Comparison and Overview of Currently Available Neurotoxins

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ABSTRACT

Background: Botulinum toxin has been in use since the 1970s. Over the last few years, the indications for botulinum toxin use have extended for cosmetic and noncosmetic applications. Three preparations of botulinum toxin type A and one preparation of botulinum toxin type B are commercially available and approved for use in the United States by the United States Food and Drug Administration. **Objective:** To review the most recent literature on all commercially available botulinum toxins in the United States, their indications, Food and Drug Administration approvals, and handling (reconstitution, storage, and dilution). **Methods:** A literature review (not Cochrane type analysis) using several databases (PubMed, MEDLINE, textbooks, Food and Drug Administration homepage, and manufacturer information) was performed. **Conclusion:** Several different preparations of botulinum toxins exist worldwide, none of which are identical or interchangeable. Manufacturer recommendations on all available botulinum neurotoxins advise the use of unpreserved saline for reconstitution. Side effects are mostly mild and always self-limited. More serious complications are associated with higher doses, improper injection techniques, and occur in patients with underlying comorbidities. (*J Clin Aesthet Dermatol.* 2014;7(2):31–39.)

Clostridium botulinum is a Gram-positive bacterium first identified more than 100 years ago. It produces a neurotoxin that has been studied extensively since its discovery. Today, seven antigenically different serotypes have been identified, two of which are used clinically: serotypes A and B. Serotype A (BTX-A) appears to be the most potent subtype among them.[1]

In 1980, Scott published the landmark paper describing the clinical use of botulinum toxin type A for the treatment of strabismus.[2] In 1987, Carruthers and Carruthers noticed that patients treated with botulinum toxin for blepharospasm experienced improvement in glabellar lines.[3] Since then, BTX-A has been approved by the United States Food and Drug Administration (FDA) for a variety of cosmetic and noncosmetic applications, including strabismus, blepharospasm, cervical dystonia, hyperhidrosis, glabellar rhytides, and, most recently, urinary incontinence from detrusor overactivity.

Botulinum toxin's most common cosmetic application is in the treatment of upper facial rhytides and dynamic lines, namely glabellar lines, horizontal forehead wrinkles, and crow's feet. In the lower face, the use of botulinum toxin was initially controversial because results were considered unpredictable. While the demarcation between the upper and lower face is somewhat arbitrary, the utility of botulinum toxin in the lower face is becoming increasingly apparent. However, the medical literature on use of botulinum toxin in the lower face and neck is sparse with most publications limited to clinician experiences only.[4]

MECHANISM OF ACTION/PHARMACOLOGY/ STRUCTURE

Botulinum neurotoxin (BTX) is a safe and attractive therapeutic treatment modality for several clinical conditions. The clinical effect of BTX is a highly specific, but reversible, inhibition of presynaptic neurotransmitter (acetylcholine) release. Botulinum toxins inhibit the exocytotic mechanisms within neurons, resulting in temporary inhibition of neurotransmitter release at the neuromuscular junction or in other tissues.

Botulinum toxins are synthesized by a variety of Clostridial species, most commonly *Clostridium*

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botulinum, but also *C. baratii* or *C. butyricum*.[5] These bacteria synthesize seven different neurotoxin strains classified as serotypes A through G. In nature, a single core neurotoxin (150-kD) is contained within a molecular complex that varies in size based on nontoxic, clinically inactive proteins classified as hemagglutinins or nonhemagglutinins.[6] These associated proteins serve as stabilizers to protect the neurotoxin molecule from pH, thermal stress, and enzymatic degradation.[7]

For biological activity, the core neurotoxin requires enzymatic cleavage by proteases to exert its activity.[8] Cleavage produces a molecule consisting of a heavy chain and light chain held together by a disulfide bond. The activated di-chain has the following three functional terminals: 1) the C-terminal (heavy chain) contains the binding domain that docks to the neuron; 2) the N-terminal (heavy chain) contains the translocation domain; and 3) the light chain contains the catalytic portion responsible for cleavage of the intracellular BTX target. All Clostridial neurotoxins consist of the same tertiary protein structure, but differ in protein sequences among the serotypes, which accounts for their different affinities, antigenicities, and intracellular targets.[9]

In order to exert clinical activity, the BTX has to gain entry into the neuronal end terminal via its translocation domain. Under physiological conditions, the core 150-kD protein dissociates from the toxin complex, binds to synaptic vesicle protein 2 using its heavy chain, and enters the presynaptic neuronal cell by endocytosis.[10] Cell surface receptors at the neuronal endplate that facilitate BTX entry are over-expressed in neurons that actively secrete neurotransmitters.[11] The acidic pH within the endocytotic vesicle cleaves the disulfide bond freeing the light chain and enabling it to traverse into the neuronal cytosol where it disrupts one or more SNARE (soluble N-ethyl-maleimide-sensitive factor attachment protein receptor) proteins on the presynaptic vesicle.[12] Without an active SNARE protein, the vesicle containing the neurotransmitter can no longer fuse with the cell membrane, thereby preventing its exocytotic release. Type A botulinum toxin binds to and cleaves the 25-kD SNARE protein "SNAP-25" (synaptosomal-associated protein), whereas botulinum toxin type B (BTX-B) binds to and cleaves "VAMP" (vesicle associated membrane protein).[13]

The duration of action or rather inhibition of neurotransmitter release varies among the serotypes based on the half-life of the light chain and the time of the neuron to restore SNARE proteins. Studies suggest that botulinum toxin type A has the longest half-life, followed by types C1, B, F, and E.[14]

The recovery of neuronal activity has been attributed to two phenomena: First, axonal sprout development in response to growth factor secretion from denervated muscle. These sprouts are active and produce temporary re-innervation in the early recovery phase. Second, during the later phase of recovery, vesicular neurotransmitter release has been found to return in the original nerve terminal.[15]

The BTX-mediated inhibition of neurotransmitter release is reversible, which has clinical advantages and disadvantages. Benefits include the flexibility to inject a given muscle based on its activity level, which may fluctuate over time; the avoidance of irreversible and more invasive interventions, such as surgery; and the resolution of possibly unintended effects after a period of time. The main limitation of the reversibility of BTX injections is the need for repeat injections. However, repeat treatments may yield sustained efficacy.

Even though the main effect of BTX-A is on alpha motor neurons inhibiting their acetylcholine release, BTX-A also inhibits neurotransmitter release from gamma motor neurons innervating muscle spindles.[16] Gamma motor neurons are known to play a critical role in maintenance of hyperactive muscle contractions, such as spasticity and dystonias.

SNARE proteins also appear to play a role in C-fiber nocioceptive neurons that are known to release glutamate and substance P. Those two substances result in vasodilation and the release of pro-inflammatory mediators, such as bradykinin, prostaglandins, histamine, and serotonin.[17] BTX-A is thought to involve inhibition of these factors, which may account for its treatment possibilities in chronic pain disorders, such as migraine headaches or neuralgias.

INDICATIONS

Botulinum neurotoxin is an effective treatment in managing strabismus, hemifacial spasm, blepharospasm, cervical dystonia, spasmodic dysphonia, hyperhidrosis, sialorrhea, gustatory sweating (Frey's syndrome), and facial rejuvenation.[18] The effects suggest that BTX injections also affect postganglionic neurons of the parasympathetic nervous system, which use acetylcholine as their neurotransmitter.[19,20]

Beyond the effects on muscle and secretions, BTX-A has been reported to reduce pain associated with cervical dystonia, temporomandibular disorders, post-herpetic and trigeminal neuralgias, and chronic migraine headaches. Although the exact mechanism of action in those conditions is unclear, it has been hypothesized that it occurs through inhibition of afferent neuronal neurotransmitter release.[21]

Facial rejuvenation. In the upper face, botulinum toxin is most commonly used to eliminate or diminish glabellar lines (procerus and corrugator muscles), forehead rhytides (frontalis muscle), and crow's feet (lateral orbicularis oculi muscle).

In the lower face, botulinum toxin may be used to treat perioral lip lines (orbicularis oris, depressor anguli oris, and mentalis muscles). In most cases, botulinum toxin is administered to this area in conjunction with dermal fillers. Injections are given in small amounts (1 U Botox® or 3 U Dysport® per site, 4–5 sites each in the upper and lower lips) while staying in close proximity (within 5mm) to the vermilion border. Results are generally not as dramatic as those in the upper face and last approximately 10 weeks. The risk of dysarthria and oral incompetence should be discussed with the patient prior to administration.[4] Due to the risk of oral incompetence, BTX-A should not be used in

the perioral region in singers, musicians, or scuba divers.

The oral commissures (depressor anguli oris muscles) are another popular area for BTX administration in the lower face. As with perioral lip lines, oral commissure rhytides should not be treated in patients where oral incompetence would have a significant impact on the patient (singers, musicians, scuba divers, etc.). One injection (2–4 U Botox® or 6–12 U Dysport®) is given on each side directly into the depressor anguli oris muscle overlying the mandibular body. Injections in the midline, directly into the mental fold, or in close proximity to the mouth should be avoided.[4]

While nasolabial folds are best treated with dermal fillers, some practitioners have successfully used botulinum toxin preparations in select patients including those with short upper lips or those who have a "canine" smile (strong raising of the medial upper lip upon smile). Nonetheless, patients must be advised that their smile pattern may be altered secondary to the BTX administration. Only small doses (1 U Botox® or 3 U Dysport® per side) should be used for treatment of nasolabial folds injected just below the nose into the lip elevator complex and the levator labii superioris alaeque nasi muscle.[4] Good candidates have deep nasolabial folds with "gummy smiles" where all incisors and some of the gingivae show upon smiling.[22]

The dimpled chin may be successfully treated with BTX administration directly into the mentalis muscle. The most commonly used dosage ranges between 5 to 10 U Botox® or 15 to 30 U Dysport® total given into two injection sites in a paramedian position (5mm lateral to the midline) overlying the inferior mandibular border. Injections administered too far laterally (depressor anguli oris muscle) should be avoided, as should injections too far superiorly (orbicularis oris muscle leading to oral incompetence and drooping mouth). Some practitioners advocate for only one injection site midline.[4]

In the neck, BTX preparations may be used to treat platysmal bands, particularly in older patients who are not good candidates for surgery, in those who do not want surgery, or in younger patients who are not candidates for cervicofacial rhytidectomies yet.[23,24] Other good candidates are patients who have retained their skin elasticity with minimal descent of submental adipose tissue. Patients to avoid are those with jowl formation or bone resorption.[25] It is important to keep in mind that BTX has no effect on skin redundancy, laxity, or fat deposits. Neck treatment with BTX appears to require higher dosages than the lower face. A total of 10 U Botox® or 30 U Dysport® per platysmal band is typically used. Some practitioners inject the neurotoxin in three to five sites per band from the mandible to the lower neck in 1cm intervals. Others inject only the central portion of the band at the cervicomental angle trying to recreate it. Injection into the strap muscles must be avoided as dysphagia, dysphonia, and neck weakness may ensue.[4]

Facial sculpting. In the upper face, BTX-A is used for chemical brow elevation and widening of the eyes. Injections into the glabella have been reported to lead to medial, central, and lateral brow elevation secondary to partial inhibition of brow depressors and increased resting tone of the frontalis muscle.[26] In the lower face, BTX-A may be injected into the masseter muscle to alter the shape of the jawline, a particularly popular application in Eastern Asian countries, most commonly in South Korea. Ten to 25 U Botox® or 30 to 75 U Dysport® are typically used on each side administered in one to three injection sites. The patient is asked to bite down while the practitioner holds the anterior and posterior border of the masseter muscle. The thickest portion of the muscle is injected over the "buckled" area.[4] Up to 50-percent reduction of muscle bulk has been reported through atrophy.[27] It is important to keep in mind that the full treatment effect requires multiple sessions with the full benefit not being evident for three to four treatment cycles (usually 1 year).[4] Adverse effects include mastication difficulty, muscle pain, dysarthria, and awkwardness with smiling.

Adjunctive botulinum toxin A. BTX-A is increasingly used in surgical and nonsurgical cosmetic procedures to prolong or enhance cosmetic results. BTX-A may be synergistically used in combination with soft tissue augmentation to achieve improved longevity of results by reducing the dynamic component of wrinkles.[28] Laser resurfacing results may be optimized by pretreatment with BTX-A, leading to longer-lasting and superior results.[29] Studies of intense pulsed dye laser showed that BTX-A treatment improves cosmetic outcomes and skin texture in treatment of cutaneous telangiectasias.[30] In surgical interventions, such as browlifts, many surgeons use BTX-A as an adjunctive procedure to increase longevity of the surgical intervention. BTX-A also reduces contractile forces on the surgical site diminishing the risk of wound dehiscence or scarring by reducing tension on wound edges. For instance, BTX-A treatment of the underlying musculature (especially the lateral orbicularis oculi muscle) 5 to 10 days prior to surgical browlifts diminishes the opposing brow depressor forces, allowing better suture suspension with less "cheese-wiring" effects. Overall, longer-lasting and improved cosmetic results are achieved with more rapid wound healing.[31]

DURATION OF ACTION

Botulinum toxins appear to have a significantly longer effect on autonomic neurons (6–9 months), such as hyperhidrosis or overactive bladders vis-à-vis to the 3 to 4 months observed for striated muscle (facial rhytides). This finding suggests that neuronal sprouting does not occur to the same degree in the autonomic nervous system as it does in striated muscle.

FORMULATIONS

Botulinum toxin formulations are neither identical nor interchangeable. They possess individual potencies and different, although sometimes overlapping, indications. Attention to different preparations to ensure proper application is required for safe use to avoid errors. In April 2009, the FDA established drug names to help the provider in differentiating several preparations and to prevent potentially serious adverse effects.[32] paragraph: For an overview and comparison of currently available botulinum

toxins, see Table 1.

Botox®/Botox Cosmetic®. Botox® (Allergan, Inc., Irvine, California) is the trade name for onabotulinumtoxinA and is approved for over 20 indications in more than 75 countries.[33] It is the original botulinum toxin type A product, which was initially purified by Shantz and later used clinically by Scott in San Francisco. In 1997, there was a formulation change to reduce the amount of immunogenic protein content. The active neurotoxin is botulinum toxin type A. This neurotoxin comprises 85 percent of the worldwide Botox® market, and most scientific articles on botulinum toxins are about Botox®.[33] In the United States, Botox® is FDA approved for the treatment of cervical dystonia, severe primary axillary hyperhidrosis, blepharospasm, neurogenic detrusor overactivity (urinary incontinence), chronic migraine, upper limb spasticity, and moderate-to-severe glabellar lines.[32] OnabotulinumtoxinA is also known as Vistabel® in Europe and Vistabex® in Italy.[34]

Dysport®/Reloxin®/Azzalure®. Dysport® (Medicis Pharmaceutical Corp., Scottsdale, Arizona) is the trade name for abobotulinumtoxinA. It was approved by the FDA in 2009 for the treatment of cervical dystonia and for temporary improvement in the appearance to moderate-to-severe glabellar lines.[32] The active neurotoxin is botulinum toxin type A. The difference between Botox® and Dysport® lies in the purification procedure. Botox® is purified by repeated precipitation and re-dissolution, whereas Dysport® is purified by a column separation method.[33] After injection, Dysport® appears to have a greater spread effect clinically leading to a more diffuse distribution of clinical effects. Dose ratios between Dysport® and Botox® have been debated in the past and likely vary greatly based on dosage. However, in a cosmetic clinical practice, a dose ratio of approximately 3:1 is most commonly agreed upon.[35]

Xeomin®/Bocoture®. Xeomin® (Merz Pharmaceuticals, Frankfurt, Germany), the trade name for incobotulinumtoxinA, is licensed in Germany. The active neurotoxin is botulinum toxin type A stripped from any complexing proteins. It is mainly used in Europe, Mexico, and Argentina for the treatment of blepharospasm and cervical dystonia. In the United States, Xeomin® was FDA approved in 2011 for temporary improvement in the appearance of moderate-to-severe glabellar lines in adults. Xeomin® appears to exhibit a dose ratio with Botox® of 1:1.[33] No significant differences in safety and efficacy have been found in studies comparing Botox® and Xeomin®. In fact, as Xeomin® is free of therapeutically superfluous complexing proteins, its purer formulation has been suggested to lead to greater efficacy with reduced risk of sensitization or antibody formation.[36] However, the clinical relevance of this has yet to be determined.

PurTox®. PurTox® (Mentor Worldwide LLC, Santa Barbara, California) is a new, purified botulinum type A neurotoxin. It has completed stage III clinical trials for FDA approval in the United States. PurTox® resembles Xeomin® (free of therapeutically superfluous complexing proteins) and aims to improve the appearance of glabellar frown lines and forehead rhytides. However, any conclusive evidence on its efficacy has yet to be determined.

Neuronox®. Neuronox® (Medy-Tox Inc., South Korea) is a botulinum toxin type A complex widely used in South Korea and Southeast Asia.[33] While limited literature is available on the efficacy and safety of Neuronox®, it appears to be effective.

CBTX-A/Prosigne®/Lantox®. CBTX-A (Lanzhou Institute of Biological Products, China) is Chinese botulinum toxin A and the only BTX-A approved in China. The formulation contains bovine gelatin protein to prevent the neurotoxin from adhering to the wall of the vial or syringe. Most other botulinum preparations utilize human serum albumin instead, but in the case of CBTX-A, the gelatin used is bovine in nature, which has the potential to trigger an immunological response and allergic reactions. In the worst-case scenario, bovine spongiform encephalopathy (i.e., "mad cow disease") may be transmitted. CBTX-A is marketed as Prosigne® in Brazil, but is not available in the United States. A double-blind, randomized, crossover study of Prosigne® versus Botox® in patients with blepharospasm and hemifacial spasm showed similar efficacy and safety profiles between Prosigne® and Botox®.[37]

CNBTX-A. CNBTX-A (Nanfeng Medical Science and Technology Development Co. Ltd., China) is a botulinum toxin type A preparation that is neither approved nor licensed in any country. It should not be confused with CBTX A/Prosigne®/Lantox®/Redux. CNBTX-A was found to contain much higher levels of neurotoxin than listed on the package insert, which could potentially lead to severe health risks to patients.[38]

MyoBloc®/NeuroBloc®. MyoBloc® (Solstice Neurosciences, Inc., Louisville, Kentucky) is the trade name for rimabotulinumtoxinB. It is the only botulinum toxin type B commercially available in the United States. Its sole indication lies in the treatment of adults with cervical dystonia to reduce the severity of abnormal head position and neck pain associated with this condition. In fact, MyoBloc® was the first FDA-approved treatment for cervical dystonia in the United States. Today, MyoBloc®/NeuroBloc® is approved in the United States, Canada, and Europe for the treatment of cervical dystonia. MyoBloc® injections for cosmetic applications have a more rapid onset of action and greater area of diffusion at the expense of more painful injections and shorter duration of effects.[39] MyoBloc® is available as a liquid with an acidic pH (5.5–6.5), which explains the increased discomfort associated with its injections. Furthermore, the units appear to be significantly less effective when compared with BTX-A units. In cervical dystonia, the ratio is approximately 50:1, whereas for glabellar frown lines, the ratio appears to be 100:1.[39,40] NeuroBloc® (Eisai Co., Ltd, United Kingdom) is the European equivalent to MyoBloc®.

RECONSTITUTION

Unpreserved saline. OnabotulinumtoxinA (Botox®) is the most studied botulinum neurotoxin. Initially, it was thought to be a fragile substance.[41] However, later studies have relativized this initial belief and confirmed persistence

TABLE 1. Comparison of botulinum toxin preparations[5]

	ONABOTULINUMTOXINA	ABOBOTULINUMTOXINA	CBTX-A	BONTA
COMMERCIAL NAMES	Botox®, Botox Cosmetic®, Vistabel®, Vistabex®	Dysport®, Reloxin®, Azzalure®	Prosigne®, Lantox®	Neuronox®
COMPANY	Allergan Inc.	Medicis Pharmaceutical Corp.	Lanzhou Institute of Biological Products, China	Medy-Tox Inc., South Korea
TYPE	A	A	A	A
COUNTRIES	Worldwide, including United States and Canada	> 65 countries, including United States and Canada	> 10 countries including China, not in the United States or Canada	South Korea, India, South America, not in the United States or Canada
ACTIVE SUBSTANCE (MOLECULAR WEIGHT)	BTX-A complex (900 kD)	BTX-A complex (500–900 kD)	BTX-A (900 kD)	BTX-A (940 kD)
STRENGTH (BTX-A: PRODUCT)	1:1	1:2–1:4		
INDICATIONS	Blepharospasm, cervical dystonia, glabellar lines, hyperhidrosis, chronic migraine, urinary incontinence, etc.	Blepharospasm, cervical dystonia, glabellar lines	Blepharospasm, cervical dystonia, glabellar lines, hyperhidrosis	Blepharospam
FDA APPROVAL	**Botox®:** cervical dystonia, severe primary axillary hyperhidrosis, blepharo-spasm, neurogenic detrusor overactivity (urinary incontinence), chronic migraine, upper limb spasticity **Botox Cosmetic®:** glabellar lines (moderate to severe)	Cervical dystonia, glabellar lines (moderate to severe)	None	None
MODE OF ACTION	SNAP-25	SNAP-25	SNAP-25	SNAP-25
PHARMACEUTICAL FORM	Powder	Powder	Powder	Powder
UNITS/VIAL	100 or 200	300 or 500	50 or 100	100
VOLUME	1.25 or 2.5mL, 10mL max	2.5mL to 5mL max		
RECONSTITUTION	0.9% NaCl	0.9% NaCl		
EXCIPIENTS	Human serum albumin, NaCl	Human serum albumin, lac-tose	Gelatin, dextran, sucrose	Human serum albumin, NaCl
STORAGE BEFORE DILUTION	2–8°C or < -5°C	2–8°C	2–8°C	2–8°C
STORAGE AFTER DILUTION	24 hours/2–8°C	4 hours/2–8°C	4 hours/2–8°C	4 hours/2–8°C

TABLE 1 continued. Comparison of botulinum toxin preparations[5]

	INCOBOTULINUMTOXINA	TBD	RIMABOTULINUMTOXINB
COMMERCIAL NAMES	Xeomin®	PurTox®	MyoBloc®, NeuroBloc®
COMPANY	Merz Pharmaceuticals	Mentor Worldwide LLC	Solstice Neurosciences Inc.
TYPE	A	A	B
COUNTRIES	United States since 2011, Germany, other European countries, Mexico, Argentina, Brazil	Pending in the United States (Phase 3 trials completed)	United States, some European countries
ACTIVE SUBSTANCE (MOLECULAR WEIGHT)	BTX-A complex (150 kD); NO complexing proteins	BTX-A complex (150 kD); NO complexing proteins	BTX-B complex (700 kD)
STRENGTH (BTX-A:PRODUCT)	1:1	1:1.5	1:50–1:100
INDICATIONS	Blepharospasm, cervical dystonia, cosmetic use in some countries including glabellar lines in the United States	TBD; currently in Phase 3 trials	Cervical dystonia
FDA APPROVAL	Cervical dystonia, blepharospasm	Pending	Cervical dystonia
MODE OF ACTION	SNAP-25	SNAP-25	SNAP-25
PHARMACEUTICAL FORM	Powder	Powder	
UNITS/VIAL	50 or 100		2,500; 5,000; 10,000
VOLUME	8mL		0.5mL; 1mL; 2mL
RECONSTITUTION	0.9% NaCl	0.9% NaCl	Not necessary
EXCIPIENTS	Human serum albumin, sucrose		Human serum albumin, NaCl, disodium succinate, water
STORAGE BEFORE DILUTION	2–8°C	< 25°C	
STORAGE AFTER DILUTION	24 hours/2–8°C		4 hours (if diluted)/2–8°C

of activity of BTX-A preparations in different situations. Most manufacturers recommend that botulinum toxins be reconstituted with unpreserved saline. RimabotulinumtoxinB (MyoBloc®/NeuroBloc®) does not require reconstitution as the manufacturer provides it as a ready-to-use liquid.

Preserved saline. Multiple studies have shown that reconstitution of onabotulinumtoxinA (Botox®) with preserved saline (containing benzyl alcohol) does not affect the potency of the neurotoxin.[42,43] In fact, the injections were less painful.[44] A consensus panel in 2004 stated that preserved saline is the preferred method of reconstitution for Botox®.[25] A separate panel recognized that benzyl alcohol acts as a local anesthetic, albeit negligibly, at low volumes.[45]

Lidocaine with epinephrine. Multiple studies have shown that Botox® reconstituted with lidocaine containing epinephrine (lidocaine 1% or 2% and epinephrine 1:100,000 or 1:200,000) retained its function without a compromise in efficacy or safety.[46–49] However, there is a case report in the literature of a fatal anaphylactic reaction in a patient treated with Botox® for chronic neck and back pain reconstituted in lidocaine containing epinephrine.[50] Even though the exact cause of anaphylaxis remains unknown to date, caution should be exercised when reconstituting in lidocaine as some patients may exhibit a type-1 immediate hypersensitivity reaction to this substance. The advantage of using lidocaine with epinephrine for reconstitution of botulinum toxin lies in enhancing its short-term efficacy, accelerating the rate of onset, and reducing patient discomfort associated with injections.

Bupivacaine. Reconstitution of Botox® with bupivacaine reduces injection pain without compromising its efficacy and safety. Yen et al[51] conducted a randomized, double-blind study where Botox® reconstituted with 0.75% bupivacaine was injected into one corrugator muscle, where the contralateral corrugator was treated using Botox® reconstituted with unpreserved saline. At one week, the side treated with bupivacaine-reconstituted Botox® showed greater muscle weakness than the control side. However, at one and three months follow-ups, no differences were noted between the two sides. The faster onset of action may be attributed to a synergistic effect between Botox® and bupivacaine-induced myotoxicity.[51]

Sterile water. Limited literature is available on Botox® reconstitution with sterile water. Moore and Naumann[52] report that Botox® is effective when reconstituted in sterile water, but associated with short-lived, intense pain at the injection site.[52]

DILUTION

The package insert of onabotulinumtoxinA (Botox®) recommends dilution of 100 units in 1 to 8mL of saline (12.5 to 100 U/mL). Three hundred units of abobotulinumtoxinA (Dysport®) may be diluted in 0.6 to 2.5mL of saline (120 to 500 U/mL). Many physicians believe that the higher the dilution of botulinum toxin, the greater the chance of diffusion to unwanted sites and the shorter the duration of effects, thereby leading to suboptimal results.[34] Large volume injections result in greater diffusion and larger affected areas.[53]

IMMUNOGENICITY/ALLERGY/RESISTANCE

Antibody formation may occur in response to botulinum toxin injections. As the protein load in current preparations is lower than in earlier formulations (prior to 1998), immunological responses with antibody formation have decreased. Nonetheless, immunogenic responses may still occur in the modern formulations, particularly when large doses of botulinum toxin are utilized (most frequently in therapeutic, noncosmetic applications).[54] The overall risk of antibody formation may be minimized by using low doses with the longest feasible interval between injections.

Allergic reactions, while possible, are exceedingly rare but have been reported in the literature. Allergies may range from non-serious skin rashes over more serious skin rashes and granuloma formation to localized or even systemic anaphylactic reactions. Whether the anaphylactic reaction is from the neurotoxin itself or its reconstitution solvent is unknown.[50,55,56]

SAFETY/COMPLICATIONS

Upper face. Most adverse effects of cosmetic applications of botulinum toxin are mild and transient. The majority of adverse effects include bruising, edema, or pain at the injection site, and possibly flu-like symptoms. More serious adverse effects include brow or eyelid ptosis and diplopia from extraocular muscle weakness. Those more serious side effects are secondary to poor injection technique, improper needle placement, and lack of experience and/or understanding of the underlying anatomy and physiology, thus allowing the neurotoxin to either diffuse or be inadvertently injected into adjacent musculature.[57,58]

As botulinum toxin works through temporary chemodenervation of muscles, complications may be diminished by using more concentrated doses, which allow for more precise placement of toxin through less diffusion with a greater duration of effect.

Brow and eyelid ptosis through spread of neurotoxin into adjacent musculature are the most troublesome complications in the upper face and may last up to three months. The risk of brow ptosis (through diffusion into the frontalis muscle) may be reduced by avoiding treating patients with preexisting brow ptosis or using a very conservative dose in the frontalis muscle.

Eyelid ptosis may occur when the neurotoxin is injected into the glabella (procerus and corrugator muscles) with inadvertent diffusion into the levator orbicularis muscle. However, it is more likely from unmasking of a preexisting ptosis that becomes increasingly more evident following relaxation of the brow elevators. Higher concentrations, careful toxin placement (1cm above the superior orbital rim and 1.5cm lateral to the lateral canthus), and advising the patient to refrain from manipulating the treated area for a few hours after treatment help diminish the risk of eyelid ptosis.[33] Additionally, a thorough exam with particular attention to the eyelid position and function may prevent a dissatisfying outcome.

Other complications include headache, infection, cocked eyebrow, diplopia, ectropion, decreased strength of eye closure, and xerophthalmia.[58]

Lower face and neck. In the lower face and neck, adverse effects are usually from overzealous delivery of large neurotoxin doses. Dysphagia, dysarthria, inability to purse lips, oral incompetence, and smile asymmetry may ensue.[58] Small doses of BTX-A injected superficially and symmetrically may decrease the risk and visibility of the aforementioned complications. Neurotoxin injections too close to the mouth, directly into the mental crease, or into the orbicularis oris muscle are more likely to yield adverse effects. In contrast to BTX-A use in the upper face, administration in the lower face and neck is less forgiving of imprecise technique and dosing that may lead to adverse effects. The lower face cannot tolerate even a few misplaced units, which most commonly affect the smile pattern in an undesirable way.[4] Fortunately, all these side effects are reversible once the neurotoxin wears off after several months.

SUMMARY

Botulinum toxins have been used therapeutically for more than 30 years with very few adverse effects when used appropriately and with proper training. There are many different preparations of botulinum toxins worldwide, which are neither identical nor interchangeable. Results with one product cannot be extrapolated to another. Manufacturer recommendations on all available botulinum neurotoxins advise the use of unpreserved saline for reconstitution. However, literature reports show that preserved saline or anesthetics (bupivacaine or lidocaine with epinephrine) may be used safely instead.

Botox® (onabotulinumtoxinA) is the most commonly used botulinum toxin. It appears to be less fragile and more stable than initially thought. While it is wise to follow the manufacturers' recommendations, some of them seem somewhat excessive. In cosmetic applications, BTX-A was initially used for treatment of upper facial rhytides, but is now considered a key component of facial rejuvenation procedures as an adjunct to either nonsurgical or surgical interventions, including in the lower face and neck. Smaller doses for perioral treatment than for upper facial applications are used as the oral musculature responds more strongly to the same BTX-A dosing. Only one preparation of botulinum toxin type B (MyoBloc®/NeuroBloc®) exists. Its sole indication and FDA approval lies in treatments of cervical dystonia.

Side effects are mostly mild and always self-limited. More serious complications are associated with higher doses, improper injection techniques, and occur in patients with underlying (mostly cardiovascular or neurological) comorbidities. Complications may be minimized by site- and patient-specific dosing.

An understanding of functional facial anatomy, the importance of precise injections, correct dosing, and familiarity with the neurotoxin and its indications are critical in providing safe treatment with optimized patient outcomes.

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