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Botulinum neurotoxin type A in facial aesthetics

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Injection of botulinum neurotoxin type A has rapidly become the most common non-surgical cosmetic procedure performed, due to its exceptional safety profile, as well as its ability to rejuvenate and enhance a number of facial areas. There are several marketed botulinum neurotoxin preparations, but products are not interchangeable as each possesses distinctive characteristics that are attributed to the unique toxin purification and manufacturing processes. These differences can emerge in the form of potency, duration of effect and the potential for migration outside targeted tissue, causing unwanted effects. However, although there are established preclinical pharmacologic and therapeutic differences between products, there are few well-controlled clinical comparisons in facial aesthetics. It is important for clinicians using these products to understand these differences as they relate to achieving desired outcomes for patients who seek improved facial aesthetics.

Keywords: botulinum neurotoxin type A, botulinum neurotoxin type B, cosmetic, facial aesthetics, wrinkle

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1. Introduction

Since the effectiveness of botulinum neurotoxin type A (BoNTA) in reducing glabellar frown lines was first reported in the medical literature in 1992 [1], BoNTA has revolutionized non-surgical and surgical approaches to facial aesthetics. In the US alone, BoNTA has become the most common non-surgical cosmetic procedure, with 3.2 million procedures performed in 2005 [101]. Such use has increased by 4700% since 1997, more than that of any other non-surgical cosmetic procedure (**Figure 1**) [101]. These data are particularly noteworthy when considering that the increase in blepharoplasty, a permanent alternative to BoNTA to enhance the eyes, has been negligible (32%) during that same time period [101]. Although the use of BoNTA to improve facial aesthetics first began in the upper face to reduce dynamic wrinkling, such as frown lines and crow's feet, its use has expanded considerably to include the mid-face as well as lower face and neck, not only simply for dynamic wrinkle reduction in these areas, but for a more overall extensive role in facial aesthetics [2].

There is more than one botulinum neurotoxin (BoNT) preparation that is available (see Section 3); however, they are not interchangeable, as each product possesses unique characteristics, such as molecular size and structure, that are likely to result from differing toxin purification and manufacturing processes. This paper provides an extensive review of BoNTs for aesthetics use, including the similarities and differences among them. Some preclinical and therapeutic trial data are also presented to provide a further understanding of pharmacologic differences among products.

2. Mechanism of action

BoNTs are derived from the bacteria *Clostridium botulinum*. There are seven distinct serotypes of BoNTs that have been identified (A, B, C1, D, F, E and G). Only serotypes A and B have established clinical use, partly due to their longer duration of action compared with some of the other serotypes [3,4]. All of the BoNTs are

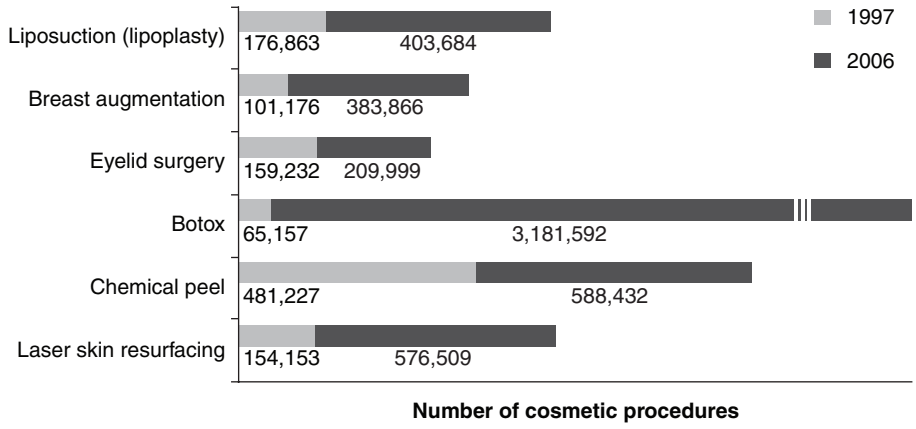

Figure 1. Common surgical and non-surgical cosmetic procedures performed in the US: 10-year comparison, 1997 – 2006.
Reprinted with permission from the American Society for Aesthetic Plastic Surgery [101].

synthesized as ~ 150-kDa single chain polypeptides, which are non-covalently bound to non-toxin hemagglutinins, which impart stability and thereby prevent degradation, to form protein complexes [5]. During the fermentation process, BoNTA forms complexes that range in size from 300 to 900 kDa [5,6]. It has been suggested that the size and structure of the toxin complex plays a role in the ability of the toxin to migrate away from its site of injection to other non-targeted tissues, with larger complexes being less likely to migrate [7-9].

BoNTA enters the neuron by binding to the synaptic vesicle protein SV2 [10], whereas botulinum neurotoxin type B (BoNTB) binds to synaptotagmin I and -II [11]. To gain maximum biologic activity, the 150-kDa toxin is proteolytically cleaved into a 50-kDa light chain and a 100-kDa heavy chain. The heavy chain mediates neurospecific binding and transport of the light chain across the endosomal membrane into the cytosol. Once internalized, the light chain is translocated into the cytoplasm of the motor neuron and selectively cleaves one or more soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins, which are necessary for acetylcholine exocytosis. BoNTA specifically cleaves synaptosomal-associated proteins of 25 kDa (SNAP-25), whereas BoNTB cleaves synaptobrevin [5]. As a result, the affected cholinergic terminals are inhibited from releasing acetylcholine. This induces temporary denervation and relaxation of injected muscles, although the storage and synthesis of acetylcholine are unaffected. When the BoNT is injected into the small muscle of the face, a reduction in muscle tone occurs, with a subsequent reduction in furrows and lines caused by dynamic muscular activity and surrounding tissue bulk.

3. Available botulinum neurotoxin products

Given the wide range of conditions in which muscle contractility plays a pivotal role, BoNTs have been approved for a variety of therapeutic uses: these include blepharospasm, cervical dystonia, dynamic equinus foot deformity in children with cerebral palsy, focal spasticity, hemifacial spasm, hyperhidrosis and strabismus [12,13]. The effectiveness of BoNTA is also being evaluated in other conditions, including detrusor overactivity, benign prostatic hyperplasia and pain (including migraine headache).

Table 1 lists the BoNT products that are widely available internationally. Not all products are approved for cosmetic use. Botox® (BoNTA; also known as Botox Cosmetic®, Vistabel® and Vistabex®; Allergan, Inc.) is the most commonly used BoNTA product worldwide for facial aesthetics. In the US, Botox has been approved for glabellar lines since 2002 and it is approved in Canada for multiple facial lines. Approval is being sought for Dysport® (BoNTA; Ipsen, Ltd.) for glabellar lines in the US under the brand name Reloxin®. BoNTB (Myobloc®; Neurobloc®; Solstice Neurosciences, Inc.) [14] is used for aesthetic purposes, but to a much lesser degree than BoNTA.

Other BoNTA products are available in select geographic regions. Xeomin® (Merz Pharmaceuticals) comprises only the naked 150-kDa toxin with no non-toxin associated proteins. It is approved for cervical dystonia and blepharospasm in Germany only and there is a Phase III trial for glabellar lines underway in Germany. Neuronox® (Medy-Tox, Inc.) is approved in Korea and is available in North Africa, some Eastern European countries, Russia and Mexico. Chinese BoNTA, also referred to as CBTX-A (Lanzhou Institute of Biological Products), is approved for use in some Asian countries and in certain countries in South America under the name Prosigne®, although not for cosmetic applications. At the time of writing, no studies on the aesthetic use of these products have been published in the medical literature.

3.1 Uniqueness of products

BoNTs are biologic products, derived from different strains of *C. botulinum* using varying proprietary methods of isolation, purification and final manufacturing steps [15-17]. Products are of different concentration, toxin complex size, neurotoxin protein content and sometimes serotype (in the case of BoNTA versus

Table 1. Characteristics of widely approved BoNT preparations.

Characteristic	Botox®, Botox Cosmetic®, Vistabel®, Vistabex®	Dysport®, Reloxin®	Myobloc®, Neurobloc®
Manufacturer	Allergan, Inc.	Ipsen, Ltd.	Solstice Neurosciences, Inc.
BoNT serotype	A	A	B
Where approved for cosmetic use (glabellar lines)	47 countries worldwide (the Americas, Europe, Asia, New Zealand); approved for multiple facial lines in Canada	17 countries (including Argentina, Brazil, Columbia, Germany, Honduras, Mexico, New Zealand, Russia, Uruguay, Ukraine, Vietnam; seeking approval in the US)	Not approved for cosmetic use; approved for cervical dystonia worldwide (North and South America, Europe, New Zealand)
Toxin complex size (kDa)	900	Various (500 – 900)	700
How supplied	Vacuum-dried powder	Lyophilized powder	Solution
Concentration per vial	100 U	500 U	2500, 5000 or 10,000 U
Neurotoxin protein content (ng/vial)	5	≤ 12.5	38 – 71

Information from [12,14-18].
BoNT: Botulinum neurotoxin.

BoNTB; **Table 1**) [15,17,18]. For example, following final manufacturing, a vial of Botox contains 100 median lethal dose (LD_{50}; causing lethality in 50% of a population of mice) units and Dysport contains 500 LD_{50} units. The LD_{50} defines the unit of activity for both Botox and Dysport. Interestingly, there is no parity between 1 U of Botox and 1 U of Dysport. Indeed, there is no international reference standard for BoNT LD_{50} units of activity, so doses are neither equivalent nor standardized among preparations [19]. In general, the literature reports that Dysport 3 – 5 U produces a similar therapeutic effect as Botox 1 U [20,21]. Because of significant differences among BoNT products both in preparation and in clinical outcomes (Sections 4.2, 4.3 and 5.1), they are not considered interchangeable.

Factors that influence the clinical utility of the various BoNTs include clinical efficacy, such as: duration of effect; adverse event (AE) and tolerability profile; and cost. Clinical studies have shown that at doses that produce comparable degrees of muscle weakening, preparations of BoNT can vary in their duration of effect [22-27] and AE profiles [21,28]. For example, in a randomized, crossover trial [28] in which 212 patients with essential blepharospasm received one treatment with double-blind Dysport (mean dose: 182 U) and one with Botox (mean dose: 45 U), the mean duration of effect was similar for both treatments (7.98 weeks with Botox, 8.03 weeks with Dysport; $p = 0.42$). However, AEs occurred significantly more frequently with Dysport, with 51 out of 212 patients (24%) reporting AEs versus 36 out of 212 patients (17%) with Botox ($p < 0.05$). Similarly, in a Cochrane systematic review of randomized, controlled trials comparing different formulations of BoNT for cervical dystonia [21], a 1:3 Botox:Dysport ratio conferred comparable clinical efficacy and duration of effect, but a higher percentage of AEs (most notably, dysphagia) was observed with Dysport compared with Botox (see Sections 4.2, 4.3 and 5.1 for more detail on differences among products in the aesthetic clinical setting).

An electrophysiologic evaluation was conducted, in which 60 healthy subjects received injections of Botox (10, 15 or 20 U) or Dysport (50, 75 or 100 U) in the frontalis muscle at constant volumes [29]. The compound muscle action potential of the frontalis was measured immediately following and 7, 30, 60 and 90 days after injection. The two products exhibited different patterns of denervation and reinnervation over time, producing different dose ratios at different time points, confirming that the products cannot be easily converted to obtain equivalent results.

There have been fewer published studies on clinical comparisons with other BoNTA products such as CBTX-A (Prosigne) and Xeomin. Only two studies appear in the published English language literature regarding the efficacy and safety of CBTX-A. One of these studies [30] was a retrospective study that compared Botox with CBTX-A at a 1:1.5 ratio in 785 patients with focal dystonias. A more recent study in 2007 compared a 1:1 Botox:Prosigne ratio in 26 patients with blepharospasm and hemifacial spasm and observed comparable duration of effect and AE rates [31].

In a study in 14 healthy volunteers, the paralytic effect of Xeomin 4 U was comparable to Botox 4 U after injection into the extensor digitorum brevis [32]. In a comparative Phase III study in 304 patients with blepharospasm, Xeomin and Botox exhibited similar efficacy and safety over the evaluation period of 112 days at mean doses of 39.6 U and 40.8 U, respectively [33]. Ptosis was the most commonly reported AE, occurring in 6.1 and 4.5% of patients receiving Xeomin and Botox, respectively. In a trial of 463 patients with cervical dystonia, non-inferiority with Xeomin was observed relative to Botox at a 1:1 dose over the 16-week trial [34].

3.1.1 Migration profiles

Regardless of clinical application, once injected into a muscle, BoNT distributes to produce the desired local muscle-weakening effect. The clinical use of a BoNT product is dependent on its ability to act locally in the targeted (injected) muscle for an extended period of time. Migration of toxin beyond the target tissue is generally not desired, as it can lead to unintended muscle weakening or leakage into the systemic circulation, thereby causing AEs. For example, in the cosmetic treatment of glabellar lines, migration of BoNT out of the injected corrugator or procerus muscle can lead to blepharoptosis, which is believed to result from the migration of BoNT through the orbital septum to the levator muscle of the upper eyelid [35]. Given the unique safety and efficacy profiles observed among various BoNT products in the therapeutic setting, scientists have sought to evaluate the pharmacologic preclinical differences among agents in an attempt to predict clinical outcomes. Most of this work has involved the three most widely used BoNTs (Botox, Dysport and Myobloc).

3.1.1.1 Preclinical observations

Preclinical dose–response experiments have attempted to compare the pharmacologic properties among BoNTs using the mouse hind limb or digit abduction score (DAS) assay to determine local muscle weakening [36-39]. Mice are suspended briefly by the tail to elicit a startle response, characterized by extension of the hind limbs and splaying (i.e., abduction) of the hind digits. Following intramuscular injection with various doses of BoNT into the gastrocnemius muscle, the DAS is measured on a 5-point scale (0 = normal response to 4 = maximum reduction in digit abduction). The median effective dose of muscle weakening (intramuscular ED_{50}), a measure of clinically relevant potency, is defined as the dose of BoNT that produced 50% of its maximal weakening effect; equal to a DAS of 2 [8,39]. The intramuscular ED_{50} and intramuscular LD_{50} can also determine the safety margin (e.g., how much toxin can be injected before it spreads from the injected muscle to the systemic circulation). Therapeutic margin, defined as the ratio of 'threshold dose' (the lowest dose showing toxin spread by causing weakening of local, non-targeted muscles) to ED_{50}, is an index of the propensity of BoNT to migrate outside of the injected muscle (e.g., how much toxin can be injected before it spreads from the injected muscle) [8]. Estimates of these values from DAS experiments are shown in **Table 2**. These results demonstrate distinct pharmacologic profiles among products in terms of potency and potential for local and systemic migration and underscore the notion that these BoNTs are not interchangeable, nor may they be converted.

3.1.1.2 Clinical evaluations of migration in the dermatologic setting

A recent 2007 study compared the migration characteristics of Botox and Dysport in patients with forehead hyperhidrosis in an attempt to confirm clinically, in a design relevant to cosmetic applications, the differences in migration patterns between these agents [40]. A total of 20 patients received Botox 6 U (divided into two injection sites, one intramuscular and one intradermal) on one side of the forehead and Dysport 15, 18 or 24 U on the other side, similarly divided into two injection sites. All injections were of identical volume (0.06 ml) and were blinded and randomized. The areas of anhidrotic halos, a measure of BoNTA migration, were highlighted with an iodine–starch application, photographed and calculated. From week 1 to month 6 following treatment, Dysport-produced halos were significantly larger than those produced from Botox at all dose ratios studied. In the 12 patients with symmetrical forehead wrinkles, although the area of anhidrosis with Botox treatment was smaller, the inhibition of maximum frontalis contraction was greater than in the area that received Dysport.

To compare the radius of diffusion between Botox and Myobloc, eight patients with symmetrical moderate-to-severe forehead wrinkles at full contracture received intramuscular Botox 5 U on one side of the frontalis and Myobloc 500 U on the other [25]. Evaluations of a wrinkle-reduction area were measured and calculated using a digital micrometer on traced scanned images daily for 14 days following treatment. At each time point, Myobloc produced a greater radius of toxin diffusion than Botox.

Taken together, the observations described in Section 3.1 provide some understanding as to why the various BoNTs cannot be considered easily converted or interchanged. However, the relevance of these observations as they pertain to the aesthetic use of BoNT must be confirmed with data from well-controlled comparative evaluations in the aesthetic clinical setting.

4. Clinical efficacy in facial aesthetics

4.1 Upper face

Table 3 lists the randomized, controlled trials that have established the efficacy of BoNTA as monotherapy for facial aesthetics. The majority of these trials evaluated the efficacy in the upper face with the Botox brand. Doses of 20 U divided among 5 injection sites initially proved Botox efficacy in glabellar lines, with effects lasting up to 4 months in duration. Higher doses (up to 40 U in women and 80 U in men) have been used, increasing the duration of effect in some patients up to 6 months without increasing AEs. For treating crow's feet, Botox doses of 12 – 18 U per side of face are effective for up to 5 months. A smaller, single-center study using three-dimensional *in vivo* profilometry confirmed the long duration of action (6 months) of Botox 12 U in this facial area in 25 patients with crow's feet [41].

Several randomized, placebo-controlled trials have been published supporting the efficacy of Dysport for cosmetic use in the upper face (i.e., glabellar lines; **Table 3**). Some studies suggest that the optimal dose of Dysport for this area is 50 U [42,43].

4.2 Botox versus Dysport

Only one randomized, controlled study comparing the efficacy of Botox with Dysport has been reported as yet and this study involved glabellar rhytids [27]. This was a single-center study in

Table 2. Calculated mean ± standard error intramuscular median effective dose, median lethal dose and safety and therapeutic margins for various BoNT products in animal models derived using the digital abduction scoring assay.

	Botox®	Dysport®	Myobloc®
ED_{50} (U/kg)	3.5 ± 0.58	16.3 ± 1.62	23.7 ± 2.04
LD_{50} (U/kg)	69.2 ± 2.89	168 ± 6.29	103 ± 2.55
TD (U/kg)	30.0	20.5	9.0
SM (LD_{50}/ED_{50})	19.8 ± 3.38	10.3 ± 1.09	4.35 ± 0.39
TM (TD/ED_{50})	8.57	1.26	0.38

Information from [39].

BoNT: Botulinum neurotoxin; ED_{50}: Median effective dose; LD_{50}: Median lethal dose; SM: Safety margin; TD: Threshold dose or lowest dose causing weakness of local, non-targeted muscles; TM: Therapeutic margin.

which 62 females with moderate-to-severe glabellar lines received double-blind Dysport 50 U or Botox 20 U, each divided into five injections (one in the procerus muscle and two each in the corrugator muscles). Maximal efficacy (i.e., ≥ 1 grade improvement of severity of glabellar lines at maximal frown on a scale of 0 – 3) peaked at 8 weeks in both groups, although the effect was prolonged with Botox. At 16 weeks, 53% of patients receiving Botox had at least a 1-grade improvement versus 28% of Dysport-treated patients ($p = 0.04$). Mean patient-assessed attractiveness and patient satisfaction scores, rated on a 7-point scale with 0 equal to 'not at all' and 6 equal to 'extremely', were significantly higher for Botox at week 12; by week 16, results were similar between groups.

Given the paucity of randomized, controlled comparisons, an earlier, uncontrolled, open-label study conducted in Korea should be noted, which compared the effects of Botox and Dysport for wrinkles in 20 patients [44]. There were six patients who received Dysport doses ranging from 20 – 80 U and 14 patients received Botox in doses ranging from 5 – 20 U, depending on the grade of wrinkles. Patients received four injections in the lateral canthal area, five in the glabella, four in the forehead, one in the nasal dorsum and two in the nasolabial fold. In general, the efficacy of Botox and Dysport were similar in reducing wrinkle severity (72.7 and 64.3% reductions, respectively; $p > 0.05$), although Dysport was associated with a higher rate of AEs at this dose ratio (Section 5.1).

4.3 BoNTA versus BoNTB

Several small studies have compared the efficacy of Botox versus Myobloc for facial rhytids [22-26]. Botox–Myobloc dose ratios in these studies ranged from 1:50 to 1:150. These studies and user experience [45] have shown that, although BoNTB may have a faster onset of action, its duration of action is substantially shorter than that of BoNTA (~ 2 months for BoNTB) and it is associated with more discomfort on injection.

4.4 Other areas of the face and neck

In addition to the observations from randomized, controlled clinical trials, there are a vast amount of experiential use and clinical trial data indicating the efficacy of BoNTA in diminishing facial rhytids and in aesthetic sculpting in other areas of the face (**Table 4**). The majority of these reports also describe experience with Botox. Indeed, the use of BoNTA has expanded downward, from the upper face to the mid-face and from the lower face to the neck. In the US, consensus recommendations have been published on the use of BoNTA (Botox) for facial aesthetics. These include guidelines for product reconstitution, the injection site and dosing considerations for various treatment areas in the face and neck, as well as patient post-treatment instructions [2]. Similar recommendations have not been published for other BoNT products. **Figures 2** and **3** provide examples of the effects of BoNTA in off-label cosmetic uses.

4.5 Part of a multimodal approach

As the field of cosmetic enhancements expands, so does the combined use of these agents to achieve the most pleasing results. Data from randomized, controlled trials suggest that the combination of BoNTA (Botox) with dermal fillers [46,47], peri-orbital laser resurfacing [48] and broadband light treatment [49] as more effective than these modalities alone. In addition, BoNTA can augment the effects of facial aesthetic surgical procedures [50]. The combined use of BoNTA with hyaluronic acid fillers is an increasingly common approach to facial aesthetics. In a randomized, controlled study in 38 patients with moderate-to-severe glabellar rhytids, the combined use of BoNTA (Botox) plus Restylane® (non-animal stabilized hyaluronic acid; Q-Med AB; n = 19) was superior to Restylane alone (n = 19) at rest and at frown on the facial wrinkle scale [47]. At rest, the improvement observed with BoNTA plus Restylane versus Restylane alone was significant up to 32 weeks (percentage improved on facial wrinkle scale from baseline, 47 versus 6%, respectively; $p = 0.0072$).

5. Safety in facial aesthetics

Given the origin of BoNTA, there were, not surprisingly, initial concerns regarding its safety for therapeutic purposes. However, an abundance of evidence has found the approved formulations of BoNTA to be exceedingly safe, particularly at the very low doses used for facial aesthetics. Most importantly, when used for aesthetic indications, all AEs that do occur are reversible, with no long-term sequelae.

Table 3. Randomized, controlled trials of BoNTA brands as a single therapy for facial wrinkles (MedLine search).

Ref.	Comparators	Study design	Area evaluated	Study duration	Main outcome measures	Results
[51]	Botox® 20 U (5 injection sites) n = 203 Placebo n = 61	Multi-center, double-blind, placebo-controlled	Moderate-to-severe glabellar lines	120 days	Investigator ratings of glabellar line severity at maximum frown and rest (0 – 3 scale; 0 = 'none' to 3 = 'severe') Responder rate (rating of 0 or 1) Patient assessment (9-point scale) Safety	Significant improvements with BoNTA versus placebo in efficacy outcomes up to 120 days Notable AEs versus placebo included headache (15% for both groups); blepharoptosis (5.4 versus 0% for placebo)
[52]	Botox 20 U (5 injection sites) n = 202 Placebo n = 21	Multi-center, double-blind, placebo-controlled	Moderate-to-severe glabellar lines	120 days	Investigator ratings of glabellar line severity at maximum frown and rest (0 – 3 scale; 0 = 'none' to 3 = 'severe') Responder rate (rating of 0 or 1) Patient assessment (9-point scale) Safety	Significant improvements with BoNTA versus placebo in efficacy outcomes up to 120 days Similar AEs between groups; blepharoptosis reported by 1% of BoNTA patients
[53]*	Botox 20 U (5 injection sites) n = 405 Placebo n = 132	Multi-center, double-blind, placebo-controlled	Moderate-to-severe glabellar lines	1 year (3 consecutive treatments)	Investigator ratings of glabellar line severity at maximum frown and rest (0 – 3 scale; 0 = 'none' to 3 = 'severe') Responder rate (rating of 0 or 1) Patient assessment (9-point scale) Safety	Significant increase in response with consecutive treatments Incidence of headache and blepharoptosis decreased with additional injections; blepharoptosis had duration of 27 – 29 days for mild-to-moderate intensity, respectively
[54]	Botox 10, 20, 30, 40 U divided unevenly among 7 injection sites n = 80 females	Single-center, double-blind, dose-ranging	Moderate-to-severe glabellar lines	1 year	Investigator and patient ratings of glabellar line severity at maximum frown and rest (0 – 3 scale; 0 = 'none' to 3 = 'severe') Responder rate (rating of 0 or 1) Safety	10 U sub-optimal at 120 days; non-significant differences 20 – 40 U No increase in AEs with 40 U dosing
[55]	Botox 20, 40, 60, 80 U divided unevenly among 7 injection sites n = 80 males	Single center, double-blind, dose-ranging	Moderate-to-severe glabellar lines	1 year	Investigator ratings of glabellar line severity at maximum frown and rest (0 – 3 scale; 0 = 'none' to 3 = 'severe') Responder rate (rating of 0 or 1) Patient assessments of attractiveness and satisfaction on 7-point scale and degree of improvement on 9-point scale Safety	40 – 80 U were optimal doses; mean time to relapse ranged from 17.6 weeks with 20 U dose to 24.2 weeks with 80 U dose All treatment-related AEs mild to moderate in severity; headache and brow twitch most common (3.7% patients)
[57]	Botox 16, 32, 48 U divided among 8 injection sites n = 59 females	Single center, double-blind, dose-ranging	Moderate-to-severe horizontal forehead rhytids	48 weeks	Investigator and patient ratings of wrinkles (0 – 3 scale; 0 = 'none' to 3 = 'severe') Responder and relapse rates	Significant dose–response trends, with maximal effect with 48 U dose

*Patients initially participated in the two studies mentioned above.

AE: Adverse event; BoNTA: Botulinum neurotoxin type A; FLTS: Facial Lines Treatment Satisfaction.

Table 3. Randomized, controlled trials of BoNTA brands as a single therapy for facial wrinkles (MedLine search) (continued).

Ref.	Comparators	Study design	Area evaluated	Study duration	Main outcome measures	Results
[56]	Botox 3, 6, 12, 18 U divided into 3 injections per eye (6 injections total) n = 31-33 per group Placebo n = 32	Multi-center, placebo-controlled, dose-ranging	Moderate-to-severe crow's-feet	180 days	Investigator ratings of crow's feet severity at maximum smile (0 – 3 scale; 0 = 'none' to 3 = 'severe') Responder rate (rating of 0 or 1) FLTS Questionnaire	18 U and 12 U had longest duration of efficacy (150 days); 120 days for 6 U and 30 days for 3 U Significant differences versus placebo in FLTS at all visits for all doses Most common treatment-related AEs were injection site bruising (8%) and headache (5.6%)
[66]	Dysport® 30 U (3 injection sites) and 50 U (5 injection sites) n = 221 Placebo n =75	Multi-center, double-blind, placebo-controlled	Moderate-to-severe glabellar lines	16 weeks	Responder rate at maximum frown at week 4 Investigator ratings of glabellar line severity at maximum frown and rest (0 – 3 scale; 0 = 'none' to 3 = 'severe') Patient global satisfaction at week 16	At week 4, both doses were significantly better than placebo Significant patient satisfaction versus placebo at week 16 BoNTA AEs included ptosis (1); elevation of outer eyebrow (5)
[42]	Dysport 25, 50 or 75 U (5 injection sites) n = 102 Placebo n = 17	Multi-center, double-blind, placebo-controlled	Moderate-to-severe glabellar lines	180 days	Responder rate at rest and at maximum frown Patient satisfaction (4-point scale)	For all 3 doses, significant differences versus placebo at 30 days, but not 180 days (120 days not evaluated) Patient satisfaction data suggests 50 U optimal dose AEs included 2 cases of headache; no ptosis seen
[27]	Botox 20 U (5 injection sites) n = 31 Dysport 50 U (5 injection sites) n = 31	Single center, double-blind	Moderate-to-severe glabellar lines	16 weeks	Investigator ratings of glabellar line severity at maximum frown (0 – 3 scale; 0 = 'none' to 3 = 'severe') Responder rate (rating of 0 or 1) Investigator global improvement Patient satisfaction (7-point scale) AEs	Response rate at 12 weeks similar between groups but significantly higher at 16 weeks with Botox versus Dysport Significantly better patient satisfaction scores with Botox versus Dysport at 12 weeks No ptosis reported, 1 case of mild bruising with Botox

*Patients initially participated in the two studies mentioned above.

AE: Adverse event; BoNTA: Botulinum neurotoxin type A; FLTS: Facial Lines Treatment Satisfaction.

Table 4. Facial areas where successful use of BoNTA for facial aesthetics has been described.

Area*	Refs
Upper face	
Glabellar complex	[2,42,43, 51-55,66]
Horizontal forehead rhytids	[2,57]
Crow's feet	[2,56]
Mid face	
Lower eyelid	[67]
Widening of the palpebral aperture	[68]
Bunny lines	[2]
Nasal flare	[69]
Nasolabial folds (gummy smile)	[69,70]
Perioral rhytids	[2]
Correction of asymmetry	[69]
Lower face	
Mouth frown and melomental fold	[50]
Chin (including peau d'orange)	[2,71]
Deep smile lines	[69]
Masseteric hypertrophy	[72,73]
Neck	
Platysma	[2,70,74]
Horizontal lines	[69,70]

*The list of facial areas and supporting references is not inclusive, but rather a representation of available data and information.
BoNTA: Botulinum neurotoxin type A.

The most common AEs to be reported in BoNTA facial cosmetic trials are headache (10 – 13%), respiratory infection (3.5%) and blepharoptosis (3%) [42,51,52]. In a 1-year analysis of the two Botox pivotal trials, the incidence of headache and blepharoptosis decreased with additional injections and the duration of blepharoptosis was ~ 27 days [53]. AEs do not seem to be dose related [54-57].

In separate retrospective analyses, there was no evidence of cumulative AEs with either Botox [58] or Dysport [59] with repeated treatment cycles for aesthetic use. In the study evaluating Botox, the charts of 50 patients at one clinic who received at least 10 treatments for hyperfunctional facial lines with Botox were reviewed. AE data were gleaned from 853 treatment sessions (with a median of 19 per patient), in which patients received a median dose per session of 40 U, for a median cumulative dose of 690 U. The median interval between injections was 17 weeks, with injection sessions ranging over a median of 5.95 years. There were 8 patients who reported a total of 10 AEs. Treatment-related AEs occurred in 9 out of 853 sessions (1.1%); 5 of the AEs were considered to be probably or definitely related to BoNTA treatment and consisted of 4 cases of eyebrow or eyelid ptosis and 1 case of dysphagia. All were mild-to-moderate in intensity and resolved with no sequelae.

In the Dysport retrospective analysis [59], 945 patients received 4103 treatment cycles of Dysport. Dosing was constant over time, with median doses of ~ 50 U in the glabella, 50 U in the frontalis and 50 U in the peri-orbital region. All of the patients had received at least 3 treatment cycles, with 58% receiving 5 treatment cycles. The AE rate per treatment cycle decreased from 4.1% at the first treatment to 2% in the fifth cycle. AEs included bruising (1.25% per treatment cycle) and eyelid or brow ptosis (0.46% per treatment cycle) that was generally mild [59].

5.1 Botox versus Dysport

In the randomized, controlled comparative study evaluating Dysport 50 U and Botox 20 U for glabellar lines [27], both products were similarly well tolerated, with few AEs reported (AEs reflected injection-site reactions such as bruising and lump). In the Korean study, in which Dysport doses of 20 – 80 U and Botox doses of 5 – 20 U were evaluated [44], AEs occurred significantly more often with Dysport (6 out of 6 patients [100%]) than with Botox (5 out of 14 patients [35%]; $p < 0.05$). A total of 11 AEs occurred in this study and included lagophthalmos (n = 3), tingling (n = 3) and eyelid swelling (n = 5), all of which were transient.

6. Resistance

The therapeutic use of BoNTs has resulted in cases of primary resistance (immediate lack of effect) and secondary resistance (lack of effect with repeated use, usually due to development of antibodies to BoNTA) [60,61]. Factors that contribute to secondary resistance to BoNT include high single doses, booster injections and short inter-injection intervals [62]. Determining rates of resistance has been difficult, as studies often do not capture these data and few cases are reported overall. A reformulation of Botox in 1997 to reduce the neurotoxin complex protein concentration from 25 to 5 ng per 100 U significantly reduced the rate of antibody-induced resistance in cervical dystonia [60].

Clinical trials in facial aesthetics did not find notable correlations between antibody development and clinical effect; however, they did find that repeated injections prolong the duration of BoNTA aesthetic effect [53]. Antibody testing was assessed in 258 patients receiving 3 injections of BoNTA over 1 year [53]. Of the patients receiving BoNT injections who had antibody-evaluable samples, 88% tested negative at 2 time points. Among those who tested positive for antibodies, all responded clinically to aesthetic BoNTA.

However, a diminished effect to injected aesthetic BoNTA has been documented anectodally [26]. Until recently, no reports of antibody-induced resistance with the aesthetic use of BoNT had been reported in the medical literature [63]; this case involved a 20-year-old Korean woman who received Botox 30 U for masseter hypertrophy [63].

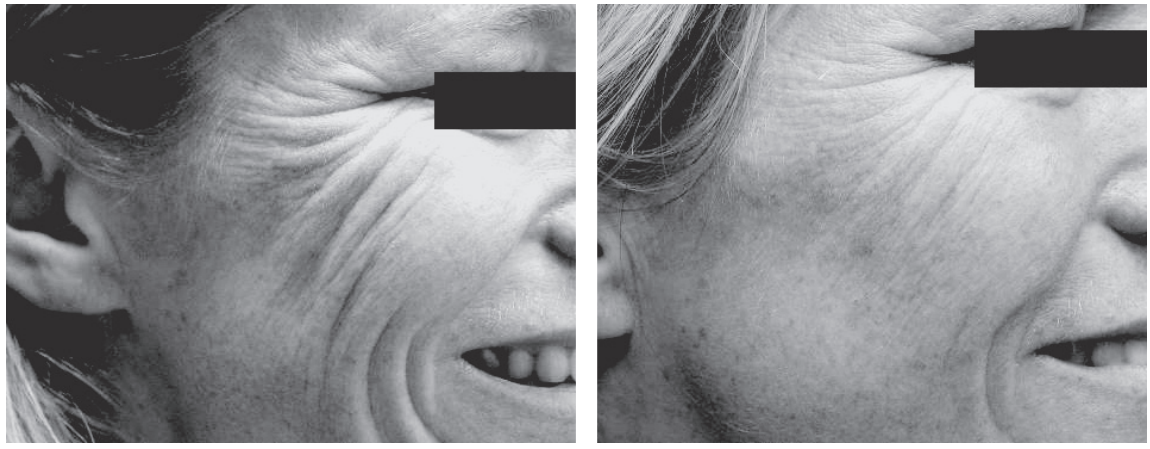

Figure 2. Reduction in deep zygomaticus lines with botulinum neurotoxin type A (Botox®). Dosing: triangular configuration of 3 injections of Botox 2 U per side in m. zygomaticus major and minor. Before injection and 2 weeks after injection.
Photographs courtesy of K De Boulle.

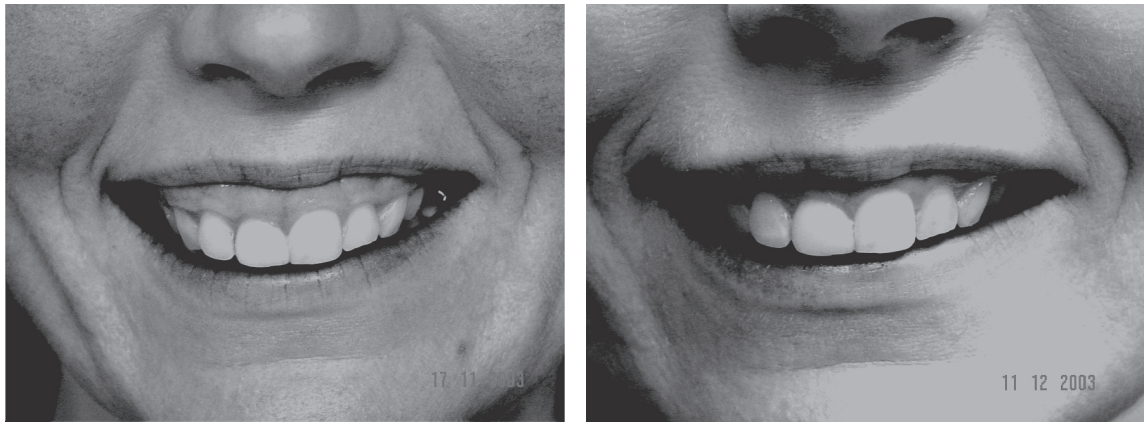

Figure 3. Correction of 'gummy smile' with botulinum neurotoxin type A (Botox®). Dosing: Botox 2 U per side in central levators of upper lip. Before injection and 4 weeks after injection.
Photographs courtesy of K De Boulle.

The patient received BoNTA injections every 4 months for 3 series of injections. Following the fourth series, the targeted muscle paresis lasted only 1.5 months and by the sixth series, no effects were evident. Two separate mouse protection assays were positive, indicative of the presence of anti-BoNTA antibodies.

Non-responsiveness to aesthetic BoNTA may also be related to improper storage, dose, reconstitution or injection technique. Switching to another BoNT product in patients with a diminished response to one product has been shown to be effective [26].

7. Agents in development and unapproved BoNTA products

Other BoNTA products are being investigated for therapeutic and aesthetic use. One such product is Puretox® (Mentor Corporation), a BoNTA formulation that is being evaluated by Mentor and obtained via an exclusive licensing agreement with a research foundation. Phase II dose-finding studies for cosmetic use were initiated in late 2005.

Given the popularity of BoNTA for cosmetic use, many companies are capitalizing on these successes by claiming to be inexpensive generic BoNTA products. These products are often advertised on the Internet and physicians may receive information about these products through salespeople, direct mailings and even professional meetings. These offers claim to save physicians considerable money over branded BoNTA products. However, given that these agents are neither bioequivalent nor have received regulatory approval in most countries, physicians cannot ascertain the proper dosing, efficacy or safety of these products for their patients. An example of a safey concern is found in an open-label study involving patients with focal dystonia and muscle spasm in which

CBTX-A was less potent than Botox, but was also associated with cases of skin rash, which the authors attributed to the purification process [30].

In addition to unlicensed BoNTA products, counterfeit products have also emerged in the marketplace recently. These agents bear similar packaging to brand-name products, but their efficacy or safety cannot be ascertained and some may not even contain BoNTA. To safeguard patient well-being, it is paramount that physicians establish conservative policies regarding the purchase and use of BoNTA. These include using only those branded products that have received regulatory approval in their country of practice, selecting BoNTA products with which they have experience and comfort in using and ordering products locally (i.e., within their own country or region) from reputable pharmaceutical sources.

8. Conclusion

The popularity of BoNTA injection for cosmetic use is a testament to the excellent results that can be achieved with a wide safety margin. A wealth of aesthetic experience has been accumulated with BoNTA (in particular, Botox). Data have emerged in recent years on the cosmetic use of Dysport in the upper face. BoNTB is also effective for facial lines, but has limited clinical usefulness due to its shorter duration of action relative to BoNTA. Other BoNTAs have been marketed for other indications and additional products are in development. Clinical trial data and experience are needed to determine the overall clinical utility of these agents for cosmetic use. Various preclinical reports, as well as observations from clinical trials in therapeutic indications, suggest that pharmacologic differences exist among products that preclude their interchangeability or conversion via a simple dose ratio. However, there is a paucity of comparative clinical data among BoNTA products, particularly with respect to aesthetic use. Head-to-head clinical trials of adequate sample size are needed to adequately compare the various clinical profiles of BoNTA when used for aesthetic indications. Clinicians who use more than one product must develop expertise with each.

9. Expert opinion

The cosmetic use of BoNTA has gained rapid acceptance in the last several years. Based on increasing data in the literature in the form of clinical trials and experiential use, cosmetic applications for BoNTA in facial rejuvenation and enhancement appear limitless. In the hands of the experienced injector, BoNTA can comfortably and simply be used to enhance most ageing caused by the dynamics of the facial musculature and asymmetry aspects of the upper, mid and lower face and neck, with no downtime for the patient. Moreover, the long-lasting (yet non-permanent) effects of the product enhance its versatility and convenience of use.

However, as the use of BoNTA moves away from the upper face and ventures further away from the realm of proof of efficacy and safety as determined in randomized, controlled trials, treatment becomes less standardized and more of an art form. Thus, training on the use of these agents (through workshops and courses offering live demonstrations) is very important and allows physicians to gain the necessary skills to provide facial rejuvenation and enhancement while achieving the desired natural and relaxed look. It cannot be overemphasized that clinical expertise with specific products is key to achieving positive facial aesthetics. The vast majority of literature and practical experience that has been gained thus far with the Botox brand, and the results achieved with one BoNTA product, cannot be extrapolated to other BoNTAs. This point becomes increasingly important as more BoNTA products are marketed in the future.

When developing an individual treatment plan for a patient, a number of factors should be considered, including the patient's aesthetic goals, target area, sex, muscle mass, skin thickness and anatomical variation [2]. Physician technique and experience with BoNTA injections are important components in successfully implementing a treatment plan and achieving a patient's aesthetic goals. Product selection, dose and precise placement of injections can affect outcomes. For example, the physician should have the expertise with BoNTA to create the type of brow a patient desires, whether it is a medial brow lift, a quizzical look or 'cat's eyes'. Although it is beyond the scope of this article to describe all of the possible considerations that are related to the components of a treatment plan, the concept is mentioned here to remind the reader that achieving a natural and relaxed look with BoNTA is as much an art as it is a science.

As more experience has been gained with both BoNTA application and other cosmetic enhancements, it has become evident that these procedures are often better used together than individually for complementary and even synergistic effects. The total dermatocosmetic approach addresses the dual effects caused by dynamic changes (i.e., increased muscle activity), as well as static changes associated with ageing (i.e., sun damage, loss of volume in facial tissues, and so on) to restore a youthful-looking anatomy. BoNTA is an invaluable component in combination with dermal fillers, intense pulsed-light therapy, radiofrequency and other rejuvenation techniques. In theory, by reducing muscle activity, BoNTA may augment the duration of effect of fillers by allowing them to stay in place longer, although studies are needed to confirm this theory.

A recent report on identical twins suggests that BoNTA may prevent wrinkle formation [64]. In addition, evidence from the two pivotal trials shows that repeat injections prolong the duration of BoNTA effect [53]. If used at regular low doses at the very beginning of the ageing process, BoNTA can assist in maintaining a low resting tone, allowing muscles to keep natural curves and not expel the deep fat.

From more than a decade of personal experience in the use of BoNTA for cosmetic indications, there seems to be two subgroups of patients who react to repeated injections: those individuals in whom the duration of the effect is prolonged and those who always need the same interval between treatments to

maintain a constant cosmetic enhancement. A possible theoretical explanation for this difference could be that, in the first group, collateral axonal re-sprouting at the neuromuscular junction may be impaired by early and regular use of BoNTA. Thus, the injected facial muscles never have the opportunity to attain maximal contraction and the resultant ability to form lines. In the second subgroup, BoNTA does not affect the speed and ability of re-establishing axonal re-sprouting. In these patients, constant re-injection at regular intervals is necessary to maintain the effects of BoNTA. Additional research is needed to confirm the notion of wrinkle prevention. If true, it would tremendously expand the usefulness of BoNTA.

It seems readily accepted that injections with BoNT for cosmetic indications have become the cornerstone of the general cosmetic treatment plan. Often for individuals commencing aesthetic enhancement, the immediately pleasing results with this cosmetic 'low hurdle' approach may be the impetus for initiating additional self-enhancing procedures, such as weight loss and so on. Interestingly, a recent pilot study in 2006 has suggested that BoNTA (Botox) has a positive effect on depressive mood [65]. Thus, although used for cosmetic purposes, BoNTA may indirectly enhance aspects of health; however, more data are needed in this regard.

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