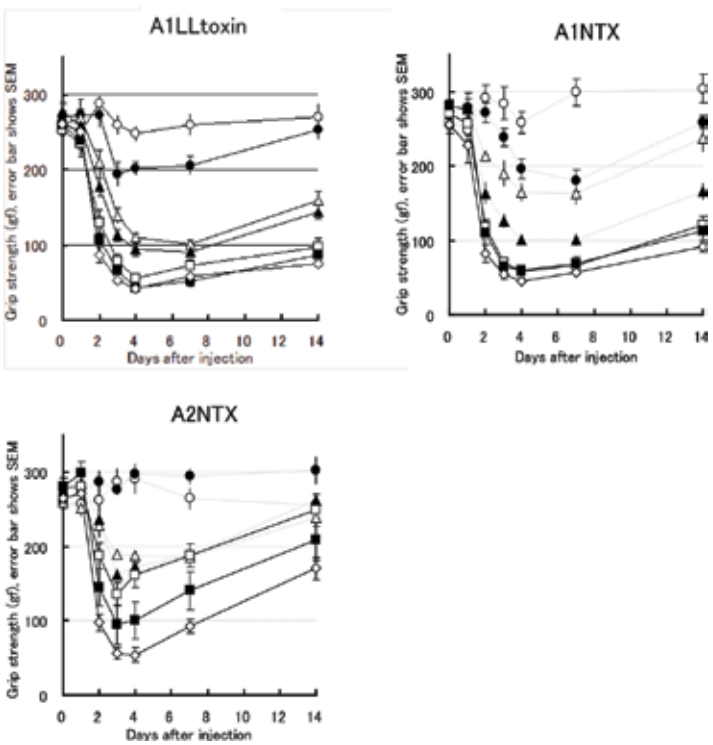

Figure 1. Time-course of the grip strength of the contralateral foreleg after toxin injection. Rats received A1LLtoxin, A1NTX, or A2NTX injection in the left foreleg (each at ○: 1 U, ●: 4 U, △: 8 U, ▲: 12 U, □: 16 U, ■: 20 U, and ◇: 24 U). The grip strength was measured in the right foreleg of each rat at 0 (before administration), 1, 2, 3, 4, 7, and 14 days after injection. Each point is the mean ± S.E.M. (n = 5). These data are cited from Toxicon (Trii, *et al.*, Vol; 57(1), [2011] pp. 97) with the permissions of ELSEVIER.

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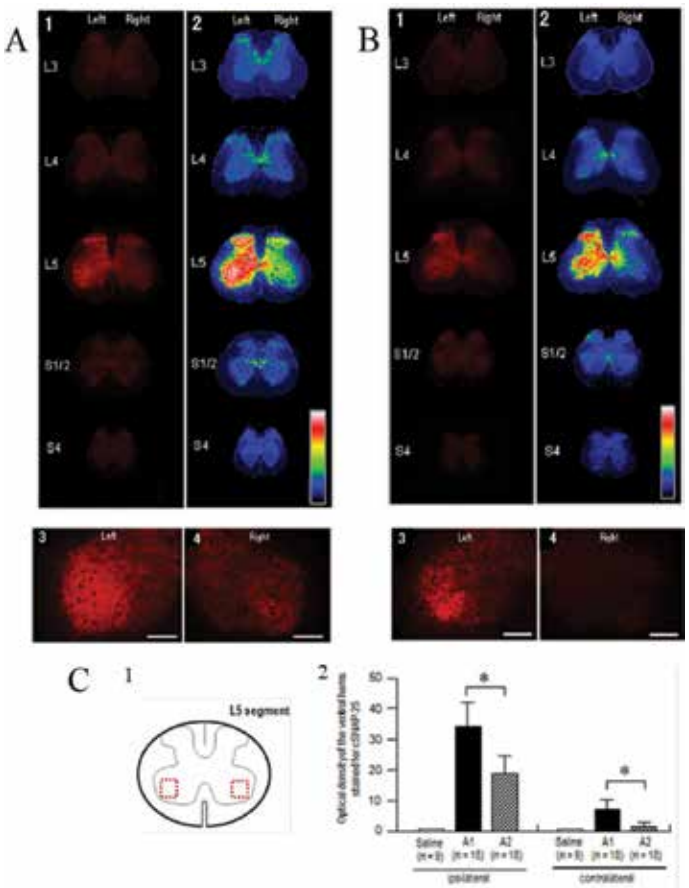

Figure 2. Appearance of botulinum toxin type A-cleaved SNAP-25 (cSNAP-25) in the spinal cord after intramuscular injection of A1- and A2NTX. Densitometric analysis on the spinal ventral horns stained for cSNAP-25. A, B: Immunohistochemical detection of cSNAP-25 was carried out in the spinal cord 4 days after unilateral injection of A1- or A2-NTX (10 U) into the left gastrocnemius muscle. (A: A1NTX, B: A2NTX) 1, 2: Displayed are multiple transverse spinal cord sections stained for cSNAP-25 in the toxin treated rats (1) and their graded color-converted images (2), in which labeling intensity is indicated in a standard pseudocolor scale from blue (lowest level) through green, yellow, red, and white (highest level). 3,4: Photomicrographs of the ventral horns stained for cSNAP-25 ipsilateral (3) and contralateral (4) to peripheral toxin injection. Scale bars = 200 mm. C: Densitometric analysis on the spinal ventral horns stained for cSNAP-25. 1: The scheme shows the transverse spinal cord section at the L5 segment, in which measured areas in the bilateral ventral horn are indicated by dashed open boxes colored in red. 2: Optical densities of the ventral horns stained for cSNAP-25 in rats treated with saline (n = 3), A1NTX (A1) (n = 6), or A2NTX (A2) (n = 6). For each animal, measurements were made in the ventral horns of three spinal cord sections ipsilateral and contralateral to the toxin-injected sites. Values are means ± SD. *P < 0.05, A1 versus A2; Mann–Whitney U-test. These data are cited from frontier in neurology (Torii, *et al.*, [2014] pp. 97. 2014; 5:98) with permissions of frontier media.

reduction in the grip strength before injection, were calculated. The TD_{50} values of A1NTX, A1LL toxin, and A2NTX were 7.54, 6.35, and 15.62 U/head, respectively. These results indicated that A2NTX required higher dosage than A1 toxins to relax on the contralateral muscle and suggested that A1 and A2 toxins have different diffusions in the body.

Why do these toxins make a difference in diffusion in the body? The pathway of A1 and A2 toxins was physiologically investigated in the immunohistological study [38].

Spinal cords (bilateral ventral and dorsal horns), in which A1NTX and A2NTX were injected into the gastrocnemius muscle, were strained using botulinum toxin type A-cleaved SNAP-25 (cSNAP-25). The L5 nerve dominantly innervates the gastrocnemius muscle. The A1NTX was observed to have a strong immunoreactivity for cSNAP-25 in the ventral and dorsal horns of the spinal cord not only at the segmental level of L5 ipsilateral to the peripheral toxin injection site but also to a lesser extent on the contralateral side (**Figure 2A**). The A2NTX was observed to have a strong immunoreactivity at the L5 spinal segment ipsilateral side as A1NTX but to a lesser extent on the contralateral side than A1NTX (**Figure 2B**). In addition, the ventral horns stained for cSNAP-25 at the L5 spinal segment in the toxin-treated rats were compared by optical density measurements. In both the ipsilateral and contralateral ventral horns, cSNAP-25 labeling in rats injected with A1NTX was spread wider than with A2NTX (**Figure 2C**).

The diffusion of A2NTX in the body summarized the previous reports as follows (**Figure 3**). After unilateral intramuscular toxin injection, the catalytically active toxin can be axonally transported to the spinal cord through motor and sensory nerves. Subsequently, the toxin can spread throughout the gray matter of the spinal cord, including the bilateral ventral and dorsal horns, via a transcytosis (cell-to-cell trafficking) mechanism by which a ligand penetrates the neuron at one side, followed by its movement and release at the opposite end, with possible uptake by second-order neurons. Differential delivery routes by which injected A1NTX and A2NTX affect contralateral muscles have also been proposed as A1NTX is transported almost equally to the contralateral muscles via this neural pathway and the blood circulation, while A2NTX is mainly transported to contralateral muscles via the bloodstream only at higher doses.

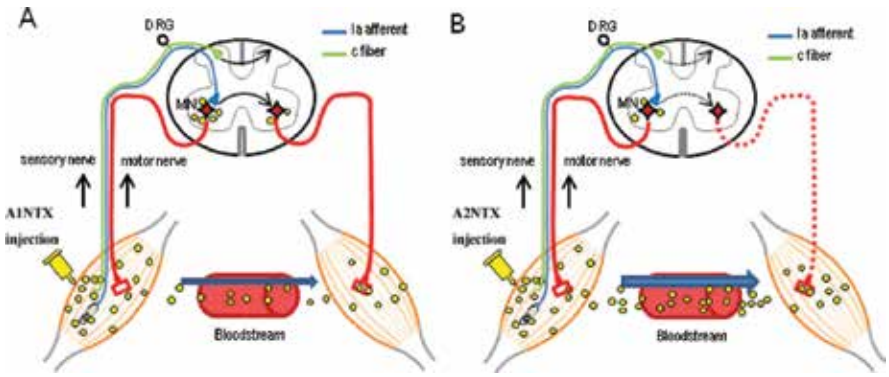

Figure 3. Possible mechanisms for the central actions of intramuscularly injected botulinum toxin type A in the spinal cord. Following unilateral intramuscular A1NTX (A) or A2NTX (B) injection, the catalytically active toxin can be axonally transported to the spinal cord through motor and sensory nerves. Subsequently, the toxin can spread throughout the gray matter of the spinal cord, including the bilateral ventral and dorsal horns, via a transcytosis (cell-to-cell trafficking) mechanism by which a ligand penetrates the neuron at one side, followed by its movement and release at the opposite end, with possible up take by second-order neurons. Differential delivery routes by which injected A1NTX and A2NTX affect contralateral muscles have also been proposed as A1NTX (A) is transported almost equally to the contralateral muscles via this neural pathway and the blood circulation, while A2NTX (B) is mainly transported to contralateral muscles via the bloodstream only at higher doses. These data are cited from frontier in neurology (Torii, *et al.*, [2014] pp. 5:98) with permissions of frontier media.

4. Therapeutic application of botulinum toxins A1 and A2 in Parkinson's disease

Parkinson's disease (PD) is one of the most common movement disorders and is characterized by a progressive degeneration of nigrostriatal dopaminergic signaling, which leads to the unbalanced release of acetylcholine in the striatum [10]. The disturbance of these neuronal circuits elicits parkinsonian motor symptoms with muscular dysfunctions, such as resting tremor, spontaneous dystonia, akinesia, sialorrhea, urinary dysfunction, and pain [10, 39]. While palliative therapies for PD subjects having sialorrler and urinary dysfunction using onabobotulinamtosinA (nealy equal to A1NTX) are going in bedside [12], there is currently a lack of curative therapies using ANTXs.

Several studies demonstrated that the intrastriatal injection of A1NTX reduces pathologic behavior in the rat 6-hydroxydopamine (6-OHDA)-induced Parkinson's disease model (rat 6-OHDA PD model) [11, 40]. These studies demonstrate the feasibility of clinical A1NTX application to treat PD without adverse side effects such as memory dysfunction [11, 40]. However, it is not clear which A1NTX has the greatest efficacy for treatment of PD. Therefore, we first compared the effect of A1NTX with that of A2NTX on pathogenic rotation behavior and in vivo cleavage of striatal SNAP-25 in the 6-OHDA PD rat model.

As a result, intrastriatal treatment of 6-OHDA-lesioned rats with A1NTX or A2NTX significantly reduced the pathogenic rotation behavior in a dose-dependent manner (**Figure 4**). The highest tested dose of A1NTX (1 ng) conferred significant reduction of pathogenic behavior, as did all tested A2NTX doses (0.1, 0.5, and 1 ng). These results suggest that A2NTX has more potent inhibition of ACh release in the striatum than that of A1NTX [40]. Indeed, intrastriatal injection of the 6-OHDA-lesioned rats with A1NTX or A2NTX caused a dose-dependent

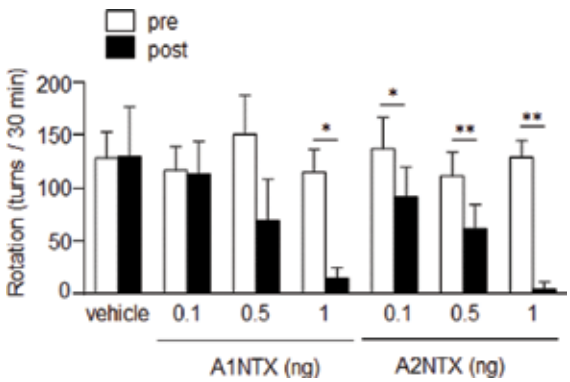

Figure 4. Effects of intrastriatal injection of A1NTX (0.1, 0.5, or 1 ng/rat; n = 6 per dose), A2NTX (0.1, 0.5, or 1 ng/rat; n = 6 per dose), or vehicle (n = 7) on methamphetamine-induced rotation behavior. All rats received ANTX or vehicle injected into the lesioned striatum induced by 6-OHDA injection. For the tests, pre (white columns) represents before injection of ANTX, and post (black columns) represents after injection of ANTX. Data represent means ±S.E.M.; statistical significance is determined as pre versus post in a paired Student's t-test; *$p < 0.05$; **$p < 0.01$. These data are cited from biochemical and biophysical research communications (Itakura et al., Vol;. 447(2), [2014] pp. 312 with the permissions from ELSEVIER).

decrease in the level of full-length SNAP-25 in the striatum [40]. These results support the observed effects of A1NTX and A2NTX on rotation behavior (**Figure 4**). Additionally, we investigated the localization of cleaved SNAP-25 and choline acetyltransferase in the ANTX-treated striatum by performing fluorescent immunocytochemical analysis [40]. These results indicate that A2NTX has greater efficacy for SNAP-25 cleavage in striatal terminals than that of A1NTX. Therefore, their dose-dependent efficacies in the striatum appear to differ, although the therapeutic effects of both toxin species on reducing pathologic rotation behavior in a PD rat model are likely due to their cleavage of SNAP-25 [40].

Several side effects have been reported after therapeutic treatment with ANTXs for cervical dystonia and cosmetic cases, such as dysphagia and respiratory compromise [28, 29]. Our studies also demonstrated that the effects of botulinum toxin could spread from the injection site to other areas of the body causing symptoms similar to those of botulism [41]; A1NTX, but not A2NTX, was transported via axons to the contralateral side after injection into the foreleg muscles as described in Section 3. These results suggest that A2NTX may have a wider safety margin than that of A1NTX for therapeutic applications for PD. Thus, we investigated side effects after intrastriatal injection of either A1NTX or A2NTX in the rat 6-OHDA PD model [42].

To investigate the distribution of A1NTX or A2NTX in the striatum, an immunofluorescent analysis of the cleaved SNAP-25, which is produced by ANTXs, is performed. The area of survey is shown in **Figure 5A**. Compared to the treatment with vehicle control (**Figure 5B**), the treatment with A1NTX increased the cleaved SNAP-25 in both the ipsilateral and contralateral striata (**Figure 5C** and **E**). In contrast, for A2NTX, the cleaved SNAP-25 signals were observed only in the ipsilateral striatum (**Figure 5D** and **F**). These results indicated that A2NTX was retained at the injection site, whereas A1NTX was diffused into the contralateral striatum.

Indeed, the previous study showed that ANTXs were retrogradely transported by central neurons and motoneurons and were then transcytosed to afferent synapses. The SNAP-25 cleaved by ANTXs was observed in the contralateral hemisphere after unilateral ANTX injection to the hippocampus [12, 43]. Moreover, this finding is supported by our findings showing that A1NTX injected into the foreleg muscles was transported via axons to the contralateral side more readily than A2NTX as indicated in Section 3.

Furthermore, we evaluated changes in body weight as an index of the adverse effects of ANTX application. Body weights were measured 1 and 9 days after the 1.0 ng ANTX injection. Treatment with A1NTX resulted in significant loss of body weight compared to both the vehicle and A2NTX groups (**Figure 6**). Together with **Figures 3** and **5**, these results suggest the possibility that A1NTX, but not A2NTX, diffuses into the contralateral hemisphere leading to dysfunction in food/water intake.

Why does the difference between A1NTX and A2NTX arise in a rat PD model? Interestingly, A2NTX enters neuronal cells faster than A1NTX [44]. Additionally, we found that A1NTX and A2NTX have distinctly different distributions in the peripheral neuromuscular system in Section 3. Unfortunately, these findings only are not sufficient to explain the differences of ANTX subtypes in vivo. Thus, further studies are needed to elucidate the variation among ANTXs from the views of genetic, immunological, and neurological aspects.

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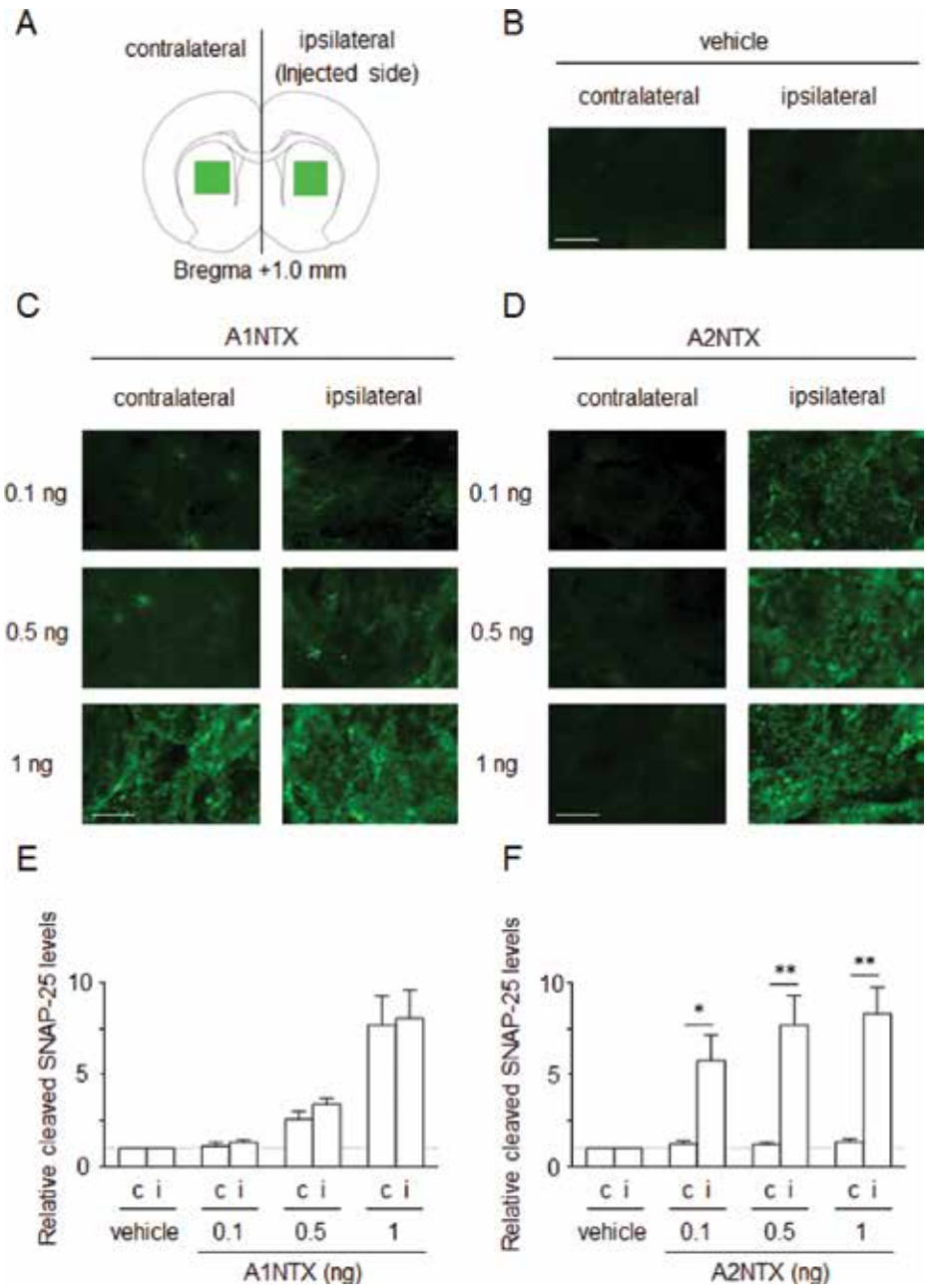

Figure 5. Distribution of A1NTX and A2NTX in the striatum. In (A), green squares represent the brain regions analyzed for the following experiments. Immunofluorescent analysis of cleaved SNAP-25 in the striatum following the intrastriatal injection of vehicle (n = 3) (B), ANTX1 (0.1, 0.5, 1.0 ng/rat; n = 3 per dose) (C) and A2NTX (0.1, 0.5, 1.0 ng/rat; n = 3 per dose) (D) are shown. Semiquantification of the cleaved SNAP-25 signals are shown for the contralateral (indicated as "c") or ipsilateral (indicated as "i," injected side) striatum relative to the vehicle-treated group (E and F). scale bars = 50 μm. Data represent means ± S.E.M.; statistical significance was determined as contralateral versus ipsilateral using a paired Student's t-test; *$p < 0.05$, **$p < 0.01$. These data are cited from Journal of Veterinary Medical Science (Itakura et al., Vol. 76(8), [2014] p. 1191 with the permissions from The Japanese).

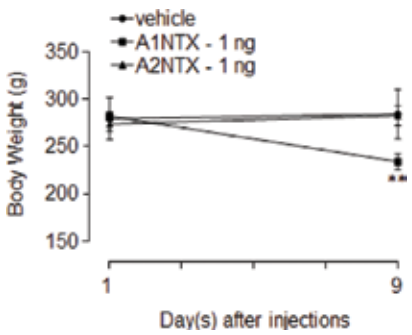

Figure 6. Loss of body weight induced by A1NTX injection. At one and 9 days after vehicle (n = 5), 1.0 ng A1NTX (n = 5) or 1.0 ng A2NTX (n = 4) injection, body weights were measured for all groups. Data represent means ± SE; statistical significance is determined as ANTX-treated groups versus vehicle using a Student's t-test; **p < 0.01. These data are cited from Journal of Veterinary Medical Science (Itakura et al., Vol. 76(8), [2014] p. 1191 with the permissions from The Japanese Society Veterinary Science).

5. Conclusion

Considering the available evidence, it can be concluded that (1) the isolates associated with infant botulism were epidemiologically divided into NTXA gene cluster types. And, A1NTX and A2NTX have marked a difference in antigenicity. (2) A2NTX caused less muscle flaccidity of nontoxin-treated muscle than A1 toxins. The variation in the amino acid sequence between A1NTX and A2NTX causes the difference in the spreading pathways. (3) A2NTX provides anti-PD effectiveness more effectively and confers greater safety than those of A1NTX. These findings might open a new therapeutic avenue for not only PD subjects but be useful also for application to other parkinonisms.

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Conflict of interest

The authors declare no conflict of interest.

Abbreviations

6-OHDA	6-hydroxydopamineACh acetylcholine
ANTX	*Clostridium botulinum* neurotoxin subtype A

CNS	central nervous system
A1NTX	ANTX subtype A1
A2NTX	ANTX subtype A2
GPS	gelatin phosphate buffer (pH 6.2)
HA	hemagglutinin component
KD values	the affinity constant calculated as dissociation (kd) rate constant/association (ka) rate constant
LD_{50}	50% lethal dose
mAb	monoclonal antibody
OD	optical densities
Orfx	unknown function open reading frame gene
PD	Parkinson's disease
SNAP-25	synaptosomal-associated protein of 25 kDa

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Chapter 4

Adjuvant Botulinum Toxin Harmonisation in Minimally Invasive Facial Aesthetic Surgery

Chedly Bouzouaya and Ronald Feiner

Additional information is available at the end of the chapter

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Abstract

Botulinum toxin (BT) has been utilised as a non-invasive injectable treatment for aesthetic facial enhancement since 1992 after Carruthers JDA and Carruthers JA published their study observing that BT improved glabellar lines. Numerous aesthetic indications were steadily devised, enabling aesthetic medical practitioners to utilise BT as a stand-alone treatment strategy. However, while effective stand-alone BT treatments are functionally limited to targeted attenuation of muscular hyperactivity. Furthermore, BT treatment outcomes are of relatively short duration, requiring repeated treatments at approximately 12–16-week intervals to maintain effectiveness. Minimally invasive treatment strategies such as volumising filler injections, thread lifting, injectable/photo-thermal biomodulation and blepharoplasty are relatively durable aesthetic treatments that can be enhanced by adjuvant BT treatments. Accordingly, rather than relying on the commonly isolated utilisation of BT, the authors suggest a more comprehensive treatment model, whereby the synergistic interplay between minimally invasive treatments and adjuvant BT is demonstrated to advance and harmonise aesthetic outcomes.

Keywords: botulinum toxin, adjuvant, harmonisation, minimally invasive, blepharoplasty, fillers, fat grafting, suture lifting

1. Introduction

In 1817–1822, the German physician/poet Justinus Kerner described "botulinum toxin" as a "sausage poison" and "fatty poison". Bacterium often caused poisoning by growing in improperly handled or prepared meat products. Kerner first conceived a possible therapeutic use of botulinum toxin. In 1870 Müller, another German physician, coined the name "Botulism" [1].

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Botulinum neurotoxins are produced by various strains of Clostridium botulinum, resulting in seven known serotypes, of which A and B had been developed for routine clinical use [2].

Botulinum toxin (BT) has been utilised as a non-invasive injectable treatment for aesthetic facial enhancement since 1992 after Carruthers JDA and Carruthers JA published their study observing that BT improved glabellar lines [3]. Numerous aesthetic indications were steadily devised, enabling aesthetic medical practitioners to utilise BT as a stand-alone treatment strategy.

However, while effective, stand-alone BT treatments are functionally limited to targeted attenuation of muscular hyperactivity. A recent review of the literature found that duration of effect was between 2 and 6 months, with most patients experiencing loss of maximal contraction for 3–4 months. Treatments may last between 3 and 4 months, and occasionally up to 6 months [4].

Minimally invasive treatment strategies such as volumizing filler injections, thread lifting, injectable/photo-thermal biomodulation and blepharoplasty are relatively durable aesthetic treatments that can be enhanced by adjuvant BT treatments.

Accordingly, rather than relying on the commonly isolated utilisation of BT, the authors suggest a more comprehensive treatment model, whereby the synergistic interplay between minimally invasive treatments and adjuvant BT is demonstrated to advance and harmonise aesthetic outcomes.

Ingeniously designed minimally invasive interventions have transformed treatment rationales for improving facial aesthetics.

These interventions have evolved since the 1980s and encompass both minimally-invasive surgical and non-surgical techniques. Prior to this evolution only invasive surgical interventions could be proposed to patients seeking facial rejuvenation and aesthetic enhancement.

Open surgical interventions such as face lifting (rhytidectomy), brow lifting, blepharoplasty, rhinoplasty and neck lifting were often overly zealous, with radical excisions and resections designed to achieve long term outcomes. Sub-nasal resection and open lip lifts were developed for enhanced vermillion display. Facial implants followed for the purpose of facial contouring. However successful the surgery may have appeared to be, patients had to endure the penalty of relatively high risk-high complication procedures [5].

Moreover, patients were to become disappointed that these radical surgical techniques did not fulfil the expectation of permanency as a trade-off for the post-operative pain, lengthy recovery and unsightly scars. Additionally, outcomes were often unnatural and morphed the patients into an appearance that sometimes barely resembled the original self.

Regrettably, this type of surgery persists to this day, championed by surgeons who have not accepted or incorporated less aggressive, innovative techniques into their treatment repertoire.

Signature features that characterise the inherent uniqueness of a person are often sacrificed, rendering that person curiously unrecognisable. Many celebrities have been divested of a certain special character, ultimately extinguishing the very celebrity they sought to maintain through rejuvenation surgery.

Since the 1990s an evolution of minimally Invasive and non-invasive treatments has occurred.

These treatments are mostly performed outside a hospital and without a general anaesthetic setting.

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Pain, downtime, side effects and complications are greatly reduced and outcomes are far more natural. The rejuvenation occurs without the penalty of misrecognision. The intention is for rejuvenation and beautification without a radical change to the appearance.

Of all the non-invasive techniques that have been devised, the administration of injectable botulinum toxin (BT) remains the most famous intervention.

There are many reasons for the popularity of this phenomenon. In the hands of expert injectors, the treatments are expedient, well tolerated, affordable and the outcomes subtlety beautifying. The side effect profiles and complication rates are low and if present are usually easily corrected at a subsequent visit.

The downside is that while effective and pleasing, the treatments generally last for only 12–14 weeks and are limited in what can ultimately be achieved or promised in a "stand alone" treatment approach.

Indeed, patient expectations can often exceed what can be attained with BT alone.

Accordingly, the authors suggest that BT can be utilised to even great effect adjuvant, to other minimally invasive procedures.

Any intervention to create aesthetic enhanced features must recognise that humans prefer attractive faces over unattractive ones. This preference for attractive faces exists from early infancy and applies across age, gender and ethnicity. Facial beauty can be defined several facial features. Important are the central facial features of eyes cheeks and mouth. The spatial relations between facial features dates back to antiquity, when the Ancient Greeks believed beauty was represented by a golden ratio of 1:1.618 [6].

2. Upper and lower blepharoplasties

Blepharoplasty addresses redundant skin on the upper lids and fat prolapse (plus or minus skin) on the lower lids. The hallmark of a meticulously performed upper and/or lower blepharoplasty is to create natural framing of the patient's eyes. Attractive eyes along with a well contoured, proportioned midface and generous lip display, are the key central features of attractive face aesthetics.

In circumstances where adjuvant fine-tuning of the outcome with BT can be anticipated preoperatively, BT can often be administered at the time of blepharoplasty while patients are still sedated. Thus, if required and pre-treatment planning is secure and properly factored, an experienced surgeon can use BT injection immediately in the forehead, glabella area, bunny lines, lips and neck.

However, one should avoid its use in the periocular area if a lower eyelid blepharoplasty is to be performed with skin and orbicularis excision. In these cases, it is better to wait for 3 months as BT can further weaken the orbicularis muscle causing a lid retraction and ectropion. If the blepharoplasty was performed transconjunctivally, BT can be administered as early as 3 weeks later. By that time most of the major swelling has subsided and the periocular muscles have regained their activity.

Alternatively, after a contemporary conservative blepharoplasty procedure has matured by some 6 weeks and particularly where brow ptosis is evident, BT can be injected into the brow depressors (Corrugator, Procerus and lateral Orbicularis Occuli muscles) to correct this negative aesthetic mimic. Conversely, judicious BT to the frontalis muscles may be required to gently control any hyper-elevation of the brow [7]. These positive effects on aesthetic brow balance and elevation are natural and of course far less invasive than surgical brow lift procedures.

Similarly, targeted BT treatments can be further considered in the longer term post-surgery. As the orbit ages and enlarges though bone atrophy (osteopenia-osteoporosis), the brow tends to prolapse into the widening orbit. After the utilisation of filler injection to the Retro Orbicularis Occuli Fat (ROOF) to augment superior orbital rim, BT can again be injected into the brow depressor musculature to augment brow elevation.

Medial to the orbit, the depressor supercilli, nasalis and Levator labii superioris alaeque nasi muscle can be injected with BT to address any dynamic rhytids compressing the medial orbit region.

The authors use in their practice is to treat negative expressive periorbital compression with BT. However, when dermatochalasis is evident and disturbing to the patient, a blepharoplasty procedure is indicated.

To summarise, when surgery is necessary, BT in the hands of an experienced surgeon can be used contemporaneously with the blepharoplasty surgery as earlier described. However, a simple and more conservative algorithm for ocular and periocular aesthetics is suggested, whereby a blepharoplasty is performed and followed after 6–12 weeks by adjuvant BT injection to any periocular musculo-compressive phenomena.

3. Injectable fillers

Injectable fillers are now fundamental in the minimally invasive surgeon's toolbox. While BT can relax overactive negative mimic musculature, fillers of variable rheological qualities can restore and beautify facial proportions and contours.

Fillers can be utilised to strengthen the frame of a face. Firstly, from the superior aspect of the forehead then across to the temple fossae. Secondly, descending into the periauricular region and then down to the mandibular angle across the jawline to the chin.

Rheologically volumising fillers are utilised for injection into the deeper fat pads such as the suborbicularis oculi fat (SOOF). The volumising fillers are anatomically stabilised in situ by retaining ligaments. The most commonly used fillers today are the hyaluronic fillers which can last for up to 2 years.

BT has an excellent role to releasing negative expressive vectors that compound the age related involuting face after correction with injectable fillers.

It has been suggested that in facial aesthetics optimal outcomes are predicated on practitioner appreciation of negative aesthetic muscle hyperactivity, volume depletion, and insufficient

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contours. Accordingly, improvements could be achieved through relaxing musculature, volume restoration and recontouring using BT and injectable fillers alone or in combination [8].

In the upper face, BT is fundamental, with the addition of hyaluronic acid fillers to enhance results. Typically, hyperactivity of the Corrugators, Procerus and lateral orbicularis creates a negative, overly concerned or even a perceived hostile appearance. There can be deficit of the arched female brow and elevatory upper lid loss along with forfeiture of the upper lid crease feature. Injectable fillers can augment the female forehead by restoring its aesthetic convex contour followed by positive effects of BT on frontalis muscle induced forehead compression.

The BT augmented eyebrow lateral arch can be further stabilised by injecting filler to the ROOF in the region of the lateral orbital rim.

In the midface, fillers are elemental in the restoration of aesthetic volumes and proportions. BT can be adjunctly injected into the lateral orbicularis oculi musculature to reduce superior cheek rhytids when smiling.

Age related mandibular involution ageing can significantly alter facial harmony. In the lower face was BT and hyaluronic acid in combination can be utilised to strengthen the mandible and reduce negative vector muscle hyperactivity.

Addressing this lower face deficit can be achieved by the firstly augmenting the angle of the jaw, jawline and chin with high viscosity fillers. The improvement in mandibular height and projection can prove remarkable with such filler injections. If necessary 2–3 weeks post filler injections, adjuvant BT to the mentalis and depressor angular oris musculature can further reduce chin retraction and the negative vector mimic of the downturned oral commissures.

Occasionally despite improvement of the mandible with injectable fillers, masseter hypertrophy can distort lateral lower face aesthetics and may require BT to form effective correction.

4. Fat transfer grafting

Fat harvested for grafting is an ideal filler naturally integrating into tissues. It is autologous, 100% biocompatible and a dynamic tissue composed of several different cell types. These include adipocytes, fibroblasts, smooth muscle cells, endothelial cells, and adipogenic progenitor cells (pre-adipocytes). Fat also contains Adipose derived stem cells which have proved to be particularly promising for regenerative therapies [9].

Fat harvested consequent to the liposuction of redundant fat in the submandibular region is an ideal paradigm for correcting facial proportion imbalance. Both the neck redundancy is corrected and the resultant harvested fat is utilised for correction of any malar insufficiency [10].

Post successful fat grafting BT can be utilised after 12 weeks to relax any exposed platysmal bands in the neck. In the recipient grafted malar region, BT can reduce any related negative or compressive hyperactive muscles as previously described in the adjuvant use of BT after of injectable fillers.

5. Per-cutaneous suture and thread lifting

There now are many minimally invasive per cutaneous suture or thread lifting techniques that have been devised to suspend redundant, lax skin. The authors have used the closed approach transcutaneous Serdev Suture® lifts in their clinics. These techniques were invented by Prof. N Serdev and have several overriding advantages. Of particular importance is that the technique incorporates the principal concept of stable suturing and fixation of mobile fascias to immobile periosteum, tendons and fascias. This results in suture suspension and/or volume augmentation and/or tissue repositioning [11].

Post suspension lifts, in appropriate cases, BT can be used to relax underlying musculature that has been elevated or repositioned along with the attendant retinacula and retaining ligaments.

BT judiciously injected into selected regional muscles of the face affected by stretching can be relaxed avoiding distortions and facilitating tissue remodelling to progress naturally.

Post brow, temporal and midface lifts, judicious BT injections into the frontalis, mid brow depressors orbicularis oculi can relax underlying muscle tension allowing for natural tissue remodelling and reduce patient discomfort. Similar possibilities apply to lower face and neck lifts techniques. Whether BT treatment is ongoing is a decision made on a case by case basis.

6. Photo-thermal skin therapies; collagen induction skin needling induced collagen induction; chemical peels; autologous growth factor and stem cell bio-rejuvenation

Lasers, photothermal devices, chemical peels, skin needling and more recently autologous growth factors/stem cell have all been successfully utilised in cosmetic facial skin and subcutaneous rejuvenation. Many technical modifications and advances continue to be developed.

Administration of BT injected into the negative mimic musculature some 2 weeks prior to such treatments facilitates a relaxed skin target with an even tissue plane for ideal healing to occur.

BT has an ongoing place in the post-treatment phases of these many techniques. Along with many other measures including sun avoidance and topical cosmeceuticals, relaxing negative vector hyperactive musculature can prevent the redevelopment of rhytids and autonomous negative facial expressions.

7. Conclusion

In the last decade, minimally invasive aesthetic procedures have become technically advanced and rising exponentially in popularity. Botulinum toxin injections have become the aesthetic

backbone of many cosmetic proceduralist's practices. This status notwithstanding, there are limitations in the use of BT as a stand-alone treatment. While it is well appreciated that BT is an excellent tool, it is functionally restricted to the relaxation of negative aesthetic vector muscle expression. There is much more to aesthetic improvement than through the utilisation of BT for this function alone.

In fact, BT is an excellent adjuvant treatment delivered pre and/or post several other more challenging minimally invasive procedures as outlined in this chapter. Utilised in this manner, sophisticated and often synergistic aesthetic outcomes are achievable.

Conflicts of interest

Nil.

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Chapter 5

Masseter Hypertrophy: Toxin Treatment Techniques, Causes of Complications, and Prevention

Hsien-Li Peter Peng

Additional information is available at the end of the chapter

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Abstract

Botulinum neurotoxin (BoNT) injection for the correction of masseter muscle hypertrophy is an off-label but increasingly popular procedure, especially in Asians where masseter hypertrophy is a common facial feature. This chapter outlines and organizes the various possible complications of such a procedure and discusses their incidence rates, etiological explanations, and prevention methods. Complications were separated into four main categories: nonmuscular-related, neurotoxin-related, dosage- or injection-level-related, and injection-site-related categories. The ideal dosage and injection location are also described and discussed, with particular emphasis on the injection safe zone, where all injections to the masseter should be made in order to minimize complications and maximize safety.

Keywords: masseter hypertrophy, botulinum toxin treatment, complications and treatment, prevention

1. Introduction

Global consensus on facial beauty in different races shares certain common features, including an oval facial shape and a V-shaped chin and jawline [1, 2]. One of the characteristic features of an Asian face is a square-shaped lower face caused by masseter muscle hypertrophy, making this a popular request for esthetic treatment [3–7]. Since the first report on treating masseter hypertrophy with botulinum neurotoxin (BoNT) was published in 1994 [8], BoNT injections for masseter hypertrophy have become quite popular not just among Asian patients but among Caucasian patients as well [9, 10], despite being an off-label indication.

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Generally, the treatment of masseter muscle hypertrophy with BoNT-A is very effective and quite safe, though the possibilities of side effects still exist and are prevalent enough to warrant attention [3, 5, 7, 9–12]. Recently, our group reported the collective data on 680 masseter hypertrophy patients receiving 2036 BoNT-A treatments over a 6-year period [13]. The causes of side effects after masseter toxin injection treatment were organized into four groups and summarized in **Table 1**. Each complication will be discussed separately below.

Category	Etiology/cause	Prevention/treatment	Incidence
Nonmuscular origin			
Bruising	Damaged vessels	Compression after inj.	2.5% [13]
Hematoma (rare)	Trauma to the arteriole or vein	Compression after inj.	N/A
Dizziness	Unknown	Rest	0.9% [12]
Headache	Unknown	Rest	0.58% [13]
Toxin effect-related			
Chewing weakness	Transient muscle weakness	Abates within a week	30% [13]
Temporalis m. hypertrophy	Compensatory m. overactivity	Injection over temporalis m.	N/A
Dosage-level-related			
Poor or no effect	Insufficient dosage/overly superficial inj.	Good inj. Dose/depth/toxin resistance	No effect (0.1%) [13]
Asymmetricity	Same dose on different sizes of hypertrophy m.	Adjust dose according to muscular size	N/A
Jowling/sagging	High dosage in elderly patient	Reduce dose, multiple ℞, inj. lower face depressors (platysma)	0.20% [13]
Paradoxical bulging (muscle bulging during mastication)	Superficial masseter m. fiber overactivity	Inj. over superficial masseter if not abated after 1–2 weeks Prevent inj. deep and superficial m. fibers	0.49% [13]
Injection site-related			
Sunken temporal fossa	Atrophy of the temporalis m. and downward displacement of the temporal and cheek fat pads	Prevent inj. too high Filler injection over temporal area	0.05% [14]
Loss of full smile/ asymmetric smile	Inj. too high or anterior, effect on zygomatic major or risorius m.	Inj. in the injection safe zone, and ideally keep 0.5–1 cm from each border of the safe zone Most complications resolve spontaneously after some time	0.15% [13]
Sunken lateral cheeks (infrazygomatic hollow)	Inj. too high, excess dose		0.44% [13]
Difficulty in mouth opening	Inj. too high, effect on lateral pterygoid m.		0.9% [12]
Xerostomia	Inj. too posterior, effect on parotid gland function		6.3% [14]
Neuropraxia (very rare)	Inj. too inferior, damage to marginal mandibular nerve		One case report [15]

Table 1. Summary of masseter toxin injection complications.

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2. Category 1: Nonmuscular-related side effects

This includes bruising, hematoma, headaches, and dizziness.

2.1. Bruising

Injury of small vessels during injection may cause bruising. Bruising is one of the most common but least severe side effects and usually dissipates in 5–7 days without sequelae. Our study reported a bruising incidence rate of 2.5%.

2.2. Hematoma

The masseter muscle is a relatively thick and strong muscle and is well vascularized. There are four major arteries that supply the upper, middle, lower, and medial parts of the masseter: the external carotid artery, the facial artery, the maxillary artery, and branches of the superficial temporal artery [16]. Needle penetration of these arteries and subsequent failure to apply compression may result in hematomas.

2.3. Headache

Headaches after treatment is of unknown etiology and is also quite rare, with literature usually reporting below 1% (our study reported 0.58%). It may be linked to individual physiological differences, and the same individuals who have suffered from posttreatment headaches are likely to encounter headaches again in the future treatment. Headaches may occur immediately after injection and take about 2–4 days to recover.

3. Category 2: Neurotoxin-related side effects

This includes chewing weakness and temporalis muscle compensatory hypertrophy.

3.1. Chewing weakness

Decreased masticatory force is the most commonly encountered side effect of masseter toxin injection: our study reported a prevalence of 30%. This side effect is caused by BoNT physiology and is perhaps unavoidable in cases where higher dosages are required. Reduction of mastication force starts at around 1–4 weeks after treatment and gradually improves in the following weeks. Mastication force generally returns to pretreatment levels by the 12th week of postinjection [17, 18].

3.2. Temporalis muscle hypertrophy

Since chewing weakness is the most commonly encountered side effect, it is theoretically possible, though not yet reported, for patients to develop compensatory overactivity and hypertrophy of another mastication muscle such as the temporalis muscle.

4. Category 3: Dosage- or injection-level-related side effects

This includes poor effect, asymmetricality, jowling, sagging, and paradoxical bulging.

4.1. Poor effect or lack of response

Poor effect of treatment is mostly due to insufficient dosage or a superficial placement of the toxin. However, it is also possible (though extremely rare; only one case was reported by our study) for a patient to exhibit a complete lack of response to treatment. The etiology of this may be due to individual immunity to the toxin.

4.2. Asymmetry

Asymmetry may occur if the physician fails to recognize the size differences between the left and right masseters before treatment. In many patients, unilateral preference in chewing will result in a bilateral discrepancy in masseter size. It is crucial to keep this in mind when doing pretreatment evaluations, which then allows the physician to adjust the dosage according to the patient's underlying asymmetry.

4.3. Worsened jowls or sagging

Worsened jowls is likely due to overly rapid posttreatment masseter atrophy, which results in volume reduction and sagging of the overlying soft tissue envelope [9]. The incidence of this complication is around 0.2% as reported by our study and usually occurs in patients over 40. To prevent this side effect, physicians should reduce the dose and separate treatment into multiple sessions, which will slow down the speed of muscular atrophy and provide enough time for the overlying skin to contract. Additional toxin injection into the platysma muscle may mitigate facial depressor action, making sagging less likely.

4.4. Paradoxical bulging

Paradoxical bulging, or masseter bulging during mastication [19], has an incidence rate around 0.49% as reported by our study. Excessive compensation of the untreated superficial layer of the masseter muscle may be a possible explanation for this complication. A recent published study [20] discovered a tendinous structure (deep inferior tendon (DIT)) located in the deeper part of the superficial masseter muscle layer in all cadaver specimens examined. The DIT may block toxin diffusion from the deep layer to the superficial layer; therefore, the superficial layer may be unaffected and prone to overcompensation in the event of masseter weakness [20]. The onset of paradoxical bulging is usually within 24 hours of treatment, and recovery is within 10 days [9]. If recovery has not been achieved within 10–14 days, a booster BoNT injection of about 5–10 units to the untreated superficial layer can usually correct this side effect. Injecting both deep and superficial muscle fibers should prevent this side effect [20].

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5. Category 4: Injection site-related side effects

This includes the loss of the full smile, asymmetrical smile, sunken lateral cheeks, difficulty in opening of the mouth, xerostomia, and neuropraxia.

5.1. Loss of the full smile

Also called smile limitation, our study reported incidence rates of about 0.15%. Smile limitation may be due to toxin diffusion into the risorius muscle; in a cadaver study, the risorius attaches to the anterior or middle part of the masseter in more than 97% of cases [21]. Smile limitation usually takes around 1–3 months to recover [9]. Thorough knowledge of muscular anatomy is important to prevent this complication, and the physician should set an injection safe zone at least 1 cm from the anterior border of the masseter and keep to a deep injection level.

5.2. Asymmetric smile

An asymmetric smile may be caused by paralysis of the zygomatic major muscle. This may occur if the physician injects in a position which is too high and too anterior. Keep the injections to the lower, more posterior part of the masseter muscle to avoid this complication.

5.3. Sunken lateral cheek

The sunken lateral cheek, or concave below the zygomatic arch, is caused by over hollowing of the infrazygomatic region resulting from volume loss over the upper parts of the masseter muscle. Our study reports an incidence rate of about 0.44%. Sunken lateral cheeks may be due to a high position of injection and simply keep the injection sites over the lower part of the masseter to prevent it.

5.4. Difficulty in opening of the mouth

Difficulty in opening of the mouth is a rare complication; according to one report, the incidence was 2 out of 220 treated patients [12]. This complication is caused by toxin paralysis of the lateral pterygoid muscles, possibly arising from an injection site that is high and deep enough to reach past the coronoid notch and affect the pterygoid muscle. Another possible etiology may be abnormal activity of the temporomandibular joint. For prevention, the physician should only inject the lower part of masseter muscle and keep at least a centimeter below the upper safety margin of masseter injection.

5.5. Sunken temporal fossa

Sunken temporal fossa is a rare side effect with an incidence of about 0.05%, as reported by a Chinese study in 2017 [22]. This complication is likely from a combination of two etiologies: atrophy of the temporalis muscle as a result of drug dispersion and downward displacement

of the temporal and cheek fat pads as a result of masseter relaxation [22]. This side effect appears about 1 month after treatment.

5.6. Xerostomia

Xerostomia is a complication due to toxin effect on the parotid gland. The reported incidence rate for xerostomia is around 6.3% [14]. Keeping the injection site 1 cm away from the posterior margin of the masseter (thus avoiding the usual location of the parotid gland) can be helpful for the prevention of xerostomia.

5.7. Neuropraxia

Neuropraxia [11] is a very rare complication caused by paralysis of the marginal mandibular nerve. There has only been one case report in which the patient experienced temporary marginal mandibular nerve paralysis. Symptoms improved within 2 weeks [15].

In one cadaver study, the marginal mandibular nerve runs about 0.1–1.0 cm from the mandibular border [23]. Physicians should therefore keep the injection site 1 cm from the lower margin of the masseter.

5.8. Neurapraxia

Neurapraxia is an exceedingly rare complication and is caused by paralysis of the marginal mandibular nerve. There were no cases in the author's two decades of experience with masseter toxin injections.

5.9. Other rare complications

A range of other possible adverse effects may occur but are usually only reported in single case reports. These include speech disturbance, altered gustatory sensation, incidental aggravation of venous malformation, madarosis, facial alopecia, and acute visual loss [4–6, 24–27].

5.10. Dosage

If the anterior to posterior width of the masseter is less than 3–5 cm, inject 20–30 units of onabotulinum toxin per side [28]. This amount may vary slightly by case depending on muscle size and individual needs. Repeated injection could be done every 3–6 months for optimized cosmetic outcome.

6. Injection safe zone

Before injection, physician should mark the anterior, posterior, inferior, and superior borders of the "injection safe zone." The anterior and posterior borders of the safe zone are the anterior and posterior edges of the masseter muscle, and the inferior border is the inferior edge of the

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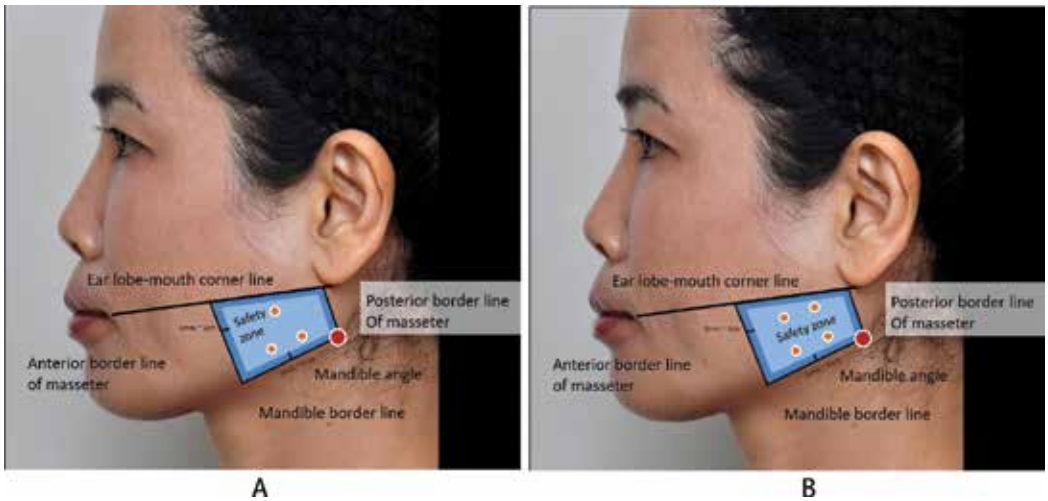

Figure 1. The recommended safety zone for masseter toxin injections is a quadrilateral with its upper border running from mouth corner to earlobe, its anterior and posterior borders the anterior and posterior edges of the masseter muscle, respectively, and its inferior border the inferior edge of the mandible. Injection safe zone, marked in lighter blue, and the ideal injection safe zone of 1cm away from each border, marked in darker blue. The mandible angle marked with large red circle. Suggested injection points mark with small red dot, with 3 points (A) or 4 points injection (B).

mandible. The upper border of safe zone runs from the mouth corner to the earlobe. Keep injections inside the safe zone and ideally in 3–4 different locations at least 0.5–1 cm from each border (**Figure 1**).

7. Conclusion

BoNT-A masseter injections can achieve satisfactory results with mostly mild and infrequent complications. However, adverse effects can still impact patient satisfaction, so an understanding of the regional muscular anatomy, appropriate dosing, injection location, and injection depth are all important aspects to consider when planning and performing treatment. In particular, the injection safety zone should be clearly demarcated by the physician before injection by identification of its four borders: upper border running from the mouth corner to the earlobe, anterior and posterior borders of the anterior and posterior edges of the masseter muscle, respectively, and inferior border of the inferior edge of the mandible. Keeping injections inside the safe zone, and ideally in 3–4 different locations at least 1 cm from any border, is crucial for the prevention of common side effects mentioned above (**Figure 1A** and **B**). Physicians should also know about the different characteristics of various complications, their etiological origin, their management, and their prevention.

Disclosure

Nothing to disclose for this report.

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Chapter 6

Botulinum Toxin in Dentistry

Diana Mostafa

Additional information is available at the end of the chapter

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Abstract

Botulinum toxin (BT) is an injectable intermuscular medication that is used as a muscle relaxant. In this chapter, we explore the applications of botulinum toxin in dentistry for either cosmetic or therapeutic purpose, such as gummy smile (high lip line), parafunctional habits, temporomandibular disorders and facial pain. It is considered as a non-invasive, conservative and affordable alternative treatment in comparison to surgical procedures. Although, the effect of BT is temporary that lasts for 4–6 months, it is preferred by most of the patients as it gives positive significant results that meet their desires with minimal side effects.

Keywords: Botox, botulinum toxin, gummy smile, temporomandibular joint disorder, asymmetric smile, reverse smile, drooping mouth corners, facial nerve palsy, migraine, excessive salivation, trigeminal neuralgia, parafunctional habits, maxillofacial fracture

1. Introduction

Botulism toxins are exotoxins produced by anaerobic, Gram-positive, rod-shaped, motile bacteria of the genus *Clostridium*, which is called *Clostridium botulinum*, *C. butyricum*, *C. baratii* and *C. argentinense* [1], which are widely distributed in the surrounding environment, including the soil and dust. Also, some food products such as honey, canned and not well cooked food may contain amounts of these bacteria [2]. These bacteria are divided into four distinct phenotypic groups (I–IV) and is also classified into seven serotypes (A–G) based on the antigenicity of the botulinum toxin produced [3]. The most common ones are Botulinum toxin type A and B. However, Onabotulinumtoxin A is marketed under brand names Botox®, Vistabel® and Vistabex®, while Abobotulinumtoxin A is marketed under the brand names Dysport® and Azzalure®. In addition, incobotulinumtoxin A is marketed under the brand names Xeomin®, Xeomeen®, and Bocouture®. Whereas, botulinum toxin

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B has approval under the brand name Myobloc® and Neurobloc®. All of them are injectable intramuscular medications that are used as muscle relaxants. In 2007, British Columbia dentists were among the first to use botulinum toxin (BT) for cosmetic treatments, and to subsequently appreciate the potential in dental therapeutic treatments [4]. Now, A growing number of dentists are practicing botulinum toxin (BT) injections for their patients for oral and maxillofacial cosmetic and therapeutic uses.

2. Mechanism of action

When BT is injected into a target muscle, it creates a temporary dose-dependent effect that weakens muscles activity. It is a neurotoxin which produces transient chemical denervation of facial muscle by binding to glycoproteins structures and inhibiting the release of acetylcholine from the cholinergic nerve endplates, which leads to decrease in the contraction intensity, flaccid paralysis and muscle inactivity. When the target tissue is an exocrine gland, the glandular secretion is blocked as it inactivates the glands innervated. However, the neuromuscular transmission is re-established by restoration of the SNARE protein complex turnover, initiation of new acetylcholine receptors, sprouting of new axonal terminals and elongation of the endplate. The effect can usually last up to 3 months and gradually returns to its full function with no adverse effects. Therefore, the blockage is transient and not permanent which make this treatment considered as a palliative approach rather than a curative option [5].

3. Preparations

BT is shipped frozen and it is recommended to be kept in at frozen temperature −5°C before use as lower temperature may affect its potency. Doses of BT utilized for the treatment of a particular purpose depend on the certain brand/preparation as the unit of one product is not similar to the other [6]. The two most commercially available types of BT are Botox® and Dysport®. About 20–25 units of Botox® are equipotent to 80 units of Dysport®. Botox® is marketed as single-use, sterile 100 Units or 200 Units vacuum-dried powder while the vial of Dysport® contains 500 units. They are reconstituted only with sterile, non-preserved 0.9% sodium chloride (normal saline solution) [7]. However, there is no established method to calculate the equivalent doses between various products of BT. The potency per unit of a product is not interchangeable with other preparations, therefore it is important for a dentist to be aware of the different formulations before their use [8].

After drawing up of preservative-free saline by an appropriate sized needle and syringe, it is important to introduce the saline slowly into the vial, then mix by gentle rotating movement to avoid foaming and denaturation of the toxin. After that, storing of BT in a frozen vial (2–4°C) is recommended [9]. However, it has been reported that higher dilutions of BT can lead to an increase of its tissue diffusion, thus influencing the therapeutic and side effects. So far, no valid studies are available to evaluate the optimal dilution for different therapeutic situations [5]. The BT potency has been reported to be effective until the fourth week after reconstitution [10].

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4. Injection procedures

The preferred syringe for BT injections is a calibrated 1.0 mL tuberculin syringe with a gauge preference of 26–30 [11]. The patient should be placed in upright raised position, the target facial muscle should be examined by inspection and palpation when the patient is making the facial expressions or just clenching to determine the exact area of the injections. The injectable areas should be sterilized with a non-alcohol cleanser such as Betadine. Topical anesthesia and ice could be applied before injection to control pain and bleeding the Botox is then injected into the desired areas. It is recommended to apply pressure to the injected area if there is bleeding. After finishing the procedure, the patient should lay upright for 2–5 min to assure his wellness and receive the postoperative instructions which are; avoiding lying down for 4 h, avoid strenuous activity for at least 24 h to avoid the risk of bruising, non-steroidal anti-inflammatory drugs will be prescribed if patients complained of pain or headache, ice packs are advisable after injection to reduce bleeding and edema. However, as the injection of BT is intramuscular, the dosage of BT injection varies between females and males, depending on the volume of muscle. Certainly, males have a larger muscle volume than females, so they require more units of BT during injection to achieve the same results as female patients [12].

Results will be conspicuous within 4–14 days and lasts for 4–6 months, depending on the muscle thickness and anatomy. Some authors [13, 14] conducted that several injections of BT could prolong its effect. One explanation of this process is that the extended muscle paralysis that occurs after numerous injections can cause partial muscle atrophy and permanent weakness in the contraction ability later on, even after the evanescence of the BT toxic effect [15]. It is important to avoid BT injections before the complete disappearance of the BT effect to prevent the antibodies formation against the toxins, which can lately lead to disappointing results [16].

5. Contraindications

1. Pregnant or lactating woman as it may harm the baby (Botox is classified as pregnancy category C drugs) [7].
2. Neuromuscular disorders as patients will be at risk of muscle weakness, diplopia, ptosis, dysphonia, dysarthria and severe dysphagia [7].
3. Patients under Ca channel blockers, cyclosporine and aminoglycosides medications as it may cause arrhythmia and myocardial infarction [7].
4. Patients with a history of allergy to any of the constituents of BT or saline solution as it may cause immediate hypersensitive reactions including anaphylaxis, serum sickness, urticaria, edema and dyspnea [7].
5. Psychological unstable patients [7].

6. Side effects and complications

Generally, the Botox treatment is safe when it is administered in proper doses and techniques. The complications of BT are divided into systemic, local, and reduced therapeutic effects due to antibody formation. The systemic complications include nausea, loss of appetite, diarrhea, abdominal pain, fatigue, malaise, flu-like symptoms such as fever and running nose, increased blood pressure, sore throat, modification of salivary consistency, difficulty in swallowing and ringing in ears [17]. While local complications vary regarding the injection site, involving infection, headache, pain at the injection site, bruising, inflammation, orofacial edema, loss of muscle strength, nerve palsy, rash, itching, ptosis, dry eye syndrome and dysphonia. In addition, improper injection technique or dose may result in asymmetrical appearance of a face or smile, some difficulties in speech, chewing and/or drinking, alternation in salivary consistency and drooping or ptosis of the lip causing obstruction of visible teeth on full smile [16].

In some cases, BT action may diffuse to sites beyond the local application site, presenting generalized muscle weakness manifesting as diplopia, dysphagia, dysphonia, ptosis or even breathing difficulties. The probability of this spread of toxin effect is considered to be high in the face as well as head and neck region due to facial planes and spaces.

The lethal dose of BT injections is not known yet, but it has been estimated to be about 3000 U [18]. The maximum dose recommended for dental applications at an injection session is about 80–100 U. Therefore, more doses could have a potentially lethal outcome.

7. Uses and indications of Botox in dentistry

Certainly, BT treatments have been amplified in popularity over the last two decades, it is getting much more attention in dentistry and frustrated many dentists, where they use BT injections for dental esthetic and therapeutic purposes. It was proven that BT injections can improve cosmetics, reduce pain and relax retrain muscles which in turn enhance the dental treatment plans.

The applications of BT injections have been classified into:

I. Cosmetic uses

1. Gummy smile
2. Asymmetric smile
3. Sad/reverse smile (Marionette lines)
4. Perioral rhytides (Smoker lines)
5. Masseteric hypertrophy (bulky jaws)

II. Therapeutic uses

1. Tempomandibular joint disorder
2. Migraine

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3. Trigeminal neuralgia
4. Facial nerve palsy
5. Bruxism and parafunctional habits
6. Salivary gland secretory disorders (excessive salivation/drooling)
7. Maxillofacial trauma and fractures
8. As adjunctive treatments in dental clinics

7.1. Cosmetic uses

7.1.1. Gummy smile

Although, displaying a small amount of gingiva is esthetically acceptable and gives a youthful appearance, a smile with more than 2 mm exposed gingiva is known to be gummy smile (GS). It is one of the most common variations among the people, with predominance of females than males. The etiological factors of GS can extensively vary, including altered passive eruption of teeth, dentoalveolar extrusion, vertical maxillary excess, short or hyperactive upper lip muscles (levator labii superioris, levator labii superioris alaeque nasii, levator anguli oris, depressor septi and the zygomaticus muscles), or combinations of one or more of them (**Figure 1**). Accordingly, proper diagnosis of the etiology will lead to the proper treatment [16]. In case of altered passive eruption, crown lengthening is the choice treatment, whether with or without bone reduction. Also, surgical lip repositioning techniques were reported to give satisfactory results. In addition, some cases need orthognathic surgery or orthodontic appliances, especially if the cause is skeletal in origin.

However, in cases of hyperactive/hyper-functional elevator labial muscles, BT injections have progressed to be popular in the correction of the gummy smile (GS) compared to other surgical procedures. The advantages of these injections are the increase of the patient self-esteem and

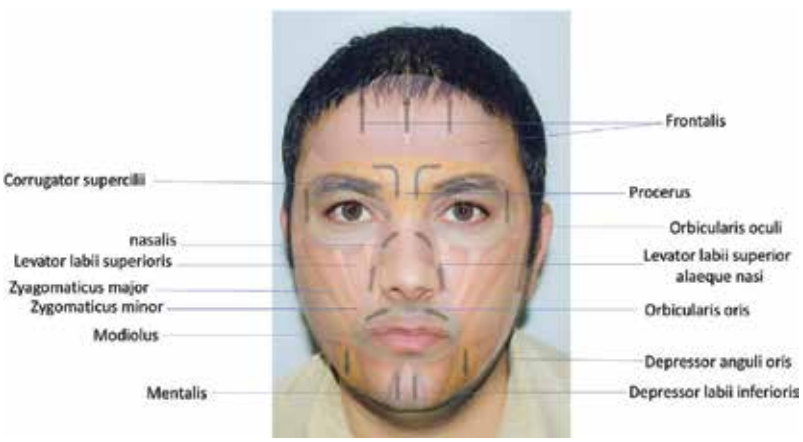

Figure 1. Facial muscles and the direction of their action (depression or elevation).

their preference because their technique is less invasive, reasonable cost and requires less time despite its short-term effect. The purpose of the BT injections is to relax the hyperactive elevator muscles, blocking excessive contractions that are excessively pull up the lip while smiling.

Injection technique: during smile, there are 2 stages in its development, the first stage happens when the upper lip raises to the nasolabial fold, the medial muscle bundles pull up the lip at the anterior teeth and the lateral muscle groups raise the lips at the posterior teeth. However, buccal fats resist the lips at the nasolabial fold while during the second stage, the lip raise superiorly by three muscle groups which are levator labii superioris, zygomaticus major and superior fibers of buccinators [19]. First, gummy smile should be diagnosed according to the classification of the exposed gingival area; anterior, posterior, mixed and asymmetrical or by Goldstein classification as low, moderate and high based on the amount of exposure of gingiva during smiling. These classifications are essential to identify the involved muscle, dose and technique of Botox injections. The muscles of injection are levator Labii superioris, superioris alaeque nasi, zygomaticus minor and major muscle, depressor septi nasi muscle and risorius muscle. The sites of injection should be determined first by palpating the muscles during smiling and relaxing movements to ensure the accurate locations of injections. There is an appropriate and effective point of intramuscular Botox injection introduced by Hwang et al., at Yonsei University College of dentistry, Seoul, Korea have where elevator lip muscles pass by, it is called Yonsei point as shown in **Figure 2** [20]. This point is located at the center of the triangle formed by levator labii superioris, levator labii superioris alaeque nasi and

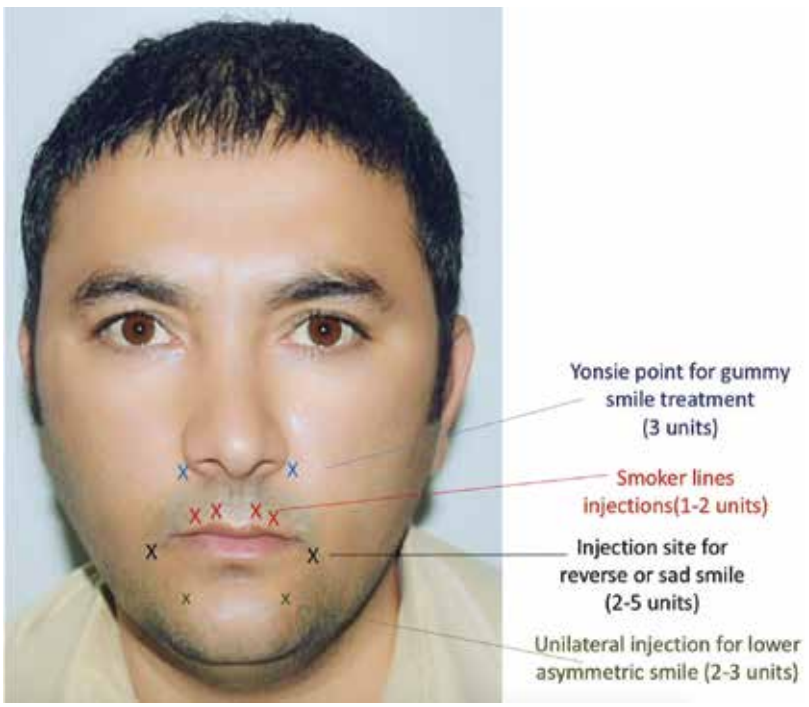

Figure 2. Sites of botox injections for the cosmetic uses.

zygomaticus minor. The doses depend on the dilution of BT with saline. However, there are different techniques for gummy smile injections, all of them have a common site of injection which is Yonsei point, where the recommended dose is 3 U at each injection site. The depth of administration should be intramuscular with the needle perpendicular to the skin surface and bevel facing upwards [16].

7.1.2. Asymmetric smile

Asymmetrical smiles (AS) are due to unilateral hyperkinetic of either lip elevator muscles or lip depressor muscles, where lip rises or depressed more on one side than the other due to the imbalance of the muscle intensity or activity between the left and right sides during smiling. Lip elevator muscles are the muscles responsible for upper AS while the depressor labii inferioris and depressor anguli oris are the muscles responsible for lower AS. However, injections of BT into the muscles of the side where the upper lip pulled to the highest side or the lower lip retracted to the lower side, shown to be effective and give positive results [20, 21].

Injection technique: for upper AS, patient should be in the right position and asked to smile, 2–3 U at the Yonsei point should be injected unilaterally to the most elevated side. While for the lower AS, patient should be asked to smile to determine which side has pulled lip down then, 2–3 U of Botox injections into one of depressor labii inferioris muscles as shown in **Figure 2** and 2 U in one of depressor anguli oris. For both techniques, the needle should passed perpendicular to the skin and enter the thickest part of the muscle. It is advisable to give small doses to correct AS, otherwise, excessive weakening and over correction may result.

7.1.3. Sad or reverse smile (Marionette lines)

The presence of the cervical commissures of the lips in combination with the rest of facial anatomical features are responsible for the old or sad appearance. This happens due to age changes as skin loses the consistency of collagen and elastic fibers and also due to the hyperactive of depressor anguli oris (triangularis muscle) that is located bilaterally (**Figure 1**), adjacent to the lip corners which causes the drooping mouth corners and gives the Marionette line appearance. However, BT injections has proved its effectiveness and give satisfactory outcomes in such cases, it relaxes the depressor muscles which in turn lift the lip corners precisely through their antagonists, improving the depressed and aged facial appearance [22, 23].

Injection technique: Botox injections are indicated for patients with horizontal and vertical platysma muscle bands and with downturned oral commissures, without the existence of submental lipodystrophy or excessive skin laxity [22]. Before injection, patient should be asked to frown, then muscles should be palpated to detect the exact sites of injection. Also, muscle can be detected by bimanual palpation at angle of mouth when the patient says "iii". The site of injection is on the trajectory of nasolabial fold to the jaw line. Bilateral superficial injections in doses of about 2–5 U of Botox is the norm (**Figure 2**). However, it is difficult to inject the depressor anguli oris muscle because its medial border overlaps with the depressor labii inferioris, and its lateral border is adjacent to the risorius, zygomaticus major, and platysma muscles. Therefore, precautions should be taken as if Botox is injected with improper

dose, possible negative outcomes may result including drooling, slurred speech and lack of facial expressions [22]. In addition, if it is injected medially, Botox may diffuse into the depressor labii inferiors causing a lower lip protrusion appearance, known as a Gomer Pyle look and if it is injected too laterally, it may reach Buccinator muscle, causing the patient to bite and traumatize the buccal mucosa.

7.1.4. Perioral rhytides (smoker lines)

These are vertical rhytides which are present in the upper lip and the lower lip region. It is caused by hypertrophic or repetitive contractions of orbicularis oris (circles the mouth) increased with age, sun exposure, strawing and smoking. Their treatment choices are Botox injections or dermal filler or both of them together. Although, BT injections will treat the vertical wrinkles around the lips, they give more eversion results and more lip fullness appearance which make them, the first choice [24].

Injection technique: ask the patient to close his lips and push them forwards, injections should be very superficial of 1–2 U at 2–4 spaced sites along the vermilion border to assure symmetry (**Figure 2**). Results do not last too long because of the repeated action of same muscles, but after injection patient may complain of difficulty in swishing, spitting, strawing, whistling, kissing and pronouncing. In addition, asymmetry may result during taking and smiling [24].

7.1.5. Masseteric hypertrophy (bulky jaws)

Masseteric hypertrophy is an asymptomatic unilateral or bilateral enlargement of the masseter muscles due to congenital cause, chronic clenching habits, asymmetric chewing habit, TMJ dysfunction and focal dystonia. Thus, causing the bulk of mandibular jaw and square appearance of the face. The traditional treatment is partial resection surgery of masseter muscle under general anesthesia, which make this choice have several complications, including hematoma, nerve paralysis, infection, mouth opening restriction and sequel from general anesthesia [25]. Botox injections of masseter muscle reported to be safe as it causes weakening in its intensity and reduction of its bulking appearance which in turn give more tapered face and jaw line contouring [26].

Injection technique: the patient should be asked to clench his teeth to determine the most bulky and prominent area in the masseter muscle for Botox injections, the injections are equally given into three points at the center of the lower third of the masseter muscle with a distance 1 cm from each other, 5–15 U in each point total of 15–45 U per side (**Figure 3**) depending on the bulk of the muscle [26].

7.2. Therapeutic uses

7.2.1. Tempomandibular joint disorder

TMJ disorder is a term used to describe orthopedic and myofascial disorders that cause disharmony in the temporomandibular joint (TMJ), masticatory muscles, and associated structures. It is associated with oromandibular dystonia, periauricular pain, cervicogenic headaches, chronic low back pain, decreased jaw excursion, jaw locking, and noise at the

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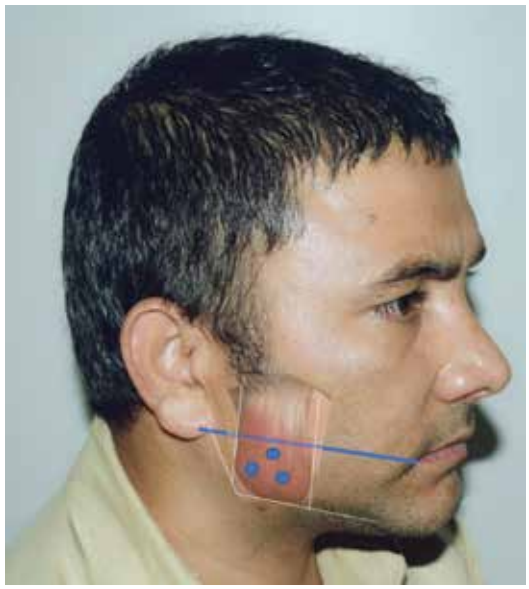

Figure 3. Injection points of Masseteric hypertrophy (5–15 U for each point).

joint with movement. The etiological factors include excessive masticatory muscle activity, parafunctional habits, trauma, psychological factors, and diseases such as arthritis [27]. In general, TMJ disorders are divided into myofacial TMJ disorder or arthrogenic TMJ disorder. The myofacial TMJ disorder is manifested by a pain from hyperfunctioning masticatory muscles causing chronic myositis. While, arthrogenic TMJ disorder is associated with pain due to intracapsular pathology [28].

The diagnosis of TMJ disorders is based on history, physical findings and clinical examination. Patient should be asked about bruxism, gum chewing, jaw soreness, morning headaches and history of trauma [26]. However, there are several therapeutic modalities for TMJ disorders, which are occlusal equilibrations, full mouth reconstructions, orthotic devices, jaw repositioning, psychological therapy, neuromuscular therapy, physiotherapy and laser. In addition, systemic pharmacological medications can play a role in its management, such as corticosteroids injections, anti-inflammatory agents, non-narcotic and narcotic pain medications, muscle relaxants and in some cases tricyclic antidepressants [29, 30]. However, some patients with arthrogenic TMJ disorder may be treated by intra-articular corticosteroid injections, arthrocentesis, arthroscopic surgery or TMJ open surgeries such as arthroplasty [28].

Some dental practitioners solve the occlusion problem and achieve ideal occlusion without treating the spasm of the masticatory muscles. Thus, will lead to the recurrence of sign and symptoms of TMJ disorders and failure of the treatment. Hereby, muscle spasm should be relieved first to reduce facial pain, then achieve the proper occlusal equilibration. However, the use of Botox is considered to be an effective supportive treatment of the myofacial TMJ disorder, especially with patients who did not achieve complete remission by conservative and pharmacological modalities. It decreases the intensity, frequency and duration of recurrent attacks [28].

Injection technique: the involved muscles are temporalis muscle and masseter muscle which are manifested as direct muscle pain while lateral pterygoid muscle involvement usually is manifested as buccal pain [31]. To identify the injected sites, first ask the patient to clench his teeth to make the injection muscles more prominent and easily detected. Injections are performed bilaterally using the proper dose of BT to reduce the contractions of these muscles as well as the facial pain. Identification of the lateral pterygoid muscle is done intraorally where needle placed between the pterygoid plate and the coronoid process of the mandible. The starting dose of Botox 10–25 U for a temporalis muscle, 25–50 U to a masseter muscle (**Figure 4**) and 7.5–10 U for the lateral pterygoid [28]. It is recommended to give low concentrations in different sites to increase the areas of injections and avoid incomplete effect. Higher doses may increase the risk of diffusion of Botox to undesired neighboring areas causing brow ptosis, blepharoptosis or diplopia if the temporalis muscle is injected too close to the orbit and if the masseter muscle is injected too close to the zygomaticus major, asymmetry may result. Also, dry mouth may occur if BT is injected into the parotid gland [32].

7.2.2. Migraine

The migraine headache is a common neurological condition that is characterized by unilateral pulsatile throbbing pain, photophobia, phonophobia, feeling of nausea or vomiting and disabling intensity, its effect lasts from 4 to 72 h and may be longer [33]. It was reported that migraine has a relation to family history and its incidence in women is three times that in men with the highest prevalence among those aged 30–39 [34]. Treatment of migraine includes abortive and preventive therapy. The treatments for mild to moderate episodes are nonsteroidal anti-inflammatory drugs (NSAD) and analgesics containing acetaminophen or aspirin. While, for severe migraine, Triptans are indicated. Not only, some patients with migraine respond poorly to triptans, but also it is contraindicated in some cases such as cardiovascular co-morbidities [35, 36]. Intravenous administration of some combination of dopamine receptor agonists (e.g., prochlorperazine), dihydroergotamine (DHE), and intravenous (IV) NSAIDs (diclofenac or ketorolac) is recommended for more severe attacks [37].

In 2000, Binder found that individuals who had cosmetic facial injections reported a pain reduction of the headache [38]. After that, they discovered that the relief of the pain often happened before the decrease in muscle contractions [39]. Botox blocks the release of peripheral nociceptive neurotransmitters, modulating the peripheral sensation and also suppresses indirectly the central pain processing systems responsible for migraine [40]. In 2010, the FDA approved intramuscular BT injections as a prophylactic treatment of migraine [41].

Injection technique: muscles to be injected by BT are procerus, corrugator, frontalis, temporalis, occipitalis and posterior cervical muscles. The FDA has approved 31 sites with total 165–195 U at which BT can be injected for treating migraines. The injections are given to corrugator in 10 U divided into 2 sites, procerus 5 U is given in one site, frontalis 20 U divided into 4 sites, temporalis 40 U divided into 8 sites (**Figures 4** and **5**), occipitalis 30 U divided into 6 sites, cervical paraspinal muscles 20 units divided into 4 sites and finally trapezius 30 U divided into 6 sites [42]. Cautions should be taken during injection of frontal sites as droopy eyelids, dry eyes and vision problem may result.

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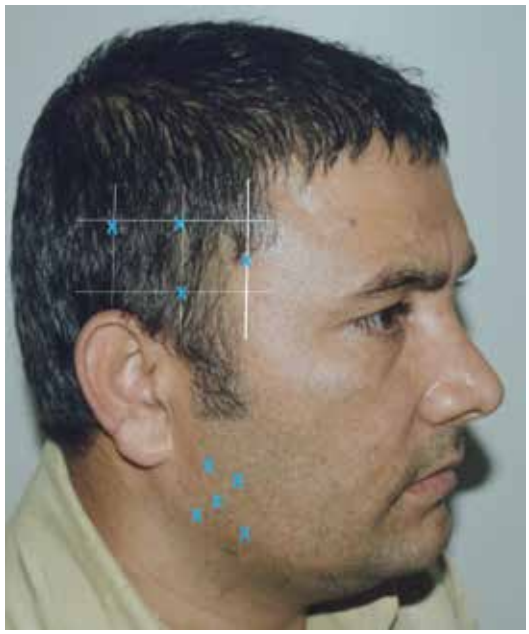

Figure 4. Botox injection points for temporalis and masseter muscles in treatment of TMJ disorders.

7.2.3. Trigeminal neuralgia

Trigeminal neuralgia (TN) is known as sudden, usually unilateral severe recurrent stabbing pain involving the distribution of one or more branches of the trigeminal nerve [43]. It is caused by compression of the nerve near its origin. The pain is usually triggered by stimuli such as chewing, washing of the face, speech and tooth brushing [33]. It occurs more in the old patient rather than younger ones. Its management based on the prophylactic pharmacological treatment with anti-epileptics including Carbamazepine, Oxcarbazepine, Bacloten, Lamotrigine, Gabapentin and ropivacaine. In case of unsatisfactory response or undesirable adverse effects, neurosurgical treatments are recommended which include peripheral techniques (cryosurgery, neurectomy, laser, acupuncture, thermocoagulation, injections of streptomycin, alcohol and phenol), Gasserian ganglion radiofrequency thermocoagulation, glycerol, balloon compression, Gamma knife and microvascular decompression. All of these surgical treatments may cause damage to the nerve except microvascular decompression, which limits the consideration of these techniques [44]. However, BT has been found to be minimally invasive and effective treatment of pain in the maxillofacial region over other invasive therapies especially, in cases of trigeminal neuralgia presenting no adverse effects [45, 46].

Injection technique: for the injection to the maxillary root, a dental needle of 0.40 × 50 mm is used through the upper edge of the zygomatic arch, midway between the external ear and the orbital rim, the needle should be pointed toward the zygomatic bone on the other side of the skull (forming obtuse angles to the front and below) at a depth of 50mm around the pterygopalatine ganglion. For the injection to the mandibular root, through the lower edge

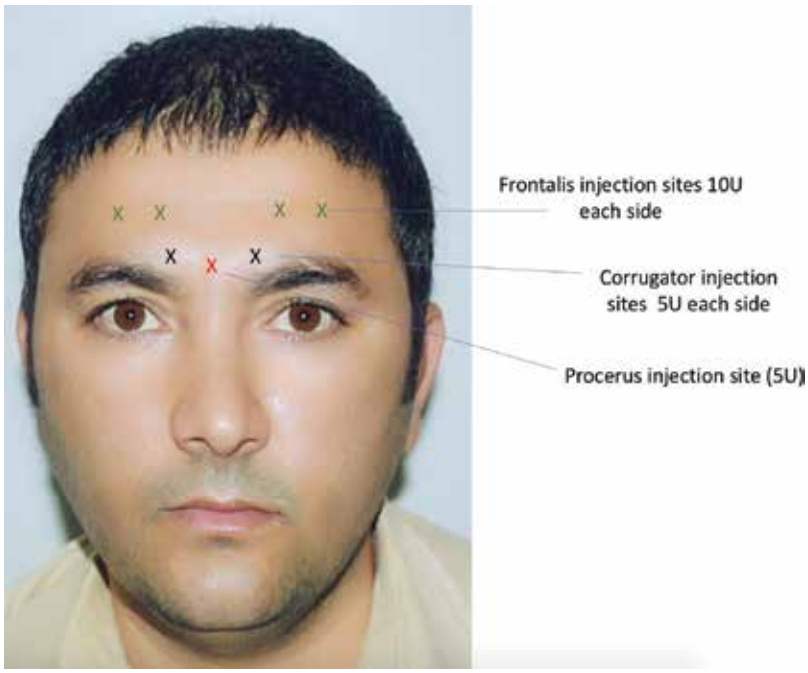

Figure 5. Injection sites of Botox into Frontalis, Corrugator and procerus muscles.

of the zygomatic arch, the position should be the same. The needle should be pointed transversely along the base of the skull toward the middle, then to be inserted below the middle of the zygomatic arch. After striking the pterygoid process, the needle should be withdrawn slightly craniodorsally about 5–10 mm where the solution is administered around the trigeminal ganglion [47].

7.2.4. Facial nerve palsy

It is a facial paralysis with resultant paresis and synkinesis of muscles on the affected side of the face, causing loss of forehead creases, loss of the nasolabial fold, lagophthalmos, brow droop, and drooping of the corner of the mouth. In contrast, muscles on the unaffected side of the face have no opposing forces [48]. Thus, lead to articulation difficulty, eating and drinking problems, asymmetry of the face and unacceptable facial esthetics causing psychological and physical disturbance in a patient's life. Treatments of facial palsy involve nerve grafts, muscle transfers, myofunctional approaches, and microsurgical patches. Although there are many treatment modalities, facial symmetry may persist. However, BT injection treatment was reported to be effective in reducing facial synkinesis, thus improving facial expression symmetry both at rest and involuntary movements [49]. One of the complications of facial nerve palsy is hyperlacrimation (crocodile tears) associated with salivation due to the abnormal connection between secretomotor fibers of salivary gland to lacrimal gland. Injection of BT into the lacrimal gland has been successfully reported in managing this condition too [50].

Injection technique: the areas of injection that are usually considered are levator labii alaeque nasi to reduce the visibility of the upper teeth; depressor labii inferioris to reduce the

visibility of the lower teeth and orbicularis oculi and frontalis to match the contralateral rhytides (**Figure 1**). Patient should be seated in an upright position with the head supported and asked to smile widely, Then, sites of injection are examined clinically, the area exhibiting the maximum pull on movement of the lower face are marked and is injected at an angle of 45° intramuscularly. The unaffected side is also injected to make balance, improve hyperkinesis and give more symmetry at rest. Titration is needed to reduce the effect of the intentional muscle function while increasing the treatment of unintended motion [51].

7.2.5. Bruxism and parafunctional habits

Parafunctional habits such as bruxism, clenching or grinding interfere with the normal occlusion causing generalized attrition, masticatory muscle disharmony, TMJ disorder, facial pain and headache. Traditionally, oral appliances such as oral splint and night guard are indicated for such cases and give good success results as to relieving some or all of the symptoms. Also, BT has been introduced successfully to reduce these symptoms. However, in comparison of BT injections to oral splint modality, both of them are equally effective and safe on bruxism [52, 53] but use of BT in sleep bruxism is more encouraging and comfortable, also a single injection has been shown to be effective for at least a month [54].

Injection technique: injection sites identified by palpation during clenching, then receive bilateral injections of Botox in three sites in the thickest parts of the masseter muscles [55] with dose range of 25–100 U per side. Exceeding the dose will paralyze the muscles of mastication and interfere with the patient's ability in chewing and talking. Also, too small doses will have no effect at all [56].

7.2.6. Salivary gland secretory disorders (excessive salivation/drooling)

The salivary gland secretory disorders cause excessive salivation, such as sialorrhea and Frey syndrome, they are due to poor oral and facial muscle control. They are common in patients with cerebral palsy or neurologic disorders, also patients have post-traumatic sialoceles and cysts, which commonly developed during cancer resection surgery. These disorders may cause perioral dermatitis, or dehydration which leads to problems in the hygiene and the psychosocial status [57]. Their treatment methods vary from a conservative medical modality to a more aggressive surgical approach, including oral motor therapy, intraoral devices, anticholinergic medications, and surgery. However, anticholinergic medications are poorly tolerated due to their adverse effects on the body, such as constipation, urinary retention, orthostatic hypotension, bradycardia, irritability and drowsiness. In addition, surgery is considered to be an invasive procedure that has complications, including increased dental caries, gingival problems, parotitis, and postoperative cysts and fistulae [58].

The secretion of saliva is under parasympathetic autonomic control with acetylcholine working as the specific neurotransmitter. Therefore, down regulation of acetylcholine by BT injections will lead to the decrease of the salivary production [59]. Lately, BT injections have been utilized to manage sialorrhea in adults with Parkinson's disease, head and neck cancer, neurodegenerative disorders and strokes without any noticeable side effects [57].

Injection technique: the injection of Botox into the parotid and submandibular glands is effective in controlling drooling [60, 61]. Botox is administered in a dose range of 30–70 U into parotid

gland. However, the significant reduction in salivary flow is usually observed at 4 weeks and fades in about 3 months, so repeated injections are necessary for such cases [61, 62].

7.2.7. Maxillofacial trauma and fractures

To avoid inappropriate muscle movements during healing period of fractured bones, BT have been introduced to be effective in this mission when there is a injury or fracture in the maxillofacial region such as the maxilla, mandible, nasal bone, zygoma and orbital bone. Also, BT injections are used as a temporary splint during fracture healing period. BT injections into the masticatory muscles in cases of jaw fractures, have been reported to prevent bone displacement and facilitate healing [63]. In 2003, Kayikçioglu et al. conducted a study in temporary paralysis of masseter muscles, to allow application of mini plates/microplates in the management of zygomatic fractures [64]. Also, some reports recommended the use of BT injections in masseter and anterior fibers of temporalis muscles as an adjunctive modality in treatment of condylar fracture [63, 65]. In addition, BT injections in the anterior belly digastric have been used successfully in the correction of post-traumatic anterior open bite [66].

7.2.8. Adjunctive to dental treatments

7.2.8.1. Implantology

BT injections have been postulated to increase the therapeutic benefits in patients with implants who have excessive functional force or have parafunctional habits. When Botox relaxes the masticatory muscles, especially the masseter and temporalis muscles, it weakens the muscles movement in immediate or delayed implant loading. Hereby, relief the abnormal forces on implants leading to successful osseointegration and good prognosis of the treatment. However, studies supporting the use of BT in implantology is rare and warrants further research [7, 67].

7.2.8.2. Orthodontic therapy

The relapse of orthodontic treatment is a common problem because not only teeth are responsible for the treatment relapse, but also the hyperactivity of facial muscles acts as a risk factor. Hereby, the BT injection of mentalis muscle and other muscles will decrease their strength and contractions which in turn avoid their disrupting to teeth alignment.

7.2.8.3. Removable prosthodontics

Some patients may suffer from difficulty in retention of their removable dentures due to hyperactivity or hypertrophy of their masticatory muscles such as masseter, lateral and medial pterygoid muscles. Therefore, weakening the contractions of these muscles by BT injections, increases the retention of the removable dentures.

7.2.8.4. Periodontal and dental health

As it is well known that there is a relation between stress and periodontal diseases as it decreases the immunity affecting the host inflammatory response to local factors, in turn increasing

the inflammation and periodontal destruction. However, a recent study has reported that BT injections improve the psychological status and release stress as Botox injections improve the facial appearance, causing increase in the immunity and health of periodontium [4]. In addition, decreasing stress and relief of grinding and bruxism habits will prevent the traumatized occlusion leading to the improvement of the dental and periodontal health.

7.2.8.5. Mandibular trismus

After a prolonged time of dental procedures, patient may complain of pain and limitation of mouth opening due to spasm of masticatory muscles. Thus, may affect the compliance of dental treatment, restrict the oral hygiene regime, difficulty in drinking and eating. Botox injections into masticatory muscles will reduce pain, paralysis the muscles and diminish the spastic activity.

7.2.8.6. Diagnostic application for toothache

In cases of chronic intermittent toothache, BT injections can be used to identify the origin of the pain and distinguish if the pain is due to muscles or teeth. The pain of pulpal origin will not be relieved when Botox is injected into the muscles [9].

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Chapter 7

Botulinum Toxin for the Treatment of Chronic Migraines

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Additional information is available at the end of the chapter

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Abstract

Migraines are the third most common disease in the world, with an estimated global prevalence of 14.7%. Migraine has a characteristic throbbing quality, of moderate to severe intensity, generally unilateral, and has associated symptoms including photophobia, phonophobia, and gastrointestinal distress. Episodic migraine occurs less than 15 days per month, while chronic migraines occur more or equal to 15 days per month. Treatment of migraine consists of abortive and preventive therapy. Acetaminophen, aspirin, and NSAIDs are often used for management of mild attacks. For more severe attacks, triptans are recommended. Intravenous administration of some combination of dopamine receptor agonists, dihydroergotamine, and intravenous NSAIDs is recommended for severe episodes. Preventive daily treatment of migraine is recommended when migraine episodes exceed 6–8 days per month, or what is tolerable to the patient. Beta-blockers, topiramate, amitriptyline, and divalproex sodium are commonly used for migraine prevention. Initial anecdotal reports in patients receiving botulinum toxin for facial cosmetic purposes noted the effects of these injections on headache and trigger point-initiated pain syndromes, which appeared to be independent of its effects upon muscle tone. Current thinking is that migraine pain results from activation of intracranial meningeal perivascular afferents with some studies suggesting the role of extracranial afferents.

Keywords: botulinum toxin, chronic migraine, headache

1. Introduction

Headache is the most common nervous system disorder. Migraine headache is one of the most debilitating forms of headache [1]. Together with anemia and hearing loss, the World

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Health Organization, migraine is one of the three most prevalent conditions but states that their affects are not dramatic, is overlooked and underestimated [2]. It affects 2–15% of the world's population for a total of 324.1 million migraine sufferers, not of episodes, with women affected three times as often as men and affects mostly socially active and productive people ranging from 25 to 55 years of age [3, 4]. The World Health Organization ranks migraine headache as the nineteenth most disabling disease and characterizes severe migraine to be as disabling as quadriplegia, psychosis, and dementia [1, 2, 5, 6]. Traditional theories regarding its cause attribute it to a vascular or central nervous phenomenon. Migraine is a primary headache, classified by the International Headache disorder in which the headache is by itself the illness. Migraine is characterized by severe headaches and is often associated with nausea, vomiting, and heightened sensitivity to sound and light at the peak of the attack. Many migraneurs even when they have consulted a physician are not satisfied with their therapy and report that typically prescribed medications are not always optimal. Currently, triptan medications are the most effective therapy for acute migraine attacks, reducing pain and associated symptoms in only up to two-thirds of patients. There is a significant need to develop more effective therapies for migraine prevention because up to 35% of affected persons suffers from 2 to 3 severe attacks per month and 25% suffers more than 4 attacks per month [7].

Patients eligible to be considered for prophylactic migraine treatment include frequent headaches, recurring disabling migraines that significantly interfere with daily routine, excessive cost of acute and preventive treatments, failure, contraindication overuse or adverse events with acute migraine therapy, patient preference, and presence of uncommon migraine conditions including hemiplegic migraine, basilar migraine, migraine with prolonged aura, or migrainous infarction [8].

Commonly used treatments for migraine prophylaxis include β-adrenergic blockers, calcium channel blockers, tricyclic antidepressants, and anticonvulsants, most of them with moderate to severe adverse effects. Botulinum toxin type A has been under use for the treatment of migraine and other types of headache. Botulinum toxin type A has been used to treat a variety of disorders including involuntary muscle contraction, blepharospasm, strabismus, cervical dystonia, and for cosmetic purposes [9]. Specifically for the treatment of dystonia and spasticity, botulinum toxin type A has shown an analgesic effect, leading to further investigation for other painful conditions such as migraine and tension-type headache.

Botulinum toxin type A has been used off-label since 2000 for the treatment of migraine headache [10]. Since then, multiple small trials report the effectiveness of botulinum toxin type A for migraine headache prevention. However, the Phase III Research Evaluating Migraine Prophylaxis Therapy (PREEMPT) 1 and 2 trials that class 1A evidence was concluded that botulinum toxin type A treatment reduces chronic migraine headache impact and improves headache-related quality of life [11–13]. After these evidence-based data were published, the Food and Drug Administration in the United States approved botulinum toxin type A for the treatment of chronic migraine headache on October 15, 2010.

Botulinum toxin has been investigated for the treatment of several headache disorders. The beneficial effect of botulinum toxin type A treatment for migraine was first noted in patients who were given the protein for cosmetic purposes treating facial rhytides and reported relief

from their migraine headaches [14, 15]. The pooled result of these studies showed that botulinum toxin type A was significantly superior to placebo in reducing headache days and multiple quality-of-life measures [16, 17].

2. Migraine

Cephalalgia has affected human beings since the beginning of time, being the earliest description among Sumerian poems circa 3000 BC, which described an individual as being "sick-headed." The first reports of a patient describing alteration in visual perceptions, or aura, came from Hippocrates. During the second century AD, Aretaeus of Cappadocia delineated the symptom structure of what is commonly referred to as migraine with aura. More recently, scientific theories to explain the pathophysiology of migraine headaches have emerged. After the introduction of the neural theory, proposing that certain disturbances within the autonomic nervous system may account for the triggering and sustenance of the headache. Dey subsequently described the phenomenon of cyclical pituitary compression of the trigeminal nerve. In the modern era, Wolff described a phenomenon of episodic extracranial vascular dilatation and constriction, leading to the formulation of the vasogenic theory of migraine.

Worldwide prevalence of migraine is estimated to be 13–17% in women and 8–14% in men. The effect of migraine on quality of life is profound. Nearly all migraineurs experience functional impairment because of their condition; more than half being severe requiring bed rest. Most migraine patients do not seek medical attention, instead relying on over-the-counter medications, because they believe that effective prescribed treatments do not exist [19].

Migraine was classified originally as classic or common. In 1988, the International Headache Society published guidelines for discriminating among 13 major types of headache because of inconsistency of headache definitions and the resulting difficulty in epidemiologic and pathophysiologic study; classic migraine became "migraine with aura," and common migraine became "migraine without aura." Migraine can be episodic or chronic but has unique combinations of neurologic, gastrointestinal, and autonomic symptoms that differentiate it from other headache conditions [19]. In the International Classification for Headache Disorders created in 1988 (ICHD-1), major headache types were categorized and distinguished primary and secondary headache disorders. Revised in 2003, the ICHD-2 defined a primary headache disorder as one for which no identifiable structural or organic cause is known. A secondary headache disorder required a known structural or systemic etiology as the cause of the headache symptom. Examples of these include intracranial bleeding, thrombosis of cerebral veins, infections (e.g., meningitis or encephalitis), tumors, dissection of cerebral arteries, and arteritis.

Migraine, tension-type headache, and trigemino-autonomic headache are the most common primary headaches. Clinical presentation, medical history, clinical, and technical examination allow us to distinguish the distinct types of headache disorders. Headache in migraine

commonly has pulsating or throbbing character and is unilateral. Attacks last 4–72 h, and usually in the moderate to severe presentations are accompanied by photophobia, phonophobia, or osmophobia, and nausea or vomiting. Typically, physical activity worsens pain. Chronic migraine is defined in the ICHD-2 as migraine headache at least 15 days per month for 3 months, with attack duration lasting more than 4 h. Whereas episodic migraine only lasts less than 15 headache days per month according to the most recent revision by the International Chronic Headache diagnostic criteria in 2014. Other forms of chronic daily headache include chronic tension type headache, hemicrania continua, new daily persistent headache, and chronic cluster headache.

The spectrum of migraine headaches has been coded by the International Headache Society in its last revision (ICHD-III-β-2014) accepted seven subtypes, with notable subforms (**Table 1**). Migraine can be classified into two major subtypes, namely with or without aura. Migraine without aura is the most prevalent subtype and may involve a higher frequency of attacks and greater disability than migraine with aura.

Migraine is a paroxysmal headache disorder, with periods of relative quiescence between acute headache episodes. Headaches typically manifest with moderate to severe throbbing head pain lasting hours to days, in a hemicranial and frontotemporal distribution; however, bilateral and posterior cervical pain can occur. Associated symptoms may include nausea, vomiting, anorexia, malaise, photo- or phonophobia, and blurred vision. Transient neurosensory perceptions prior to or concomitant with the pain phase occur in migraine with aura.

Diagnostic criteria for migraine without aura include at least five attacks, lasting 4–72 h, with at least two of the following characteristics: unilateral location, pulsating quality, moderate or severe intensity, aggravation by or cause avoiding of routine activity. Also nausea or vomiting or both during the headache of photophobia or phonophobia, in general, cannot be attributed to any other disorder.

Approximately 30% of migraineurs experience auras; for migraine with typical aura, criteria include at least two attacks including an aura consisting in the presentation of fully reversible visual symptoms including positive features (e.g. flickering lights, spots or lines) or negative features (loss of vision) or fully reversible sensory symptoms including positive features (e.g. pins and needles) or negative features (numbness), or fully reversible dysphasic disturbance. Migraneurs also develop at least, two of the following: homonymous visual symptoms or unilateral sensory symptoms, at least one aura symptom developing gradually over greater than 5 minutes or different aura symptoms occurring in succession over greater than 5 minutes, and each symptom lasting greater than 5 minutes and less than 60 minutes. The headache fulfilling criteria for migraine without aura begins during the aura or following the aura within 60 minutes must be considered a migraine with typical aura, and as the other classification, it cannot be attributed to any other disorder [20].

2.1. Pathophysiology of migraine

The pathophysiology of migraine is complex and is still a focus of research. Contrary to the previous vascular theory of migraine, which held that migraine resulted from constriction

1.1. Migraine without aura
1.2. Migraine with aura
1.2.1. Typical aura with migraine headache
1.2.1.1. Typical aura with headache
1.2.1.2. Typical aura without headache
1.2.2. Migraine with brainstem aura
1.2.3. Hemiplegic migraine
1.2.3.1. Familial hemiplegic migraine
1.2.3.1.1. Familial hemiplegic migraine type 1
1.2.3.1.2. Familial hemiplegic migraine type 2
1.2.3.1.3. Familial hemiplegic migraine type 3
1.2.3.1.4. Familial hemiplegic migraine, other loci
1.2.3.2. Sporadic hemiplegic migraine
1.2.4. Retinal migraine
1.3. Child. Chronic migraine
1.4. Complications of migraine
1.4.1. Status migrainosus
1.4.2. Persistent aura without infarction
1.4.3. Migrainous infarction
1.4.4. Migraine aura-triggered seizure
1.5. Probable migraine
1.5.1. Probable migraine without aura
1.5.2. Probable migraine with aura
1.6. Episodic syndromes that may be associated with migraine
1.6.1. Recurrent gastrointestinal disturbance
1.6.1.1. Cyclical vomiting syndrome
1.6.1.2. Abdominal migraine
1.6.2. Benign paroxysmal vertigo
1.6.3. Benign paroxysmal torticollis

Adapted from the International Classification of Headache Disorders-III-β-2014, International Headache Society, 2014.

Table 1. Classification of migraine headache disorders.

and dilation of blood vessels innervating the head, migraine is now recognized as resulting fundamentally from a hypersensitive central nervous system that has difficulty properly modulating pain. In the current neurovascular model, the vascular changes that occur are

recognized as secondary phenomena. The primary components involve interactions among the brainstem, the cortex, and the trigeminovascular system. The brainstem is involved in descending modulation of pain, neuronal inhibition that traverses the cortex is the recognized cause of migraine aura, and resulting sensitization and activation of trigeminal afferents are the source of pain. Culminating from this sequence is a release of neuropeptides, dilation of meningeal blood vessels, neurogenic inflammation, and both within attacks and over time central sensitization manifests. Intracranial blood vessels and meninges are pain sensitive. Sterile neurogenic inflammation may evoke migraine pain.

Neurogenic inflammation includes vasodilation, plasma protein extravasation, mast cell activation, and release of proinflammatory mediators. The activation of meningeal nociceptors releases various neuropeptides, including calcitonin gene-related peptide (CGRP) and substance P from trigeminocervical nerve endings. CGRP is a potent dilator of cerebral and dural vessels and has found to be elevated in migraine attacks. Substance P is involved in plasma extravasation in the dura mater during primary headache attacks, and neurokinin receptor antagonists can inhibit neurogenic dural inflammation but have not been found to have effect in acute migraine attacks. In contrast, a clinical trial with CGRP receptor antagonist was successful in treating acute migraine attacks [21]. Also, a sensitization of peripheral and central trigeminovascular neurons seems to take place in migraine.

Sensitization of the peripheral trigeminovascular neurons could mediate the throbbing, and sensitization of the central trigeminovascular neurons that propel cutaneous allodynia often observed during migraine attacks. The pathophysiology influences a cascade of interacting events within the nervous system resulting in headache [22, 23].

Another potential mechanism involves the synaptic vesicle glycoprotein 2A protein (SV2A), a synaptic vesicle protein isoform with high affinity for botulinum toxin type A that is involved in the binding and subsequent internalization of the toxin into peripheral neurons. Botulinum toxin interacts with peripheral nociceptive neurons and inhibits release of nociceptive mediators from peripheral nociceptors such as glutamate, substance P, and calcitonin gene-related peptide.

Migraine runs in families and has a strong genetic component, and the best example is familial hemiplegic migraine, an autosomal-dominant subtype of migraine with aura that includes motor weakness. Three genetic mutations corresponding to three variants of familial hemiplegic migraine have been identified of which genes code for the ion-channel transport: *CACNA1A* on chromosome 19, *SCN1A* on chromosome 2, and the *ATP1A2* gene on chromosome 1. However, contributors to other more common forms of migraine have not been firmly established [23].

2.2. Treatment of migraine

Typically, medications for acute attacks include simple analgesics or NSAIDs for mild to moderate attacks as abortive treatments. For moderate to severe attacks, ergot derivatives were originally prescribed but now are replaced by triptans, with a greater receptor specificity

and greater effectiveness for more severe attacks. Opioids are reserved for rescue therapy when other medications are contraindicated. Acute medications have limited efficacy and are only useful for short-term symptom relief, and some of them result also in adverse side effects; further, they do not offer prophylactic benefits and have diminished effectiveness if taken over long periods of time. Long-term prevention is the preferred treatment approach to migraine.

The primary goal of migraine prophylactic treatment is improving quality of life through decreased frequency and intensity of headache, improved function and decreased disability, and reduced use of medications with improved efficacy of acute therapy. Although some of the proposed etiologic factors are out of the patient's control, such as heredity [24] and cyclical hormone changes in females [25], others are amenable to lifestyle changes. Examples of such include stress, smoking, intake of certain foods such as meats and cheeses (high nitrites), nuts, chocolate, caffeine withdrawal and alcohol consumption, lack of exercise, sleep pattern, quality and duration, and, in females, menstruation, oral contraception, and estrogen replacement therapy. Some medications also have hypothesized to initiate or increase the frequency of migraine attacks, such as nitroglycerin, some calcium channel blockers, tetracycline, and sildenafil citrate.

Preventive therapy should be offered to patients with migraine reported six or more days per month, with four or more days of headache with some impairment or three or more days with headache with severe impairment requiring bed rest. Situations in which should be considered include patients with 4–5 migraine days per month, with 3 days with some impairment or 2 days with severe impairment [4]. Currently, anticonvulsants, antidepressants, beta-blockers, calcium channel antagonists, conventional or selective nonsteroidal anti-inflammatory drugs, and serotonin antagonists have been used as prophylactic treatments for migraine. Unpleasant side effects can occur with each of these types of drugs. They include drowsiness, fatigue, dizziness, sexual dysfunction, weight gain or loss, constipation, nausea, dry mouth, and insomnia. None of the abovementioned drugs have been approved by the US Food and Drug Administration or labeled as such for use in headache treatment or prevention [26].

There exists a great demand for long-acting acute and prophylactic therapies that are effective, well tolerated, and devoid of significant systemic toxicities or adverse effects. The interest in the use of botulinum toxin type A as an alternative therapy has gained popularity.

3. Botulinum toxin and chronic migraine

There are seven botulinum toxin serotypes (A, B, C1, D, E, F, and G) with an eighth serotype (H) described by some authors as a hybrid of known serotypes F and A [27]. All serotypes inhibit acetylcholine release, although their intracellular target proteins, physiochemical characteristics, and potencies are different. Botulinum toxin type A has been the most widely used and studied for therapeutic purposes.

Botulinum toxin binds to the motor and sympathetic nerve terminals. It enters the nerve terminals and inhibits the release of acetylcholine. This inhibition occurs as the botulinum neurotoxin cleaves one of several proteins integral to the successful docking and release of acetylcholine from vesicles situated within nerve endings. This results in blocking neuromuscular transmission at the neuromuscular junction. After direct intramuscular injection, botulinum toxin produces partial chemical denervation and paralysis of the muscle, resulting in a decrease of muscle activity [28].

The precise mechanism by which botulinum toxin type A alleviates headache pain is unclear, but the inhibition of release of glutamate and other neuropeptides suggests that its antinociceptive properties are distinct from its neuromuscular activity. A peripheral trigger point theory emerged when Binder first noticed the positive effect of onabotulinumtoxin-A while conducting clinical trials for frontal lines and noticing that frontal migraine symptoms improved with either corrugator supercilii muscle paralysis by botulinum toxin type A injection or corrugator muscle resection for the treatment of hyperfunctional facial lines [18, 29–30]. Nonsurgical treatment of migraines includes avoidance of triggers, such as alcohol and caffeine, and pharmacologic control with medications [31]. Current data suggest that botulinum toxin type A modifies the sensory feedback loop to the central nervous system by clocking intrafusal fibers, resulting in decreased activation of muscle spindles. This effectively alters the sensory afferent system by reducing the traffic along IA spindle afferent fibers [32]. Botulinum toxin type A also appears to inhibit the release of glutamate and calcitonin gene-related peptide from primary nociceptive fibers, reduce the firing of wide-dynamic range neurons within the dorsal horn of the spinal cord, and reduce the activity of central nociceptive neurons, as demonstrated by decreased expression of immediate early genes (c-Fos) after nociceptor stimulation [28]. A reduction in afferent sensory activity coming from pericranial and cervical muscles and inhibition of peripheral and central trigeminal sensitization may be the potential mechanisms by which botulinum toxin type A exerts its therapeutic effect in migraine, tension-type headache, and other primary headache disorders [33].

Jakubowski explored neurologic markers that might distinguish migraine patients who would benefit from botulinum toxin treatment from those who would not. The prevalence of neck tenderness, aura, photophobia, phonophobia, osmophobia, nausea, and throbbing was similar between responders and nonresponders. However, during clinical investigation of pain semiology, 92% of nonresponders describes a build-up of pressure inside their head or an exploding headache. Among responders, 74% described their head to be crushed, clamped, or stubbed by external forces, what we understand as imploding headache, and 13% perceived an eye-popping pain (ocular headache). Exploding headaches could explain the pain mediation by intracranial innervation; thus, we infer that extracranial botulinum toxin application will not correspond to a responsive individual. Imploding and ocular headaches respond to botulinum neurotoxin application, suggesting that the migraine pain involves extracranial innervation as well [34].

The physiologic mechanism of migraine treatment suggested by Guyuron is the decompression of peripheral nerves, decreasing peripheral nerve inflammation and excitability, leading to newer treatment techniques such as migraine surgery. Botulinum toxin exerts its mechanism chemically, whereas surgery releases such anatomical entrapments mechanically [15].

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4. Patient evaluation

A correct diagnosis of patients presenting with chronic headache requires a systematic approach to obtain the necessary information. This may be difficult because of the anxiety, feeling of helplessness, and other mood disorders that obscure migraine symptoms. A careful interview and documentation of headache history and examination can aid in reaching a precise diagnosis and classification.

During the interview, some descriptors will guide in precise identification, such as progression of headache through present time, age of onset, frequency and duration of the attacks, severity of headache episodes, quality of the pain, presence or absence of aura, inciting factors, mitigating factors, systemic reactions, craniofacial disorders, systemic illnesses, medication history, family history of headache, and social history.

Although the patient only complains primarily of cranial discomfort, a complete physical examination is warranted on the initial visit. Cardiovascular, ophthalmologic, or neuromuscular symptoms could be missed; ear, nose, throat, scalp, and neck should be thoroughly examined. Specific aspects of the physical examination that should be systematically examined include: vital signs and affect, cardiopulmonary evaluation, auscultation of the carotid, vertebral arteries, cranium and orbits for bruits, range of motion, tenderness, crepitus of the neck, and jaw/temporomandibular joint. The head, neck, and back should be palpated for trigger points, masses, bruises, or thickened or tender blood vessels. Neurological examination should rule out papilledema and focal signs, such as visual field deficits, pupillary asymmetry, sensory deficits of the face, trunk, or extremities; asymmetric gait or motor weakness.

Ancillary tests may provide additional clinical information. Neuroimaging in the form of computed tomography (CT) or magnetic resonance imaging (MRI) has become an invaluable resource for physical diagnosis. Lumbar puncture may be indicated during a severe headache to detect subarachnoid hemorrhage or meningitis, and can be diagnostic of meningeal carcinomatosis or lymphomatosis, and to detect high or low cerebrospinal fluid pressure. Electroencephalography (EEG) has been used to screen for structural cerebral abnormalities via the detection of altered electrophysiological patterns, but the American Academy in Neurology failed to find in 1995 sufficient evidence supporting the utility of EEG in the routine evaluation of headache. If clinical evidence suggests the possibility of organic brain pathology, only CT and MRI are suggested [20].

5. Safety, indications, and contraindications

According to the Food and Drug Administration in the United States, botulinum toxin type A is indicated for the prophylaxis of headaches in adult patients with chronic migraine. Safety and effectiveness have not been established for the prophylaxis of episodic migraines.

Adverse effects are most commonly related to the injection, with systemic adverse effects being very rare. Injection-related adverse effects are mild and transient and rarely lead to discontinuation of therapy.

Serious and/or immediate hypersensitivity reactions have occurred, including anaphylaxis, serum sickness, urticarial, soft tissue edema, and dyspnea.

Individuals with peripheral motor neuropathic diseases, amyotrophic lateral sclerosis, or neuromuscular junction disorders (e.g. myasthenia gravis or Lambert-Eaton syndrome) should be monitored closely and have an increased risk of clinically significant dysphagia and respiratory compromise.

Botulinum toxin type A contains albumin, which based on effective donor screening and manufacturing processes carries an extremely remote risk of transmission of viral diseases or Creutzfeldt-Jakob disease.

Specifically, for chronic migraine, it has been shown in double-blind, placebo-controlled efficacy trials, which the discontinuation rate was 12% in the Botox®-treated group and 10% in the placebo-treated group. Discontinuations due to an adverse event were 4% in the Botox® group and 1% in the placebo group. The most frequent adverse events leading to discontinuation were neck pain, headache, worsening migraine, muscular weakness, and eyelid ptosis.

The most common reported adverse reactions following injection of Botox® for chronic migraine include neck pain (9%), headache (5%), migraine (4%), eyelid ptosis (4%), musculoskeletal stiffness (4%), muscular weakness (4%), bronchitis (3%), myalgia (3%), musculoskeletal pain (3%), injection site pain (3%), facial paresis (2%), muscle spasms (2%), and hypertension (2%). Other adverse reactions that occurred more frequently in the Botox® group compared to the placebo group at a frequency less than 1% include vertigo, dry eye, eyelid edema, dysphagia, eye infection, and jaw pain.

Safety and effectiveness in patients younger than 18 years have not been established. Clinical studies also did not include sufficient subjects older than 65 years to determine whether the response to treatment is different from younger patients.

6. Dosage and administration

Indication specific dosage and administration recommendations should be followed. In treating adult patients for one or more indications different than migraine, the maximum cumulative dose should generally not exceed 360 Units, in a 3-month interval.

Onabotulinumtoxin-A vacuum-dried vials should be reconstituted prior to injection with sterile, non-preserved 0.9% Sodium Chloride Injection USP. For chronic migraines, the recommended dilution is 200 Units/4 mL or 100 Units/2 mL, with a final concentration of 5 Units/0.1 mL. The recommended dose for treating chronic migraine is 155 Units administered intramuscularly rather than intradermal, avoiding the periosteum, eyelid region, and visible superficial blood vessels while using a sterile 30-gauge, 0.5-inch needle as 0.1 mL (5 Units) injections per each site for a total of 31 injection sites in the head and neck, divided across seven specific head/neck muscle areas. A 1-inch needle may be needed in the neck region for patients with thick neck muscles; the use of needles longer than 1-inch increases the risk of

complications such as pneumothorax, vascular injury, and spinal cord damage. Even though the FDA approved dosage is 155 Units distributed in 31 sites as in the PREEMPT trials, the total dose has ranged from 25 to 300 Units over several injection sites.

With the exception of the procerus muscle, which should be injected at one site (midline), all muscles should be injected bilaterally with half the number of injection sites administered to the left and half to the right side of the head and neck; even if the patient has strictly unilateral headaches. The recommended re-treatment schedule is every 12 weeks. **Figure 1** shows the recommended injection sites for chronic migraine.

Botox dosing by muscle for chronic migraine is as follows: (1) frontalis muscle—20 Units divided in four sites distributed bilaterally; (2) corrugator muscles—10 Units divided in two

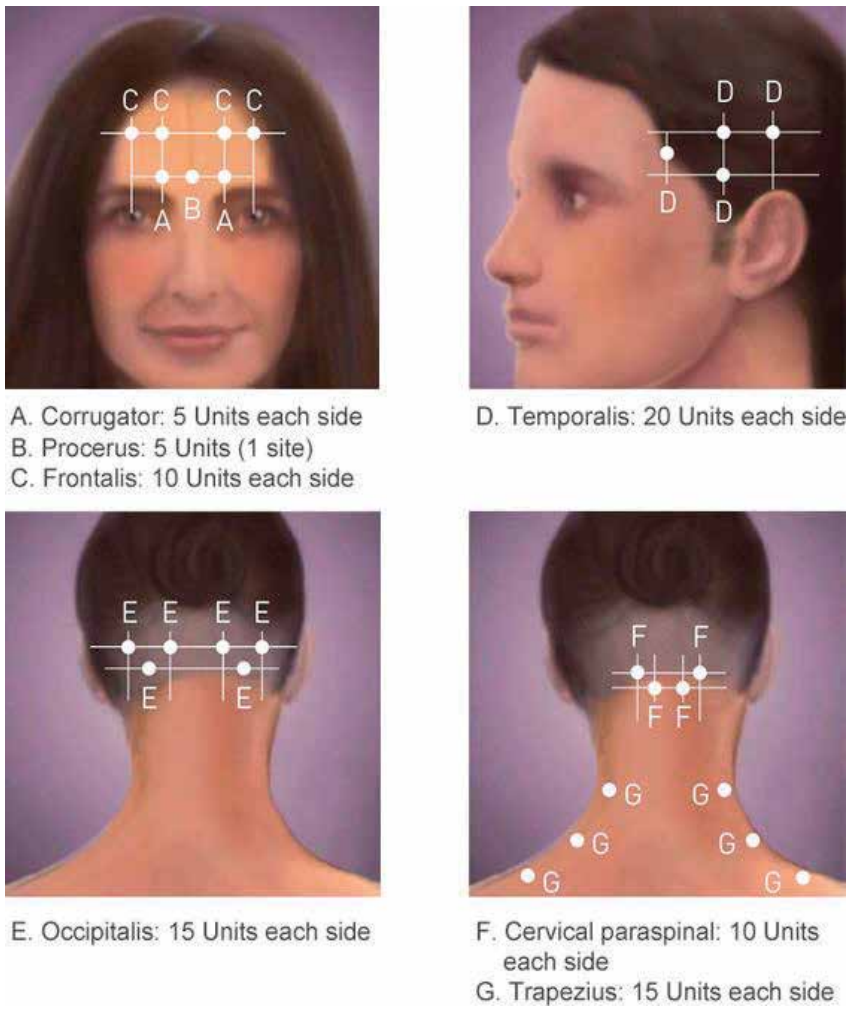

Figure 1. Injection sites.

sites distributed bilaterally; (3) procerus muscle—5 Units in one site; (4) occipitalis muscle—30 Units divided in six sites distributed bilaterally; (5) temporalis muscles—40 Units divided in eight sites distributed bilaterally; (6) trapezius muscles—30 Units divided in six sites distributed bilaterally; and (7) cervical paraspinal muscle group—20 Units divided in four sites distributed bilaterally.

The target of these injections is superficial to the peripheral sensory nerves, namely the trigeminal nerve branches, the occipital nerves, and the cervical sensory rami from C3 to C5, rather than the muscles themselves.

Upper cervical-occipital muscles, especially the splenius capitis and splenius cervicis, may trigger migraine. Frequently, these muscles also contribute to pain and headache by irritating the adjacent greater occipital nerve and causing the concomitant neuralgic symptomatology. Thoracic paraspinal and periscapular muscles are frequently symptomatic and can also trigger headache. Unwanted weakness of the supraspinatus and infraspinatus muscles, which form part of the rotator cuff, allows the humeral head to rise, while injected trapezius and levator scapulae may cause the acromion to sag inferiorly and anteriorly. This can result in painful shoulder impingement 8–10 days after injections.

Onabotulinumtoxin type A reaches its clinical effect at 7–10 days and plateaus at 3 weeks. The neuromuscular blocking action of BTX-A lasts 3–4 months; however, the reduction of pain can last substantially longer, and an effect more specific for migraine may continue to develop beyond 2–3 months after the injection session [35].

A combination of a fixed-dose/fixed-site injection plan and a follow-the-pain method is appropriate. Following this premise, Guyuron has identified trigger points and has proposed another method of peripheral nerve decompression, through surgical release of mechanical entrapment, reducing effectively migraine severity and frequency with surgical deactivation of peripheral trigger points. Even though other trigger point identification methods have been described, botulinum toxin type A injections can serve as a prognosticator of migraine surgery success because of its significant positive association with surgical outcomes [36–38].

Follow-the-pain injection sites are identified by history and examination of the cervical-shoulder girdle and temporomandibular musculature being most useful for patients with tension-type headaches. These sites include the frontalis, temporalis, occipitalis, trapezius, splenius capitis, suboccipital, and cervical paraspinal muscles. Guyuron has identified four major and several minor trigger sites, and botulinum toxin type A injections for trigger site identification and migraine surgery planning are administered at one trigger site per visit based on the constellation of symptoms. The frontal trigger site (Site I) involves the supraorbital and supratrochlear nerves. In the temporal trigger site (Site II), the zygomaticotemporal branch of the trigeminal nerve is compressed by the temporalis muscle and the tight deep temporal fascia. In the rhinogenic trigger site (Site III), contact points among the septum, turbinates, and concha bullosa or sinus inflammation can irritate the trigeminal nerve, obviously when the symptoms suggest Site III as the main trigger, this site should not be injected. In the greater and/or third occipital nerves (Site IV), the semispinalis capitis muscle, fascial bands, and occipital artery could irritate the nerves triggering migraines. The minor triggers consist of the auriculotemporal nerve (Site V) and the lesser occipital nerve (Site VI) [39].

When only a follow-the-pain approach is used in patients with migraine or migrainous headache, there is a risk for a poor cosmetic outcome and/or shifting of the headaches to the previously unaffected side. Even in these cases, cosmetic effects in the frontal region need to be obtained, which also assure good compliance with continued treatment; but asymmetric injections can be given in the temporalis, occipitalis, splenius capitus, cervical, and subcervical paraspinal muscles. The doses injected in the cervical-shoulder girdle muscles are low to prevent any possible weakness that could cause headache. Patients need to be carefully assessed for associated cervical dystonia, which requires injection of the dystonic muscles. Current available data do not appear to indicate a dose-response benefit from BTX-A injection therapy.

For patients with migraine or migrainous headache features identified by history, treatment with a fixed-site approach may be required for successful results.

Therefore, further randomized, placebo-controlled clinical trials are needed to identify the optimal dosing regimen and injection sites. However, some studies have reported greater efficacy with repeated dosing [8, 14].

Migraine improvement can be monitored with the use of a diary or another self-reporting method. Progress is indicated by reduction of oral prophylactic medications, improved response from abortive therapies, as well as reduced frequency, intensity, and severity of migraine headache symptoms. Nonpharmacologic headache therapies, such as biofeedback, cognitive-behavioral pain management strategies, and relaxation therapies, which were previously ineffective, may prove more successful after BTX chemodenervation and should be reconsidered as adjuncts to treatment [35].

Currently, only Botox® has been approved by the FDA for the treatment of chronic migraines, but in studies of botulinum toxin type A, Dysport® has shown efficacy when administered for tension-type headache using a dosage of 200–500 Units per application.

After injection, patients should be informed that (1) from time to injection to symptomatic benefit is between 3 and 14 days, peaking at 3 weeks, (2) duration of benefit is 12–16 weeks, (3) maximum effect may take several treatments, (4) duration of reduction in headache symptoms may not be synchronous with the return of muscle function, and (5) postinjection site blebs in the forehead region will disappear within a few hours and will reduce the hyperfunctional lines of the face in 3–5 days.

Patients should be instructed on keeping headache diaries, which document the frequency and location of headache, severity, and medications used over a 4-month period. The Migraine Disability Assessment (MIDAS) can be used as a measure of treatment success. Objective measurements of treatment effectiveness are important, so that clinical response can be evaluated and future treatment sessions can be modified as necessary [30].

7. Efficacy

Guidelines indicate that quality clinical trials in patients with migraine should always be double-blind, randomized, placebo-controlled trials [40]. Clinical evidence supporting the

use of botulinum toxin injections is mixed. Binder demonstrated that 51% of migraineurs reported complete response, and an additional 38% reported partial responses for a mean of 4.1 and 2.7 months, respectively [30]. Data from the double-blind phase of studies demonstrate significant improvement with onabotulinumtoxin A versus placebo observed over 24 weeks of treatment, demonstrating that the benefits persist over 56 weeks of treatment using measures of headache impact (HIT-6) and quality of life questionnaires (HRQoL). Patients who switched from placebo to onabotulinumtoxin A at 24 weeks experienced significant improvements from baseline at a rate of change not different than observed among patients that received onabotulinumtoxin A from the start of the double-blind period. This indicates that efficacy persists even if the treatment is delayed.

All patients improved, as indicated by a change from baseline in the frequency of moderate to severe migraines. Botulinum toxin type A is a safe treatment that significantly reduces migraine frequency and severity. It is still discussed to what extent the way of application of botulinum toxin may influence its efficacy, and in most studies, the fixed-site approach has been employed. This creates a systematical approach injecting the same predetermined sites with predetermined doses. For the follow-the-pain method, the authors conducting trials have been acknowledged that their results do not confirm efficacy but may be useful for chronic migraines [11].

Silberstein published the first placebo-controlled, double-blind study in migraine patients with 123 patients who were randomized into three groups and treated with placebo, 25, or 75 Units of Botox®. The treatment with 25 Units was superior to placebo in reducing the frequency of the attacks but no different than the 75 U group [8].

Multiple studies have shown a tendency to reduce headache days in distinct period of time but have failed to reach criteria for statistical significance [34, 41–43].

Dodick has found a statistically significant difference in an analysis of 228 patients without prophylactic medication [12]. The breakthrough of onabotulinumtoxin-A in the treatment of chronic migraine came in 2010, when Phase III Research Evaluating Migraine Prophylaxis Therapy (PREEMPT) study group published the results of the PREEMPT I and PREEMPT II trials, totaling 1384 patients who were included in a 28-day baseline screening period, a 24-week double-blind, parallel-group, placebo-controlled phase, and a 32-week open-label phase. Both studies completed three injection trials, with the same study design but different endpoint conclusions.

In the PREEMPT I trial, significant differences were found in the reduction of headache and migraine days but missed the amount of migraine episodes. The PREEMPT II confirmed the efficacy in the reduction of headache days [12, 16]. The positive results of the two PREEMPT trials led to approval of onabotulinumtoxin-A in September 2011 by the US FDA and many other registration authorities worldwide.

Medication overuse is a major problem in chronic migraine patients, and the PREEMPT pooled data show effectiveness in a reduction in headache days and a reduction in medication overuse. Beside reduction in headache frequency and severity, botulinum toxin also improves quality of life [3, 4]. In a more recent study [44], it was demonstrated that monthly headache

days, migraine days, days with nausea/vomiting, and days with intake of pain medications were significantly reduced after the first treatment, maintaining such effect throughout the entire study period. Also health-related and migraine-related quality of life improved after the treatment. Patients also had a decrease in depression symptoms, theoretically mediated by improving quality of life.

In all the mentioned studies, approximately a 10% of patients did not respond to treatment with botulinum toxin. The development of antibodies, intrinsic worsening of migraine, and an initial placebo effect have also been discussed as causes of resistance to treatment [45].

8. Conclusions

Migraine is a major cause of disability worldwide. Chronic migraine can reduce quality of life and is one of the most prevalent conditions. Physicians treat day-to-day and must precisely diagnose and effectively treat headache disorders. An adequate examination will guide to a specific disorder, so the indicated therapeutic plan can be started to assure patient satisfaction. Acute medications have shown variable efficacy, and patients commonly seek preventive therapy to avoid the inconvenient impairment chronic migraines that can cause. Botulinum neurotoxin, even if not fully comprehended in its precise pathophysiology for the treatment of pain, has provided relief from headache pain, reducing severity, frequency, and duration of episodes and improving quality of life. Currently, the FDA has approved only a fixed-point technique; together with the follow-the-pain injection, technique can relief migraine for 12 weeks or more. Further studies have to be conducted to demonstrate the mechanism of action pathways and to perfect the administration, but currently, botulinum toxin is a safe, effective, and with minimal adverse effects to be considered in migraine therapy.

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Chapter 8

Urological Applications of Botulinum Toxin A

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Additional information is available at the end of the chapter

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Abstract

Botulinum toxin A (BoNT-A) has seen in the last two decades an increased level of application in urological practice, first FDA approved in 2011 for neurogenic detrusor overactivity and then later in 2013 for refractory overactive bladder. Hundreds of studies have been published in literature assessing the chemical structure of botulinum toxins and how urothelial injections in the lower urinary tract and vesical instillations can be employed in the management of a spectrum of urological conditions particularly voiding dysfunction. The consensus is still out on toxin A preparations, mode and pattern of application whether instilled or injected intradetrusally, units used, as well as time to onset and duration of effect of injections and is still a dense research topic. This is reflected in the continuously changing and differing grades of recommendations between societies of urological practitioners such as the EAU and AUA, among others. This chapter discusses both the FDA-approved and experimental applications of botulinum toxin A in urology, indications, techniques, and points of debate.

Keywords: overactive bladder, neurogenic detrusor overactivity, intradetrusor injections, voiding dysfunction

1. Introduction

Urological applications of botulinum toxins are not new, but their approval and mass use are overdue. Though it was first used safely on humans in the 1970s, the journey for emergence of the urological uses of botulinum toxin isolates was only recognized in the first decade of the twenty-first century. The introduction of the use of botulinum toxin type A (BoNT-A) in its various preparations revolutionized and extended the spectrum of conservative and minimally invasive treatment modalities of a spectrum of voiding and sexual dysfunction conditions. Despite that, and although it is being increasingly recognized in guidelines, botulinum

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toxin remains of limited approval in urological applications by regulatory bodies such as the US Food and Drug Administration.

The general principles of the mechanism of action of botulinum toxins apply similarly in urological applications of the toxin. By binding presynaptically to sites on the cholinergic nerve terminals, it decreases the release of acetylcholine causing a level of neuromuscular blockade. This initially relaxes whichever muscular site is injected, commonly being the bladder intradetrusally, leading to relaxation of the target muscle injected by exerting an effect on the efferent detrusor pathways. In the case of the detrusor muscle, this will lead to a decrease in its contractions and increase in accommodative capacity. Eventually, the effect of the toxin wears off, to which the hypotheses of how are varied, and the injections are to be repeated if the desirable effect is to be achieved.

In literature, the use of botulinum toxin in urology was first described in 1988 for the treatment of detrusor sphincter dyssynergia and gained momentum in more trials in the late 1990s. The studies concentrated on the use of botulinum toxin type A, with little to no evidence showcasing the effect of other types. The experimental applications first concentrated on treating voiding dysfunction disorders, especially those of neurogenic causes such as spinal cord injury and multiple sclerosis, and later went on to include the management of lower urinary tract symptoms and chronic pelvic pain. Though first initially US FDA approved for human application in 1989, it was not until 2011 that the FDA approved onabotulinumtoxinA (BoNT-A) (BOTOX®; Allergan; Irvine, California) for the treatment of urinary incontinence and detrusor overactivity secondary to neurological conditions such as spinal cord injury where conservative therapy with anticholinergic medication was inadequate or intolerable. BoNT-A was later then approved for treatment of overactive bladder symptoms in adults with an inadequate response to anticholinergic medication [1].

Estimates of the burden of urological conditions such as overactive bladder have varied in published reports. In one review, it was estimated that around 16% of adults in the USA experienced some degree of urgency symptoms with or without incontinence, irrespective of gender [2]. This number contrasts greatly to that deduced from a Finnish study published earlier and conducted in a similar manner where the prevalence of overactive bladder symptoms among adults was estimated at around 8% of the surveyed population [3]. Irrespective of the prevalence, the US drug market for overactive bladder medication was placed at USD 3 billion, including anticholinergic medication and beta-agonists [4]. This market does not include the US reported sales of USD 1.38 billion for Botox Therapeutic, the neuroscience and urology division of Allergan which is the parent company of BOTOX concerned with the treatment of chronic migraines, urological conditions, and others [5]. With an average price tag of around USD 1300 per cycle of BOTOX injection intradetrusally on average three to four times the price of a 30-day supply of anticholinergic medication, questions had to be raised on the cost-effectiveness of this mode of intervention.

In 2006, a study from the UK demonstrated that BoNT-A injections for overactive bladder irrespective of the pathology were more cost-effective over a 1-year duration than standard care with regular office follow-ups and anticholinergic medication or clean self-intermittent catheterization. This effectiveness was reproduced by other studies from the USA, Europe, and the UK for a 5-year cost-effective and sensitivity analysis comparing BoNT-A injections to

conservative management and surgical intervention [6–9]. All of this economic evidence, coupled with numerous trials demonstrating the effectiveness of botulinum injections for treatment of overactive bladder symptoms and other emerging uses, has popularized its application among urologists and has led to its inclusion as second- and third-line management modality in numerous urology care guidelines reviewed and published by authorities such as the European Association of Urology (EAU), American Urological Association (AUA), and International Continence Society (ICS).

This chapter will review the urological applications of botulinum toxin, particularly toxin type A, the different injection modes, and FDA supported, guideline supported and emerging, experimental, or deemed "off-label" uses.

1.1. Mechanism of action of botulinum injections in the urinary tract

In general, botulinum toxin is a very potent neuromuscular blocker. Each serotype exerts the neuromuscular effect by working on a different molecular level. For example, botulinum serotype A works by cleaving SNAP-25, a presynaptic protein involved in the fusion of vesicle-containing neurotransmitters, while serotype B exerts its effects to another vesicle-associated membrane protein (VAMP). This chapter will not discuss the molecular details of each serotype, and most effects and mechanisms of action mentioned will pertain to serotype A which is the most common serotype used for intradetrusor injections.

1.1.1. Botulinum injection in the bladder

In the bladder, as in other injection sites, botulinum toxin primarily acts by binding in presynaptic targets impairing acetylcholine release and thus decreasing detrusor muscle contractions by reducing the amount of acetylcholine that binds with M2 and M3 muscarinic receptors in the detrusor muscle [10, 11]. Thus, it achieves its main function by relaxing the detrusor muscle. However, many studies have proposed and to an extent showed evidence that intradetrusor botulinum toxin injections, particularly toxin A, achieve relief from certain chronic symptoms of detrusor overactivity and pain through several other mechanisms:

1. Other than through exerting a direct effect on motor function of the bladder muscle, it has an indirect sensory effect via afferent sensory pathways of the urinary bladder. Botulinum toxin injections reduce levels of sensory reception in the bladder suburothelium and, in turn, desensitize to an extent the afferent output by unmyelinated C-fibers that arise because of the damage to the pathways consisting of myelinated Aδ fibers usually carrying signals to higher brain regions involved in micturition. This eventually results in reduction of the activity of the spinal arc pathway that through activity of C-fibers causes detrusor contractions [10, 12].
2. Researchers have demonstrated that botulinum toxin A also exerts a detrusor inhibitory effect through inhibiting ATP release as well as acetylcholine. This was supported in both animal and human bladder isolates with idiopathic detrusor overactivity [11].
3. Additionally, through inhibition of urothelial ATP release, research suggests that intradetrusor botulinum injections may have antinociceptive effects not related to their

effect on efferent nerves. In the case of chronic inflammation or neural injuries, this effect could reduce sensitization in the bladder that provokes afferent activity usually causing detrusor overactivity and, instead, leads to relaxation of the detrusor muscle [11, 13].

4. Inhibition of other neurotransmitter molecules and sensory receptors such as glutamate, substance P, calcitonin gene-related peptide (CGRP), and TRPV1 has been demonstrated in basic research and clinical trials, contributing to the sensory effect of botulinum bladder injections [14].

5. A number of studies addressed the effects of botulinum injections on muscular composition in general and in the detrusor muscle specifically. In one study on injections of botulinum toxin for cervical dystonia, repeated type A injections lead to some minor muscle fiber alterations proposed to later cause muscle weakness [15]. In the urinary bladder specifically, botulinum type A injections reduced fibrosis and bladder nerve growth factor levels, but not necessarily the level of inflammation or edema [16, 17].

Cumulatively, botulinum injections in urothelial tissue result in relaxation of the detrusor muscle. This effect is not immediate and is time-restrained by the induction and the slow recovery of the neuromuscular junction plate from the paralytic effects of the injection. As the recovery begins, the detrusor relaxation effects begin to decrease. For a maintained and sustained effect, repeated injections are necessary. Unfortunately, recipients of repeated intradetrusor injections do not always continue to exhibit similar responses to consecutive injections. A hypothesized "secondary failure" phenomenon [18] has been addressed in literature for injections in the bladder and in other sites, and the theoretical reasons attributed include the following:

1. Botulinum toxin injections have been shown to induce an immune response that results in the production of antibodies that counter the effect of the toxin [19, 20].

2. Animal studies have demonstrated a reactive increase in production of intracellular proteins after repeated injections of the toxin, possibly in a cellular effort to counter the effect of the injections [21].

3. Microscarring of injection sites, hypothetically, though recent literature rebutted this theory by demonstrating no significant detrusor muscle ultrastructure alterations after injections [22].

It is worth mentioning that there also are several studies that counter the hypothesis of diminished effect on repeated injections. The EAU guidelines side with such studies based on randomized controlled trials (RCTs) that showcased sustained efficacy on repeated injections of onabotulinumtoxin A [23]. The frequency of subsequent intradetrusor injections will be discussed onward in this chapter.

1.1.2. Botulinum injections in the urethra

BoNT-A injections in the urethra particularly at the level of the urethral sphincter have been demonstrated to reduce sphincteric tone and urethral pressure. The mechanism of action is likely similar to the action of botulinum toxin injections in detrusor muscle.

1.1.3. Botulinum injections in the prostate

The effects of BoNT-A injections in the prostate have been demonstrated through a number of clinical trials in both humans and animals. In rats, botulinum toxin injections resulted in activation of apoptosis inducing prostatic atrophy. This was also demonstrated in clinical trials where apoptosis was identified at both the stromal and epithelial levels of prostatic tissue after BoNT-A injections, which reduced prostatic tissue mass, and was shown to reduce prostatic urethral pressure [24–27].

1.2. Botulinum toxin serotypes and preparations in urology

There are seven different serotypes of botulinum toxins with several different properties and preparations. Commercially available preparations of serotypes A and B have been approved for human use, but their urological application has been limited. These include, but are not limited to, two botulinum toxin type A preparations, onabotulinumtoxinA commercially known as BOTOX® and distributed in the USA and abobotulinumtoxinA more widely known as Dysport® (Galderma; Ipsen; Paris, France). These two preparations have been extensively studied in literature and trials of urological applications. There are a few reports that compared the potency and efficacy of these two preparations of serotype A.

It should be noted there are other BoNT-A preparations and, along with other serotypes except for one preparation of serotype B, have not been in significant trials for application in urological conditions. The reasons may be the unavailability of these serotypes in abundant commercial quantities or, in some cases like serotype F which has a short duration of action, may be deemed impractical or ineffective for intradetrusor injections, especially when considering the desired durable neuromuscular effect by botulinum injections in the bladder detrusor muscle [11, 28]. Similarly, one preparation of serotype B has been shown to exhibit effects of a shorter duration than onabotulinumtoxinA and abobotulinumtoxinA, though not in direct comparison [29].

BOTOX® is FDA approved for use in neurogenic detrusor overactivity and refractory overactive bladder where anticholinergic medication failed to resolve symptoms of frequency, urgency, and urge urinary incontinence satisfactorily or were intolerable by patients. In contrast, Dysport® is yet to be FDA approved for any urological application or included in the guidelines [30]. However, that has not limited its inclusion in a substantial number of trials for different applications including overactive bladder, idiopathic and neurogenic, as well as bladder pain syndrome, among others. Though both formulations are toxin A serotypes, they differ in their preparation and extraction methods and molecular characteristics. Hence, there are differences in quantitative dosage and potentially potency, which will be covered in a subsequent section of this chapter.

There are also other commercially available formulations of BoNT-A: incobotulinumtoxinA, marketed as Xeomin® by the German company Merz Pharmaceuticals and Chinese BTX-A marketed as Prosigne®, among others. These preparations, along with botulinum toxin B preparation rimabotulinumtoxinB (Myobloc®, Solstice Neurosciences Inc., San Francisco, USA), are much less extensively investigated in urology literature and research but have been

utilized experimentally for certain applications. Additionally, two more new preparations of botulinum toxin A are on the horizon, including Evolus' DWP-450 (Irvine, California, USA), expected to undergo review by the FDA in 2018 and Revance Therapeutics' RT-002 (Newark, California, USA) whose FDA application filing is expected in 2019 [5]. Though yet far from being introduced commercially, research into their urological uses would not be surprising.

Recently, BoNT-A preparations have been augmented with added substances thought to improve the delivery and potency of the injections. Of these preparations were liposomal activated preparations, which have been experimented for different urological applications. The consensus is still not drawn, but data suggests no difference in efficacy or potency or need for repeat injections.

1.3. Dosage and potency

Research has extensively investigated the different dosages and regimens for botulinum toxin A injections in the urinary bladder. Differences were identified according to the preparation, as is the case between onabotulinumtoxinA and abobotulinumtoxinA, as well as the quantity of toxin per unit of each preparation measured using different modalities and the clinical implication this may have. Difference in dosing also exists for each condition and in recommendations and guidelines by different advisory bodies. It should be noted, however, that most guidelines only describe injection doses of onabotulinumtoxinA (BOTOX®) since it is the only FDA-approved formulation for some urological uses. Nevertheless, this has not limited research into dosage and effects of abobotulinumtoxinA (Dysport®). Additionally, dilution of the toxin and amount of liquid injected has varied, as well as the number of injection sites.

OnabotulinumtoxinA/BOTOX® comes in different dose formulations than abobotulinumtoxinA (Dysport®). BOTOX® vials are available in 100 and 200 U, whereas Dysport® vials are available in 300 and 500 U; researchers have, however, used higher doses of BOTOX® of up to 300 U. The units for each preparation are not the same nor are they interchangeable. In general, 1 U of BOTOX® is equivalent approximately to 3 U of Dysport®. However, these units are no indication of the potency of either drug. Potency of BoNT-A has rather been described using other units, including mouse units (MU) and median paralysis units (MPU), and the results of different studies have not been successful in concluding which preparation is more potent than the other, especially for bladder injections. It should be of note that the only FDA-approved doses for BOTOX® are 100 U per setting for idiopathic overactive bladder and 200 U for neurogenic detrusor overactivity. Higher doses of BOTOX® have not demonstrated clinically significant efficacy in relation to a higher incidence of adverse effects [1, 31–34].

1.4. Injection modes and sites

Botulinum toxin injections have been described to be delivered to different tissues along the lower urinary tract. In the bladder, literature investigated both intravesical instillations and intradetrusor injections, with the latter proving to be more effective in achieving the therapeutic effect of botulinum in the bladder. BoNT-A can also be delivered intrasphincterically to the urethral sphincter, either periurethrally or even transperineally as some research describes, as well as injected into the prostate.

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Literature and guidelines alike have described different numbers of injection sites in a technique called "mapping." This entails injecting the toxin in a well-spread manner to a specific number of sites on the cystoscopy. Of recent, the most commonly utilized number of injection sites varies between 20 and 30 mapped sites, equally spread between the right and left posterolateral walls of the bladder, with some sites injected more caudally. However, there has been an avoidance for injection of botulinum toxin in the bladder trigone as hypothesis suggests it may contribute to the development of retrograde ascension of urine from the bladder to the kidney, known as vesicoureteric reflux. The consensus is out on whether this hypothesis is valid; however, trigonal injections have been applied in botulinum injections for bladder pain syndrome/interstitial cystitis with no reported occurrence of reflux [35, 36].

1.5. Injection techniques

1.5.1. Preparation of the toxin

To inject the botulinum toxin preparation, it must be first dissolved and diluted from its powdered preparation in the storage vial. It is a surgeon's preference for the dilutional amount of normal saline solution to be used and depends on the number of injection sites the surgeon plans on delivering the toxin through. In order to prepare 100 U of onabotulinumtoxinA for injection, the surgeon usually injects 10 ml of sterile normal saline into the toxin vial and gently swirls the vial to ensure completely dissolving the toxin powder. If 200 U are to be used, the surgeon could use 5 ml for each 100 U vial or 10 ml for each vial [35, 36].

1.5.2. Endoscopic delivery

The toxin is delivered using an ultrafine needle placed through an introductory channel element of the cystoscope device. Generally, the patient is under sedation or general anesthesia, but injections under local anesthesia have been reported. A rigid or flexible cystoscope can be used with equal effectiveness. The bladder is filled with irrigation fluid, and the needle is mapped across the bladder urothelium to deliver a specific amount of the diluted toxin per injection site. The amount delivered is dependent on the number of sites and amount of toxin applied. Traditionally, injecting 100 U of onabotulinumtoxinA diluted in 10 ml of normal saline over 20 sites yields an amount of 0.5 ml per injection, delivering 5 U of the toxin at each site, delivered into the suburothelium or detrusor muscle.

Figure 1 provides a schematic presentation of the posterolateral view of the urinary bladder on cystoscopy, 20 injection sites equally spread in a mapped scheme in each half of the wall. The trigonal area, which stretches between the right and left ureteric orifices along an interorifice ridge, is labeled and is usually spared unless indicated [35, 36].

1.6. Safety and adverse effects

Ever since its initial approval and application, the safety of botulinum injections has been front and center and in a continuous debate. Botulinum toxin is considered one of the most potent toxins to humans and, as such, the level of caution in utilizing it is understood. However, the FDA and other regulatory bodies have approved its clinical application supported by a myriad of clinical trials demonstrating both its safety and efficacy.

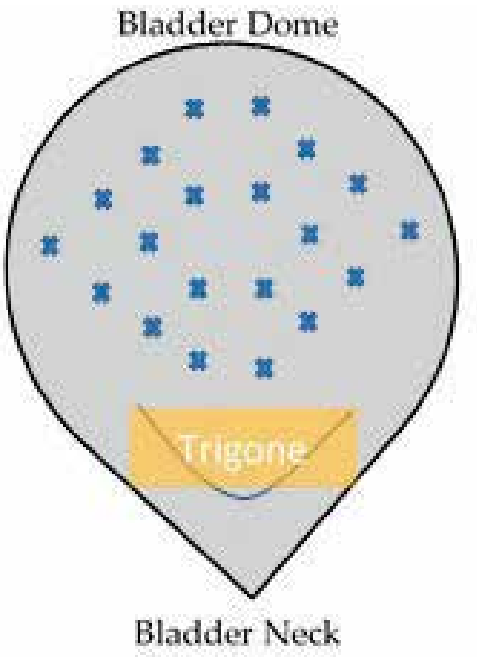

Figure 1. Twenty mapped injection sites over the bladder urothelial posterolateral walls.

In urological application, it has demonstrated to be a safe modality of treatment regardless of which condition it is being utilized for. Numerous trials have proven that the toxin does not seem to systemically spread. One concern was spinal diffusion of the toxin after detrusor injection, which has been debunked. It has also been proven that it does not cause any fibrotic or spastic changes in the urothelium, which was hypothesized as a result of injections earlier on in its application.

Though declared safe toxicologically, injection of the botulinum toxin A into the bladder urothelium does result in certain adverse events, depending on the amount or dose injected and on the disorder being treated. Common reported adverse events from the literature and acknowledgment in guidelines include:

1. Bacteriuria and urinary tract infections. However, septic illness is not significantly reported.
2. Acute urinary retention in the setting of detrusor injections in rather incontinent patients. Patients are usually counseled and consented prior to injection to the development of urinary retention postinjection that it is probable, transient, and will require temporary self-intermittent catheterization for an estimated period and a maximum of 2 weeks. In subsequent repeat injections, some practitioners may opt to lower the injectable dose below to what has resulted in urinary retention.
3. Limited hematuria.

1.7. Efficacy and follow-up

To deem botulinum injections as a viable treatment option for any urological condition with established treatment modalities, it had to prove its efficacy, durability, and comparative benefit. For each urological application, botulinum toxin A injections have been compared to established standardized modalities of treatment. In the case of treatment for overactive bladder, for example, intradetrusor BoNT-A injections provided a more cost-efficient and tolerable treatment method according to some reviews. However, the injections had questions of durability.

Understanding the chemical effect of the injectable toxin, it was well understood that it was time limited, and repeated injections will be required to attain and sustain the effect of the injection. Questions of the safety of repeated injections were satisfactorily addressed in both clinical trials and guidelines, with no evidence to warrant against it. However, reports of a decreased effect after subsequent injections of the same preparation emerged on longer follow-up trials, described as "secondary failure." The rates of this failure are not high, and the data is inconsistent. It should also be noted that urological conditions where botulinum toxin is applied are mostly of a chronic nature, and repeated injections are associated with a higher financial burden and operative morbidity for the patients; thus, it is reasonable to assume that patients may opt for more definite treatment modalities even if they were more invasive.

It has been demonstrated that a positive response to botulinum toxin could be reachieved after secondary failure by applying different preparations of BoNT-A or even using BoNT-B in certain circumstances. However, this is all experimental and not endorsed or approved by the FDA or any urological association.

Intradetrusor BoNT-A injections have been demonstrated to have an initial, subjectively, and objectively reported effect starting at 2 weeks after the injection. Numerous studies demonstrate a peak effect at 6 weeks postinjection. The effect is sustained variably, with reports extending to 9 months or even a year, but the accepted consensus is that the effect does regress at around 6 months postinjection. However, the frequency of reinjection to attain the effect is not mandated by these numbers rigidly and shows interpatient differences. Thus, most practitioners perform reinjections of the toxin on a patient-request basis. The time of onset and length of the effect of intrasphincteric, paraurethral, and intraprostatic injections differ from detrusor injections.

2. Urological applications

The urological applications of botulinum toxin A and B are numerous. As previously described, the FDA approved the use of onabotulinumtoxinA for neurogenic detrusor overactivity in cases of spinal cord injury or multiple sclerosis, idiopathic overactive bladder, and urge urinary incontinence [1]. However, the guidelines and experimental uses have extended to include numerous other urological conditions.

2.1. Neurogenic detrusor overactivity

Neurogenic detrusor overactivity (NDO) is defined as a spectrum of lower urinary tract dysfunction symptoms that result from disruption of the neural control of the bladder, and the term "neurogenic bladder" applies to the urinary bladder malfunction that ensues neural dysfunction resulting from conditions affecting the nerves, including trauma as with spinal cord injury, of which NDO is one entity and detrusor areflexia is another. The range of symptoms includes bladder overactivity, urinary retention, or even both.

Symptoms of neurogenic detrusor overactivity vary according to the onset and cause, as well as the level of the insult in the nervous system. They are generally divided into suprapontine lesions, spinal cord lesions, and peripheral neuropathies. **Table 1** lists the different common causative entities of neurogenic bladder. Each disease results in a different combination of symptoms of bladder dysfunction as a result of the neural pathway it affects and may result in overactivity. Botulinum injections are indicated only when the detrusor muscle is overactive as a result of the neural disease secondary to suprapontine and spinal cord injuries.

Whichever the causative neurological insult, quality of life measurement tools utilized in clinical research unveil a debilitating entity of bladder overactivity encountered by NDO patients. When the suprapontine neural pathways are affected, primitive voiding reflex arcs of the lower urinary tract remain intact, and the bladder becomes overactive. Overactive bladder and spasticity can result in frequency of urination, urgency, and urge urinary incontinence. If the external urinary sphincter is affected and becomes hypotonic by the neural condition, stress urinary incontinence or mixed urge-stress urinary incontinence may also occur.

Anticholinergic medications are the first line of therapy for neurogenic detrusor overactivity. However, the use of these medications is sometimes limited by patient tolerability and requirement for high doses to achieve satisfactory results, and that is often accompanied by a higher level of side effects. The EAU and ICS both recommend the use of botulinum toxin A injections as a second line of management in agreement with the FDA approval. The recommendations of the EAU are based on several randomized controlled trials that proved the efficacy of intradetrusor injections of BoNT-A for the treatment of neurogenic bladder overactivity [36]. A summary of a number of these studies can be reviewed in **Table 2**.

The FDA recommends the injection of a maximum of 200 U of onabotulinumtoxinA intradetrusally in the bladder for NDO. However, trials have reported injections of up to

Suprapontine lesions	Spinal cord lesions	Peripheral neuropathies
Cerebrovascular accidents (stroke)	Spinal cord trauma above or below T6 level	Diabetes mellitus
Parkinson's disease	Multiple sclerosis	Neurosyphilis
Brain tumors		Herpes zoster
Shy-Drager syndrome		Lumbar disk herniation and surgery
		Radical pelvic surgery

Table 1. Causes of neurogenic bladder.

Author	Year	Toxin	Patient population	Outcome	Notes
Denys et al.	2017	Abobotulinum, 750 U, 15 or 30 injections, trigone sparing	NDO and incontinence from SCI or MS	Fifteen injection sites as effective as 30 injection sites compared to placebo	
Kennelly et al.	2017	Onabotulinum, 200 or 300 U, trigone sparing	NDO patients	Safe outcomes, similar effects for both doses	Four-year follow-up study
Apostolidis et al.	2013	Onabotulinum, 50, 100, and 200 U, trigone sparing	NDO patients with urge incontinence	200 U dose most effective and durable effect	Placebo controlled. Effect reported at week 6 postinjection, measured for 52 weeks
Rovner et al.	2013	Onabotulinum, 200 and 300 U, trigone sparing	NDO due to MS or SCI with urgency incontinence	Both doses achieved comparable results in improving urodynamic outcomes of patients	Placebo-controlled phase III trials
Sussman et al.	2013	Onabotulinum, 200 and 300 U, trigone sparing	NDO due to MS or SCI with urgency incontinence	Both doses achieved comparable results in improving health-related quality of life outcomes of patients	Placebo-controlled, double-blinded. Maximal effect gained at week 6 postinjection compared to placebo
Šámal et al.	2013	Onabotulinum, 300 U, submucosally or intradetrusorally	NDO patients	Submucosal injections equally effective	
Ginsberg et al.	2012	Onabotulinum, 200 or 300 U	NDO due to MS or SCI with urgency incontinence	Both doses equally improved the number of incontinence episodes, cystometric parameters, and quality of life	Placebo-controlled, double-blinded, 52-week follow-up
Cruz et al.	2011	Onabotulinum, 200 or 300 U, trigone sparing	NDO due to MS or SCI with urgency incontinence	Both doses equally improved the number of incontinence episodes, cystometric parameters, and quality of life	Placebo-controlled, double-blinded. First effect documented at 2 weeks postinjection

Table 2. Summary of RCTs utilizing botulinum toxin in treatment of neurogenic detrusor overactivity [34, 37–43].

300 U for the control of the overactivity. Reports of higher dose efficacy being clinically insignificant considering a higher level of adverse events and complications have steered the consensus toward the FDA set dosage.

2.2. Idiopathic overactive bladder

Idiopathic or non-neurogenic overactive bladder (OAB) describes a syndromic set of symptoms of increased daytime and nighttime urination urgency and frequency with or without urgency urinary incontinence in the absence of a causative pathology. In ICS definition, OAB is a syndrome of urgency, a compelling sensation to urinate, frequency, or urinating more than eight times during waking hours, and nocturia, waking up once or more to urinate at night;

whether urinary incontinence occurs as a result of the urgency (wet OAB) or does not (dry OAB) is not essential for the clinical diagnosis.

Often, investigations for these presenting symptoms include performing a urodynamic evaluation of the bladder, where a urinary catheter connected to pressure transducers is used to fill the bladder with a saline solution in order to reproduce the urinary complaints of the patient; uninhibited involuntary bladder contractions witnessed as a result of the filling or after the patient has voided are defined as detrusor overactivity, which occurs with the majority of OAB patients. Nevertheless, many patients with idiopathic OAB do not require urodynamic assessment when the symptoms are clear-cut, and a number of clinical tools and scores can aid in diagnosing, assessing severity, and following up of treatment efficacy of the syndrome. Refractory OAB is when symptoms fail to resolve on conservative management with nonsurgical noninvasive modalities.

Botulinum toxin A for the treatment of idiopathic refractory OAB was approved by the FDA in 2013, but only in the onabotulinumtoxinA/BOTOX preparation [1]. Clinical trials on both onabotulinumtoxinA and abobotulinumtoxinA for the treatment of refractory OAB have preceded this approval, and to date, there is continuous investigation into the optimal dosage, dilution, and frequency of injections to achieve optimal relief of the symptoms.

Though the ICS recommendations for the use BoNT-A for OAB refrain from specifying a certain preparation, both the EAU and American Urology Association/Society of Urodynamics, Female Pelvic Medicine and Urogenital Reconstruction (AUA/SUFU) guidelines specifically mention onabotulinumtoxinA injections only. While the EAU guidelines mention that abobotulinumtoxinA and incobotulinumtoxinA are neither licensed nor interchangeable for onabotulinumtoxinA, the AUA guidelines go a step further in adding a note on the use of abobotulinumtoxinA compared to onabotulinumtoxinA. Citing one clinical trial that compared the efficacy of the two preparations, the AUA/SUFU guidelines on management of non-neurogenic overactive bladder declare that although abobotulinumtoxinA is of equal clinical efficacy, it is reported with a higher incidence of postinjection adverse events when compared to onabotulinumtoxinA, particularly with regard to the development of postinjection urinary retention requiring self-catheterization [44].

The recommended dose of onabotulinumtoxinA intradetrusor injection for idiopathic overactive bladder in both the EAU and AUA guidelines is 100 U diluted in normal saline and mapped across 20 injection sites. Both sets of guidelines discuss the possible rates of bacteriuria postinjection, cautions of injection in elderly patients with OAB, and necessity of repeated injections to sustain a desirable effect. The grade of recommendation for onabotulinumtoxinA injection per the EAU guidelines is listed as grade A recommendation, supported by a compelling set of randomized clinical trials, while the AUA gives the same treatment modality a standard level of recommendation with a grade B strength of evidence where the benefits of the injection outweigh the risks.

2.3. Detrusor sphincter dyssynergia

Normally, when the bladder contracts, there is a synergistic neural communication that relaxes the sphincter responsible for control of the bladder outlet. This coordination allows the bladder to perform its storage and emptying functions. However, any disruption to this synergy causes

voiding dysfunction. Certain neurological conditions affecting the suprasacral micturition centers, such as some of the causes listed in **Table 1** like spinal cord trauma below the vertebral level of T6, could lead to an entity of voiding dysfunction known as detrusor sphincter dyssynergia (DSD). In DSD, the detrusor sphincter pathologically contracts simultaneously with the detrusor muscle of the urinary bladder instead of relaxing to allow bladder emptying. Thus, instead of free urinary flow during attempts of voiding, patients only pass small amounts of urine, if any. Multiple sclerosis affecting the spinal cord could also result in DSD.

Botulinum toxin injection for the management of DSD was the first published urological application of the toxin. Since its first description in 1988 by Dykstra, intrasphincteric botulinum injections for the management of DSD have been the focus of many researchers and urologists [45]. However, the clinical guidelines such as those published by the ICS and EAU do not dive into details for recommending this modality of managing DSD in neurogenic bladder patients since it is not registered for such an application, but rather mention it as a possible entity of treatment and make no specifications on the dosage or frequency of injections. The EAU based its limited recommendation on a Cochrane review that concluded that more RCTs are necessary before further recommending intrasphincteric botulinum injections for DSD but acknowledged reports stating its effectiveness. It should be mentioned, however, that the literature describes different techniques for intrasphincteric and periurethral injections of BoNT-A for the treatment of DSD, including transurethral endoscopic injections and imaging-assisted transperineal approaches, with variable reported outcomes.

2.4. Other neurogenic and non-neurogenic voiding dysfunctions

Lower urinary tract symptoms can also be attributed to asynchrony between the detrusor and sphincteric muscles and over- or underactivity of either the bladder detrusor muscle or urethral sphincter. This may be an entity of dysfunction voiding, idiopathic or even neurogenic, such as the peripheral neuropathy causes listed in **Table 1**. Successful treatment of DSD with sphincteric BoNT-A injections allowed an insight into the treatment of these voiding dysfunction entities. It has been demonstrated to decrease patient reliance on self-catheterization and improve voiding efficiency. However, the data is experimental, and the modes, dosage, and frequencies vary, in the absence of a consensus or clear recommendation by regulatory bodies like the EAU or AUA.

2.4.1. Fowler's syndrome

Fowler's syndrome is a unique entity of voiding dysfunction. Usually affecting young women, it is defined as a disorder of urethral sphincter relaxation and urinary retention in volumes reaching up to 1 l of urine, often in the absence of sensation of bladder fullness. Though its etiology is not well understood, the introduction of sacral neuromodulation has provided a means for restoring the normal voiding function in patients of Fowler's syndrome. Guidelines on the treatment of this syndrome with intrasphincteric injections of botulinum toxin are not available, nor is there any consensus or panel discussion. However, an open-label study in 2015 reevaluated this modality of treatment after an unsuccessful trial 20 years prior. This recent trial reignited interest into a less invasive modality of treatment of this syndrome compared to sacral neuromodulation.

2.5. Bladder pain syndrome/interstitial cystitis

This chronic debilitating condition was first described over 200 years ago. The hallmark of this condition is "bladder pain" or suprapubic pain that the patient can specifically attribute its sensation in the bladder. Different terms have been used to describe the combination of suprapubic pain, urgency, and frequency, in the absence of an infective pathology. Originally named interstitial cystitis only, the term bladder pain syndrome was added to further describe this disease entity where macroscopic findings may be absent on cystoscopic examination of the bladder. Similarly, the disease has been also termed hypersensitive bladder for the same reason. Nevertheless, a subgroup of the disease exhibits positive cystoscopic findings of what is known as "Hunner's lesions." These lesions, originally thought to be ulcers in the bladder mucosa but proven otherwise, constitute what is more known as the "classic" or "ulcerative" type of bladder pain syndrome/interstitial cystitis (BPS/IC), and although first described in women, the disease afflicts both genders.

Treatment of this disease is as complex as its pathology. With an unknown cause, the aim of treatment for the most part has concentrated on symptomatic relief and prolonged periods of remission of the pain in between flares of the disease. Different modalities have been described for the treatment of the disease, with varying degrees of success, ranging from oral amitriptyline to surgical interventions. In the presence of Hunner's lesions, there are numerous reports of achievement of some degree of pain relief on resection and ablation of these lesions. However, the other lower urinary tract symptoms may not be limited.

Botulinum toxin injections have been extensively described in the literature as a modality of treating the symptoms of BPS/IC. First described by Smith et al. in 2004, it was reported to provide relief from the bladder pain. Research then demonstrated the effects of BoNT-A injections on the bladder in BPS/IC. On the microscopic level, BoNT-A in BPS/IC was shown to:

1. Decrease acetylcholine and noradrenaline release from nerves in the urothelium.
2. Decrease the level of TRPV1, which is typically elevated in BPS/IC in the bladder urothelium.
3. Decrease the levels of nerve growth factor (NGF).
4. Decrease the level of neurogenic inflammation.
5. Decrease the expression of P2X3 receptors and CGRP release.
6. Modulation of the release of inflammatory mediators from the bladder urothelium, typically upregulated in BPS/IC.
7. Decrease mast cell infiltration and apoptotic cell counts in the urothelium.

These cellular effects have been both demonstrated and reproduced in several studies; however, there was no consensus or standardization on the dose of BoNT-A injected and modalities employed to augment the injections. Nevertheless, the described effects included:

1. Marked decrease in bladder and suprapubic pain.
2. Decrease in daytime and nighttime urinary frequency.
3. Decrease in the ICSI symptom score, an index used to assess the severity of the symptoms of patients diagnosed with BPS/IC.

4. An improvement in the quality of life of injected patients.
5. An increase in the maximal bladder capacity of urinary storage known as cystometric capacity.
6. Decrease in urgency and desire to void.
7. Antinociceptive effect.

BoNT-A injections for bladder pain syndrome is not FDA approved. However, the data is compelling enough from many randomized control trials that the ICS, EAU, and AUA sought to include its application in their guidelines. Though the grades of recommendation differ in strength, BoNT-A injections for the symptomatic treatment of BPS/IC are recognized as a viable option. The EAU strongly recommends the use of BoNT-A injections for BPS/IC when more conservative measures have failed and lists in sequence the different modalities it can be used in. The AUA has a more modest recommendation for BoNT-A in BPS, listing it as a fourth-line management modality.

Both onabotulinumtoxinA and abobotulinumtoxinA have been utilized in trials of treatment of BPS/IC. There is no consensus in both the literature and the guidelines on the dose of BoNT-A to be used. It should be of note that many trials, along with the EAU guidelines on management of BPS/IC, describe an entity of management known as hydrodistension, used alone or in combination with BoNT-A injections. During hydrodistension, the bladder is filled with a considerable amount of irrigation fluid and left in the bladder for an amount of time ranging between 5 and 15 minutes. The EAU proposes in its treatment algorithm that hydrodistension can be tried alone, then submucosal BoNT-A injections with hydrodistension, and, finally, intradetrusal BoNT-A injections with hydrodistension, without specifying the injectable dose or duration of distension, reflecting the variance in the data. Additionally, trigonal-involving BoNT-A injections have been described in the treatment of BPS/IC without inducing vesicoureteric reflux and with a considerable efficacy.

2.6. Chronic pelvic pain syndrome

This broad term describes a spectrum of disorders including chronic prostatitis, which is dubbed in some guidelines as the male variant of bladder pain syndrome. As with BPS/IC, chronic pelvic pain syndrome (CPPS) is of a noninfective etiology with a significant effect on the quality of life of the afflicted patient. A number of trials described decreased levels of pain on both periurethral and transperineal injections of BoNT-A for the treatment of CPPS. The EAU guidelines do not specify a recommendation for BoNT-A into the pelvic floor or prostate for CPPS and describe it as having a "modest" effect, while the AUA pairs treatment of BPS/IC and CPPS modalities in its recommendations.

2.7. Benign prostatic enlargement

The effects of intraprostatic injections of BoNT-A have been demonstrated in research on both humans and animals and have supported the hypothesis that an induction in the apoptosis of the glandular tissue of the prostate could lead to its atrophy and relief from the obstructive

component of lower urinary tract symptoms that result from benign prostatic enlargement (BPE). BPE could be thought of as a disease of age in men, where continuous proliferation of the glandular tissue in the transitional zone of the prostate gland leads to an increase in its size and narrows the outlet of the bladder, obstructing urinary flow. There is no specific sizable enlargement that causes symptoms, and the degree of symptoms does not correlate to the size of the enlarged prostate.

To date, the gold standard of treatment of BPE is transurethral resection of the prostate by endoscopic measures, after standing the test of time against open prostatectomy and when compared to emerging modalities of treatment. However, this has not deterred research into less invasive modalities of treatment including pharmacological regimens using alpha-receptor antagonists and 5-alpha reductase inhibitors, both first lines of treatment for BPE that have been shown to offer symptomatic relief and delay the need for surgical intervention, and other endoscopic and interventional radiology modalities.

Trials that have investigated intraprostatic injections of BoNT-A for BPE have described different doses and modes of injection. Due to the multifactorial nature of the lower urinary tract symptoms in BPE, prostatic BoNT-A injections may not provide complete or significant results in the presence of associated bladder over- or underactivity as a result of the outlet obstruction resulting from the prostatic enlargement. However, among those trials, there were clinically significant results with injections into the prostate and the bladder neck, including:

1. Improvement in the maximal urinary flow rate of patients, known as the QMax on uroflowmetric studies.
2. Reduction in symptomatic scoring indices used to assess severity of symptoms associated with BPE.

Nevertheless, within these trials, the modes of injection and doses are different, and the results are inconsistent and in some instances contradictory, indicating the need for further assessment and more trials before a consensus could be made.

BoNT-A injections for the treatment of BPE are considered completely "off-label" and against the EAU guidelines on the management of male lower urinary tract symptoms resulting from BPE or benign prostatic obstruction. The EAU cites trials and a recent systemic review and meta-analysis that showed BoNT-A not to be superior to placebo in the management of BPE and, as such, recommends against the use of BoNT-A in BPE. The AUA, however, makes no reference to or acknowledgment of BoNT-A prostatic injections.

2.8. Erectile dysfunction

Erectile dysfunction (ED) describes a spectrum of disorders in which a male cannot attain or maintain an erection sufficient to perform penetrative intercourse or complete it to ejaculation. The application of botulinum toxin injections in the treatment of entities of ED such as premature ejaculation and vasculogenic ED is reported in the literature of recent but are limited to small clinical trials and case reports hypothesizing the effect of the toxin in improving the sexual performance of the affected male. It is an area of future research and consideration,

especially with certain animal trials showing complementation of the mechanism of action of botulinum toxins and the physiology of attaining an erection.

3. Conclusion

Botulinum toxin applications in urology have garnered much attention in the last two decades both on the research and regulatory levels. The effects of the toxin at the neurophysiological level of the bladder urothelium extend beyond the neuromuscular blockade leading to detrusor relaxation and have been proven to exert sensory, antinociceptive, and anti-inflammatory effects as well through mediation of neural, cellular, and inflammatory markers. Though limited to the use of botulinum toxin A with the exception of one preparation of botulinum toxin B, the urological applications of botulinum are categorized into FDA-approved, guideline-supported, and experimental or "off-label" uses. The FDA has approved the use of onabotulinumtoxinA only and in the setting of neurogenic detrusor overactivity and refractory overactive bladder after failed treatment with anticholinergic medications. Regulatory bodies like the EAU and AUA not only adhered to this approval but also endorsed clinically apparent beneficial applications of BoNT-A in conditions like DSD and BPS/IC. Experimental and off-label uses are not recommended but are still practiced with limited evidence.

Conflict of interest

None to declare.

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The aim of this book is to ensure a safe understanding and use of botulinum toxin in medicine. Known indications, contraindications, diversity, comparative effects between subtypes, limits, allergies, treatments, adverse reactions, nonresponsiveness, and new investigations will be described. Botulinum toxin can be currently used in nearly every specialty. The main areas in this book are cosmetics and dermatology, as well as dentistry, urology, masseter hypertrophy, chronic pain treatment, and others. The important aim is formation of the knowledge of anatomy, muscles to be treated and their function, risk factors, brand names, associations with operations, and other treatments.

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