Neurotoxin Update and Review, Part 1: The Science

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In the 2 decades since the approval of botulinum toxin for medical use in the United States, neurotoxin for facial enhancement has become a standard of care for the treatment of dynamic wrinkles. Transient paralysis of the musculature underlying facial rhytides can reduce the appearance of wrinkles. In recent years, refinement of technique and combination therapy with other cosmetic modalities such as lasers and filling agents has allowed practitioners to create a more natural appearance, rather than one that is expressionless. As more botulinum toxin products become commercially available, it is clear that differences exist and the nuances of dosing and injection pattern continue to be elucidated. Part 1 of this series will focus on the science of the currently available neurotoxin products. Part 2 will outline best practices for their use in aesthetic dermatology.

The use of neurotoxins for aesthetic medicine has become the most common minimally-invasive cosmetic procedure according to the American Society of Plastic Surgeons 2008 National Clearinghouse statistics.[1] OnabotulinumtoxinA (Botox Cosmetic) is the most extensively used and studied neurotoxin and has been used off-label for aesthetic use since the US Food and Drug Administration (FDA) approved it in 1989 for neurologic indications until its approval for glabellar lines in April 2002. Botox Cosmetic exerts its effect by temporarily paralyzing hyperdynamic muscles underlying facial rhytides. The safety and efficacy of Botox Cosmetic has been established both in clinical trials and practitioner experience, although injection technique, dilution and dosing, product storage, and avoidance and management of potential complications, may differ among those experienced with its use. Currently, several other neurotoxin products are available worldwide, with only a few studied for cosmetic applications and only one other product approved for facial aesthetics in the United States (Table 1).

AbobotulinumtoxinA (Dysport) is another botulinum toxin A product now approved in the United States for facial cosmesis (April 2009). RimabotulinumtoxinB (Myobloc), a botulinum toxin B product, is only approved in the United States for cervical dystonia (December 2000).[2,3] Myobloc has been used off-label in facial aesthetics and for hyperhidrosis[4-9]; however, its shorter duration of action, potential for higher diffusion rates, and increased risk of autonomic adverse effects at the required higher dosing units have limited its use in facial aesthetic medication so it will not be discussed further in this article.[10-13] IncobotulinumtoxinA (Xeomin), another botulinum toxin A was recently FDA approved for cervical dystonia and blepharospasm. It is anectdotally reported to behave similarly to onabotulinumtoxinA clinically. Off-label

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Table 1

Major Commercially Available Neurotoxins

	OnabotulinumtoxinA/ Botox Cosmetic	AbobotulinumtoxinA/ Dysport/Azzalure	IncobotulinumtoxinA/ Xeomin/Bocouture
Manufacturer or distributor	Allergan Inc, Irvine, CA	Ipsen LTD, UK; Medicis Aesthetics, Scottsdale, AZ	Merz Pharmaceutical, Germany
Bacteria strain	Hall strain	Ipsen strain	Hall strain
Molecular weight	900 kD	900 kD	150 kD
Composition	100 U BTX-A	300 U and 500 U BTX-A	100 U BTX-A
	0.5 mg HSA	0.125 mg HSA	1 mg HSA
	0.9 mg NaCl	2.5 mg lactose	5 mg sucrose
Complexing protein	5 ng/100 U	12.5 ng/500 U	None
Approval regions	USA, Canada, South America, Europe, Asia	USA, Canada, South America, Europe, Asia	Canada, South America, Europe, Asia; USA[a]

Abbreviations: BTX-A, botulinum toxin A; HSA, human serum albumin.
[a]FDA approval in USA August 2010 for noncosmetic medical usage.

use for cosmetic purposes is anticipated but will not be discussed here because it is not yet available at the time of writing. Differences in dosing and practitioner experience challenge comparisons between Botox Cosmetic and Dysport. Attempts to compare their clinical and pharmacological differences have shortcomings due to the lack of clear dosage equivalency formula. Nonetheless, clinical experience for the approved and off-label indications will reveal characteristics of each treatment that may refine therapeutic choices.

BRIEF HISTORY AND PATHOPHYSIOLOGY OF NEUROTOXINS

Botulinum toxins are produced by *Clostridium botulinum*, gram-positive anaerobic bacteria that works through multiple mechanisms to block the release of acetylcholine from the presynaptic terminal of the neuromuscular junction.[14,15] Botulinum toxins are similar in amino acid size and molecular weight (toxin type A dimers approximately 900 kD and toxin type B dimers approximately 700 kD) and are synthesized as a complex which is inactive until it is cleaved by bacterially produced proteases.[16,17] All active botulinum toxins are comprised of 2 chains, one heavy chain (molecular weight of 100 kD) and one light chain (molecular weight of 50 kD) joined by a disulfide bond.[18,19] The integrity of the disulfide bond is essential for the toxin's biological activity, making it fragile to various environmental influences. Seven distinct antigenic toxins (A, B, C, D, E, F, and G) are produced by different strains of the bacterium with only 5 being communicable to the human nervous system (A, B, E, F, and G).[20]

Although several subtypes have potential therapeutic benefits, only commercial preparations of toxins types A and B are approved by the FDA for use in humans. When injected into muscle, the toxin inhibits the release of acetylcholine at the neuromuscular junction, causing local paralysis in an area of functional denervation within a few days to a week. Botulinum toxin can also block the cholinergic autonomic innervations of the sweat glands, the tear ducts, the salivary glands, and smooth muscles. Clinical effects are dose related and transient and usually diminish several months after the natural regeneration of new nerve terminals

at the treated site.[21-23] Botulinum toxin is more effective in blocking neuromuscular junctions when the target muscle is active, whether this is produced by self-made movements or electrical stimulation.[24,25]

The release of acetylcholine at the neuromuscular junction requires the assembly of a set of soluble N-ethylmaleimide-sensitive fusion attachment receptor (SNARE) proteins at the neuronal cell membrane.[20,26] The 3 steps involved in neurotoxicity and subsequent paralysis are: (1) the botulinum toxin binding irreversibly to the presynaptic cholinergic receptors; (2) the neurotoxin is internalized via receptor-mediated endocytosis; and (3) the neurotoxin cleaves synaptosomal-associated membrane protein (SNAP-25) along with vesicle-associated membrane protein (VAMP or synaptobrevin) and syntaxin to inhibit docking, fusion, and release of acetylcholine.[18,27] The SNARE proteins are targeted by different neurotoxins: botulinum toxins A and E cleave SNAP-25; botulinum toxins B, D, F, and G cleave VAMP or synaptobrevin; and botulinum toxin C cleaves both SNAP-25 and syntaxin.[28,29]

Potency units of the commercially available neurotoxins are not interchangeable. The unit potency of these toxins is assessed with mouse intraperitoneal injection assays and expressed in units of activity with 1 U defined as the dose that produces death within 72 hours in 50% of Swiss-Webster mice assessed (LD_{50}).[30] Although this definition applies to all forms of commercially available botulinum toxin, mouse LD_{50} assay protocols vary among manufacturers.[31-33] In the literature the approximate unit equivalencies between botulinum A neurotoxins are: 1 U Botox Cosmetic equals 2 to 5 U Dysport.[2,3,22,34,35] Animal studies suggest the longest duration of action is botulinum toxin A, followed by B, F, and E, with clinical efficacy, safety, and adverse effects (such as unwanted diffusion) related to protein composition, differing dilutions, volume, target muscle selection, and injection technique.[29] The lethal dose of botulinum toxin A is between 2500 and 3000 U for a 70 kg person, which is much higher than the typical dosage used for cosmetic applications.[36]

In the 1970s and early 1980s, human trials began to be conducted using minute doses of botulinum toxin A to selectively inactivate muscle spasticity in strabismus.[37-39] Botulinum toxin A was found to be safe and effective without any notable local or systemic adverse effects leading to the approval in 1989 for ophthalmologic and neurologic use (strabismus, blepharospasm, and hemifacial spasm). Since then, botulinum toxin A has been used to decrease muscle activity in a variety of other conditions including dystonias, involuntary muscle activity, and spasticity[40] (Table 2).

Improvement of facial wrinkles inadvertently was observed when treating hemifacial spasm and blepharospasm.[41-43] When patients pointed out they were unable to frown as much as they had before treatment, systematic studies of botulinum toxin A for glabellar lines were performed with the first reports published in the 1990s.[42,44,45] In 2001, the results of a large, randomized, placebo-controlled trial revealed that botulinum toxin A was remarkably safe and effective for the treatment of facial rhytides.[46] In this trial, 264 participants with moderate to severe glabellar lines at maximum frown received 20 U of botulinum toxin A or placebo into 5 injection sites and were followed for 120 days after injection.[46] Participants who received botulinum toxin A experienced a significantly greater reduction in glabellar line severity than participants receiving placebo. Clinical results lasted 3 to 6 months and were greater in magnitude and duration in participants less than 50 years of age.[46]

US Food and Drug Administration approval of Botox Cosmetic in 2002 led to subsequent clinical studies that popularized botulinum toxin A as a safe and effective treatment for a multitude of facial aesthetic conditions. The approval of Dysport for cervical dystonia and glabellar lines in the last year provided the first practical alternative to Botox Cosmetic. Transitioning between neurotoxins requires an understanding of differences in dosing, preparation, storage, and immunogenicity.

TABLE 2

Common Clinical Uses of Botulinum Toxins

Approved	Not Approved
Aesthetic – glabellar lines	Aesthetic – all other areas
Severe primary axillary hyperhidrosis	Palmar/plantar hyperhidrosis
Cervical dystonia	Migraine headache
Blepharospasm	Back pain
Strabismus	Achalasia
Upper limb spasticity	Spastic bladder

PREPARATION OF NEUROTOXINS

OnabotulinumtoxinA, Botox Cosmetic

Each vial of Botox Cosmetic contains either 50 or 100 U of the Hall strain of *Clostridium botulinum* type A neurotoxin complex with 0.25 or 0.5 mg of human serum albumin and 0.45 or 0.9 mg of sodium chloride at an optimal pH of 4.2 to 6.8 in a vacuum-dried form without a preservative.[47] Botox Cosmetic is indicated for the temporary improvement in the appearance of moderate to severe glabellar lines associated with corrugator and/or procerus muscle activity in patients aged 65 years or younger.[48] The initial recommendation to freeze unopened vials (−5°C or colder) has been amended to allow storage in a refrigerator (2°C–8°C) for 24 or 36 months for the 50- and 100-U vials, respectively. The package insert recommends dilution with sterile preservative-free saline 0.9%; however, use of preserved isotonic saline reduces patient discomfort without sacrificing efficacy and has become standard of care.[49] It also recommends reconstituted product be refrigerated and used within 24 hours; however, it has been shown that Botox Cosmetic retains its efficacy for at least 4 to 6 weeks following reconstitution if stored at 4°C.[50,51] Most practitioners use between 1 and 3 mL of saline to reconstitute for aesthetic use.[52] A 5 U/0.1 mL preparation is prepared by 2 mL of preservative-free saline into the 100-U vial.[52,53] Consensus recommendations and the Botox Cosmetic package insert validate 100 U/2.5 ml equals 4 U/0.1 mL as the most versatile concentration.[54] Despite initial concerns about toxin stability, no reduction in efficacy has been found when the vial contents are shaken and foamed during reconstitution.[55] Reconstituted Botox Cosmetic should be clear, colorless, and free of particulate matter and should never be refrozen.

Clinical effect is determined by the number of units of toxin injected as well as the concentration.[56-58] In a dose-dilution study in which a total dose of 30 U of Botox Cosmetic was reconstituted in 1-, 3-, 5-, or 10-mL saline, no differences in safety or efficacy were seen among groups in treating glabellar lines.[59] Dosage is increased to correlate with the size of the treated muscle. For example, adult men often require up to double the dose of adult women for the same anatomic area. In general, higher concentrations mean lower injection volumes, less pain and edema, and more precise placement of product. Delivery of the same number of units in a lower concentration requires a higher volume and therefore has the potential for increased pain and a larger field of effect. This can be utilized to an advantage or may lead to unpredictable side effects nearby or far from injection placement.

AbobotulinumtoxinA, Dysport

Dysport is supplied in 300-U vials for glabellar lines and 500-U vials for cervical dystonia. Each 300-U vial contains lyophilized (vacuum-dried) abobotulinumtoxinA, 0.125 mg of human serum albumin, and 2.5 mg lactose. Like Botox Cosmetic, Dysport is approved in the United States for the temporary improvement of glabellar rhytides, is vacuum dried, and must be reconstituted before injection.[60] Since Dysport is produced by column-based purification rather than by the precipitation technique used for Botox Cosmetic, it may be stored at room temperature until reconstituted even though the package insert recommends refrigeration storage prior to reconstitution.[20] The package insert recommends refrigeration of reconstituted product at 2°C to 8°C and administration within 4 hours; however, stability studies[61] confirm at least 15 days and extrapolation from Botox Cosmetic data suggests a 4- to 6-week window of efficacy.

For the treatment of glabellar lines, reconstitution of Dysport with 1.5 mL (10 U/0.05 mL) or 2.5 mL (10 U/0.08 mL) of saline is recommended by the manufacturer. As detailed previously, the potency units of Dysport are specific to the preparation and assay method utilized. Units of biological activity of one toxin cannot be precisely converted into units of any other botulinum toxin product assessed with other specific assay methods.[22] However, in practice, most physicians assume a ratio of 2 to 3 U Dysport to 1 U Botox Cosmetic.[62] The results of a recent consensus group conference suggested a "rule of 10s" be used; that 10 U of Dysport be injected at sites where 4 to 5 U of Botox Cosmetic would be used.[63] In the authors' experience a 2 U Dysport to 1 U Botox Cosmetic ratio using a 3.0 mL dilution (300 U/3.0 mL equals 10 U/0.1 mL) gives an easily manageable unit conversion for the practitioner used to Botox Cosmetic and the volumes used will be the same.

Relative contraindications, warnings, and precautions for all neuromuscular blocking agents include: patients with preexisting neuromuscular disorders; certain medications that can interfere with neuromuscular transmission such as aminoglycosides, muscle relaxants, or anticholinergic drugs; pregnancy and lactation; patients aged 65 years or older (geriatric use); patients with surgical alterations to the facial anatomy; marked facial asymmetry; inflammation or infection at the proposed injection site(s); ptosis; excessive dermatochalasis; deep dermal scarring; and hypersensitivity to any of the toxin preparation or components in the formulation. Dysport is specifically contraindicated in patients with a milk allergy, but is safe in those with the much more common condition of lactose intolerance.

CONCLUSION

Remarkable additions recently have become part of the armamentarium of treatment options for dynamic facial wrinkles. Major differences between the neurotoxins are due to the pharmacology and formulation and may be responsible for differences in diffusion capability, onset of action, efficacy, and safety. Although precise dosing conversion and injection point locations have yet to be determined, it appears each neurotoxin is safe and effective with lasting results. Published results confirm that botulinum toxin type A has a slower onset of action, is longer-lasting, and less painful on injection when compared to botulinum toxin type B.[4,10-13] Additional clinical trials are needed to effectively determine the differences among commercial toxins, especially as new agents reach the US market. In Part 2 of this series, treatment parameters for the currently approved botulinum toxins will be outlined to maximize patient satisfaction.

This article is the first of a 2-part series. The second part on best practices for neurotoxin use in aesthetic dermatology will appear in a future issue of Cosmetic Dermatology®.

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