

Advances in the use of botulinum neurotoxins in facial esthetics

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Summary

Aim To present the latest findings and future developments in the cosmetic use of botulinum neurotoxin.

Methods Review of recent literature and new scientific developments.

Results Botulinum neurotoxin type A preparations onabotulinumtoxinA (BOTOX[®] Cosmetic/Vistabel[®], Allergan Inc.) and abobotulinumtoxinA (Dysport[®]/Azzalure[®]/Reloxin[®], Ipsen Pharma,) have been used for many years and are effective and well tolerated for facial esthetic procedures. However, advances are continually made in the esthetics field. New formulations that may exhibit reduced antigenicity are becoming available, such as incobotulinumtoxinA (Xeomin[®]/Xeomeen[®]/Bocouture[®]; formerly known as NT 201, Merz Pharma), which is a botulinum neurotoxin type A free from complexing proteins. In addition, lower facial procedures using botulinum toxin combined with fillers are becoming increasingly popular. Injection techniques and patterns are also evolving, with the aim of creating a more natural result and avoiding a “frozen” appearance. Moreover, the diversity of individuals requesting esthetic procedures is increasing, with growing interest from men and patients with a variety of skin types and colors.

Conclusions The uses of botulinum toxins for facial esthetics procedures continue to expand, with new techniques and formulations. The availability of products such as incobotulinumtoxinA may reduce the risk of neutralizing antibody development while maintaining the good efficacy and safety of existing formulations.

Keywords: botulinum neurotoxin type A, complexing protein, neutralizing antibody, BoNT-A

Introduction

Botulinum products today

Botulinum neurotoxin type A (BoNT-A) is produced from a strain of *Clostridium botulinum* and associates with complexing proteins during the fermentation process, to produce a large protein complex.¹ Other structurally distinct neurotoxin serotypes, types B to G, are also produced from strains of *C. botulinum*.¹ The

neurotoxin types differ with respect to their molecular mass, their localization in the nerve terminal, and also their therapeutic use.^{1,2} BoNT-A is a mainstay in esthetics, with facial rejuvenation being the most common nonsurgical esthetic procedure undertaken in the USA.³ Type B (BoNT-B) has also demonstrated efficacy in esthetics and cervical dystonia.^{4,5} Similarly, BoNT-A is also used in the therapeutic setting, while type C (BoNT-C) has shown beneficial effects on blepharospasm and facial hemispasm.⁶ Type F (BoNT-F) has also demonstrated efficacy in focal dystonia and torticollis; however, its use is limited by a short duration of effect.^{7,8} Only BoNT-A and BoNT-B are available as approved drugs; other serotypes have only been used in experimental settings.

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Table 1 Overview of botulinum toxin type A (BoNT-A) products marketed for esthetic use. Adapted from Carruthers A, Carruthers J. Botulinum toxin products overview. Reproduced with permission⁹

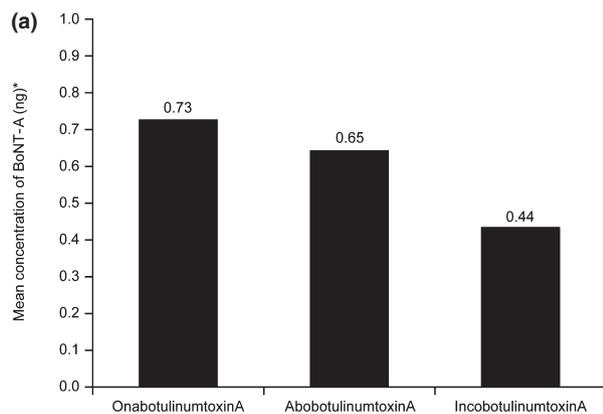
Product	Manufacturer	Active component	Strength of activity compared to BoNT-A	Storage	Approval for facial esthetics
onabotulinumtoxinA (BOTOX [®] Cosmetic/Vistabel [®] /Vistabex [®])	Allergan Inc.	BoNT-A complex (900 kDa)	1:1	2–8 °C or <–5 °C	Glabellar frown lines in USA, Canada, Europe
abobotulinumtoxinA (Dysport [®] /Reloxin [®] /Azzalure [®])	Ipsen Inc./Medicis Inc./Galderma	BoNT-A complex (500–900 kDa)	Approx 1:2 to 1:4	2–8 °C	Glabellar frown lines in USA, Europe
incobotulinumtoxinA (NT 201/Bocouture [®] /Xeomin [®] /Xeomeen [®])	Merz Pharmaceuticals	BoNT-A, free from complexing proteins (150 kDa)	1:1	Up to 25 °C	Glabellar frown lines in USA, Germany, UK and all other major European countries, Argentina, South Korea Esthetic use in China
DPS Refinex [®]	Technology Development Ltd	BoNT-A, contains complexing proteins			
Prosigne [®] /CBTX-A	Lanzhou Biological Products Institute	BoNT-A, contains bovine gelatine			Esthetic use in China and Brazil
PurTox [®]	Mentor Corporation	BoNT-A, uncomplexed (150 kDa)	2:3		Under development

Today, a number of different BoNT-A formulations are available under different names and in different countries (Table 1).⁹ The first BoNT-A product to become available, onabotulinumtoxinA (BOTOX[®] Cosmetic, Allergan Inc., Irvine, CA, USA), received Food and Drug Administration (FDA) approval for the cosmetic treatment of glabellar frown lines in 2002.¹⁰ It has since been licensed for esthetic use in the European Union and is sold for this indication under the names BOTOX[®] Cosmetic and Vistabel[®] (Allergan Inc.). The second BoNT-A formulation, abobotulinumtoxinA (Dysport[®], Ipsen Pharma, Boulogne-Billancourt, France), was licensed for esthetic use in the European Union in 2006, with US FDA approval in April 2009. For esthetic purposes, it is also marketed as Azzalure[®] by Galderma, Lausanne, Switzerland, and as Dysport[®] by Medicis, Scottsdale, AZ, USA. Importantly, the products from the two manufacturers are not interchangeable, and there is no consistent agreed-upon, fixed-dose conversion ratio.^{11–14}

In addition to these two BoNT-A formulations, a number of other products have entered the market and are available in some countries (Table 1). IncobotulinumtoxinA (Merz Pharmaceuticals, Frankfurt, Germany) has been approved under the brand name Bocouture[®] in all major European markets for the treatment for glabellar frown lines and under the brand names Xeomin[®] and Xeomeen[®] in Argentina, Mexico, and Russia for the treatment for hyperkinetic facial lines. It is free from the complexing proteins that are included

in other currently available BoNT-A formulations, and although standard clinical practice uses a 1:1 unit equivalency for incobotulinumtoxinA and onabotulinumtoxinA formulations, *in vitro* biochemical assays have shown differences in the protein load and specific activity of the different formulations (Fig. 1).^{15–17} Clinical trials have shown that, when administered at the same dose, incobotulinumtoxinA is noninferior to onabotulinumtoxinA for the treatment for blepharospasm,¹⁸ glabellar frown lines,¹⁹ and cervical dystonia²⁰ and that both preparations display comparable treatment duration and waning of effect (Fig. 2).²¹ In addition, incobotulinumtoxinA has recently been shown to be effective and well tolerated in the treatment for poststroke upper limb spasticity with a median time to waning of effect reported as 10 weeks.²² Importantly, the presence of complexing proteins does not appear to affect the rate of diffusion of the toxin from the injection site^{23,24} nor enhance stability during storage;²⁵ incobotulinumtoxinA can be stored at room temperature, whereas conventional BoNT-A preparations require refrigeration and prior reconstitution.^{9,26}

The second complexing-protein-free formulation, PurTox[®] (Mentor Corporation, Santa Barbara, CA, USA), is expected to gain FDA approval. In addition, a BoNT-B product, rimabotulinumtoxinB (which is the FDA-given name for Myobloc[®], Solstice Neurosciences Inc, Malvern, PA, USA), is approved for the treatment of cervical dystonia and has been investigated in the esthetic setting. While studies report a fast onset of action and



*Mean concentration per 100 U

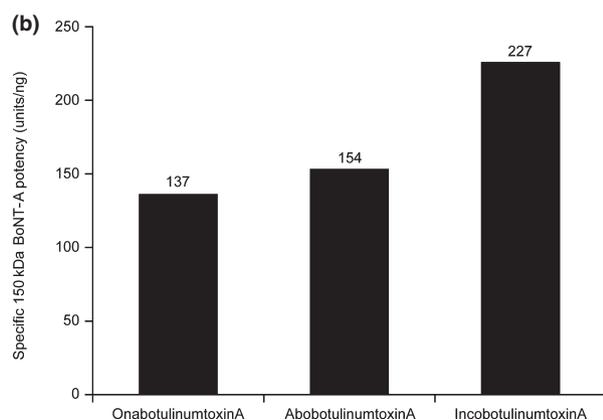


Figure 1 (a) Mean concentration and (b) specific potency of botulinum neurotoxin type A (BoNT-A) in incobotulinumtoxinA, abobotulinumtoxinA, and onabotulinumtoxinA. Drawn from data in Frevert J. Content of botulinum neurotoxin in Botox®/Vistabel®, Dysport®/Azzalure®, and Xeomin®/Bocouture®.⁶⁹

a wider spread of diffusion, its shorter duration of effect compared to BoNT-A^{27–29} makes it impractical for some applications. It may, however, have a particular use in patients experiencing secondary therapy failure because of neutralizing antibodies to BoNT-A. Additionally, the faster onset of action has been seen as beneficial when used intraoperatively for facial reconstruction.²⁷

Complexing proteins in BoNT formulations

Conventional BoNT-A products consist of a 900 kDa or smaller neurotoxin complex, which dissociates largely by reconstitution/dilution and completely upon injection into the neutral tissue pH.³⁰ The size of the biologically active neurotoxin is only 150 kDa, with the remainder being composed of haemagglutinin and

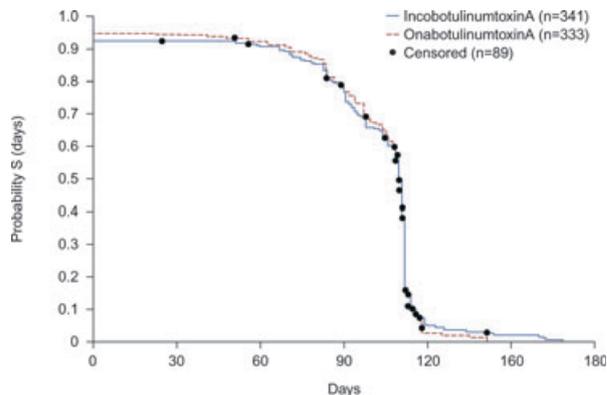


Figure 2 Duration of treatment effect of incobotulinumtoxinA and onabotulinumtoxinA combined from two phase III studies in blepharospasm and cervical dystonia. Censored = data from patients with missing data on final visit (patient with treatment effect on final visit or lost to follow-up). Reproduced with permission from Jost WH, Blümel J, Grafe S. Botulinum neurotoxin type A free of complexing proteins (Xeomin®) in focal dystonia.²¹

nonhaemagglutinin clostridial proteins.³¹ The complex is rapidly dissociated in the tissue, which suggests that the presence of complexing proteins does not affect the spread or persistence of the neurotoxin. In addition, these complexing proteins have no known therapeutic role and present a foreign protein load, which may increase the potential for eliciting an immune response.⁹ Complexing proteins have adjuvant activity on the formation of antibodies against botulinum neurotoxin that can lead to partial or total treatment failure.^{32,33} In a rabbit model, however, repeated intradermal injection of incobotulinumtoxinA was not associated with the development of neutralizing antibodies.³⁴ Antibody formation has also been linked to the dose of BoNT-A applied during each series and the interval between each injection series.^{32,33} The site of injection may also play a role, with differences noted between injections into deep muscle or near the skin. Whereas the doses used for esthetic purposes are lower than those used in therapeutic indications, secondary nonresponders have been reported with esthetic usage of onabotulinumtoxinA and abobotulinumtoxinA.^{35,36}

The initial formulation of onabotulinumtoxinA has undergone development to reduce the amount of clostridial protein in the formulation. Even with the new formulation, however, production of neutralizing antibodies can occur; the rate of antibody-induced therapy failure in patients with cervical dystonia dropped from 5–17% with the old formulation to 1.2% with the new formulation.^{37,38} When used in esthetics, the development of neutralizing antibodies to onabotulinumtoxinA is rare. Borodic (2007)³⁹ reported a

44-year-old woman who became unresponsive to onabotulinumtoxinA after 14 injections into the glabellar area over a 5-year period. Subsequent administration of BoNT-B to the glabellar area achieved a response, suggesting the presence of neutralizing antibodies to BoNT-A. In addition, antibody-induced treatment failure was recently reported in an esthetic patient treated for masseteric hypertrophy with onabotulinumtoxinA.³⁵ Despite low doses of onabotulinumtoxinA being used (30 U each side per treatment), after the fourth treatment series, paresis diminished after only 1.5 months, and after the sixth treatment, no paresis occurred.³⁵ A further four cases of antibody-induced treatment failure have since been reported:³⁶ two patients received abobotulinumtoxinA, one received onabotulinumtoxinA, and one received both abobotulinumtoxinA and onabotulinumtoxinA. Complete secondary treatment failure occurred after 3-, 5-, 10-, and 13-injection series, respectively (average single doses: 68 U abobotulinumtoxinA, 82 U abobotulinumtoxinA, 80 U onabotulinumtoxinA, and 30 U abobotulinumtoxinA/30 U onabotulinumtoxinA).³⁶ By eliciting a reduced treatment response or treatment failure, antibodies can limit the number of effective repeat BoNT-A injections an individual can receive. In theory, this could be

problematic, with the esthetic market shift toward younger recipients, as earlier initiation of BoNT-A and longer lifetime treatment may result in an increased risk of antibody formation, although this has not been demonstrated to date. Recent data suggest that incobotulinumtoxinA is less immunogenic and may result in reduced incidence of antibody-induced secondary treatment failure.⁴⁰

Efficacy of BoNT-A for esthetic use

The first esthetic uses for BoNT-A were in the upper face for the treatment for glabellar frown lines (Fig. 3). In a randomized, double-blind trial of 62 patients with moderate or severe glabellar frown lines, the occurrence of a 1-grade improvement in glabellar frown lines peaked at week 8 with both formulations (onabotulinumtoxinA 20 U, 94%; abobotulinumtoxinA 50 U, 97%) but was significantly lower with abobotulinumtoxinA at week 16 (28%) than with onabotulinumtoxinA (53%; $P = 0.04$).⁴¹ Furthermore, the incidence of relapse as measured at week 16 was 40% and 23% with abobotulinumtoxinA and onabotulinumtoxinA, respectively.⁴¹

In two large, randomized, double-blind, placebo-controlled, pivotal trials, the efficacy of onabotulinumtoxinA

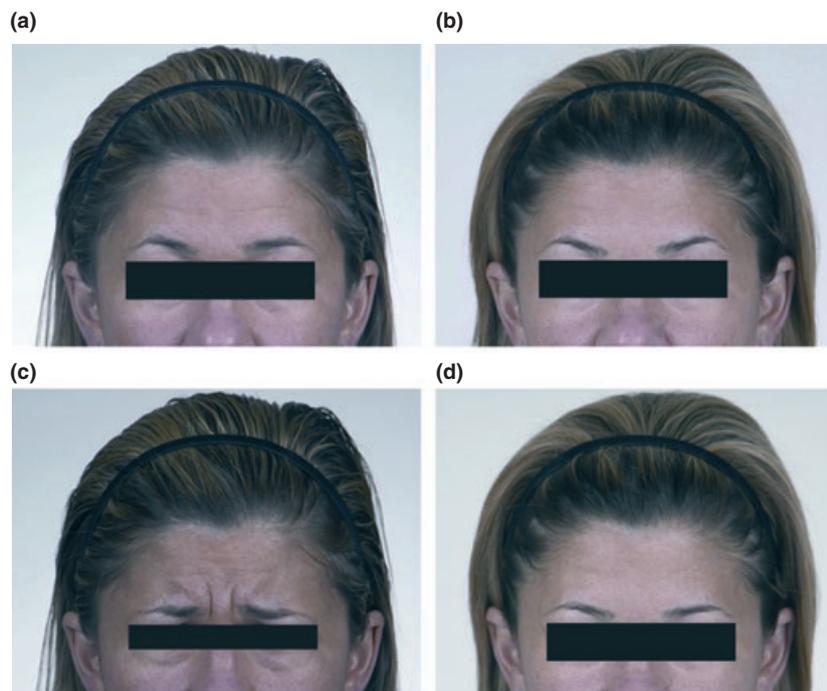


Figure 3 Before and after pictures of treatment of glabellar frown lines with incobotulinumtoxinA (a) before procedure (at rest); (b) 1 month after procedure (at rest); (c) before procedure (at maximal contraction); (d) 1 month after procedure (at maximal contraction). Courtesy of Merz Pharmaceuticals.

(20 U) was evaluated in 537 individuals—mostly women—with moderate or severe glabellar frown lines during facial animation.⁴² At every postinjection visit, participants who received onabotulinumtoxinA had significantly greater improvement in the appearance of glabellar frown lines (physician evaluation and self-report) compared with those who received placebo. These findings have been corroborated by multiple other reports.^{43–46} When three doses of abobotulinumtoxinA (25, 50, and 75 U) were compared with placebo in individuals with moderate-to-severe glabellar frown lines in a French trial ($n = 119$), the response rate at rest after 1 month was evidently greater ($P \leq 0.015$ vs. placebo) in each abobotulinumtoxinA arm (45–55%) compared with placebo (7%).⁴⁷ Response rates were increased vs. placebo after 2 weeks and were maintained at 3 months ($P < 0.001$). After 6 months, approximately one-third of patients receiving abobotulinumtoxinA were still classed as responders, but the difference vs. placebo was no longer obvious. Based on investigator judgment and patient satisfaction, 50 U was considered to be the optimal dose of abobotulinumtoxinA. This was also found to be the case in a more recent placebo-controlled trial of abobotulinumtoxinA 20, 50, and 75 U in the United States.⁴⁸ In a recent noninferiority study evaluating the efficacy and safety of incobotulinumtoxinA and onabotulinumtoxinA in the treatment for glabellar frown lines, it was reported that both BoNT-A preparations were equally as effective in improving the appearance of glabellar frown lines over at least 12 weeks, with response rates of 96.4% and 95.7% after 4 weeks and 80.1% and 78.5% after 12 weeks, respectively.¹⁹ The comparable efficacy and duration of effect of incobotulinumtoxinA and onabotulinumtoxinA have also been observed in the treatment for mimical smile lines.⁴⁹ In addition, in a small, investigative trial of subjects with glabellar frown lines, the treatment response to incobotulinumtoxinA has been reported as “good” or “very good” by 90% and 89% of patients after 2 weeks and 12 weeks of treatment compared to baseline.⁵⁰

Advances in BoNT-A esthetic procedures

The field of esthetics is developing rapidly as clinicians refine their techniques to provide the best possible outcomes. Today, an increasing number of procedures are performed on the middle and lower face rather than just on the upper face.¹¹ Optimal technique includes consideration of a number of variables, including the treatment goal, skin thickness, and anatomic variation⁵¹ (Fig. 4). Similarly, differences in skin type and color, facial shape, and esthetic and cultural ideals must be taken into account when planning esthetic procedures.

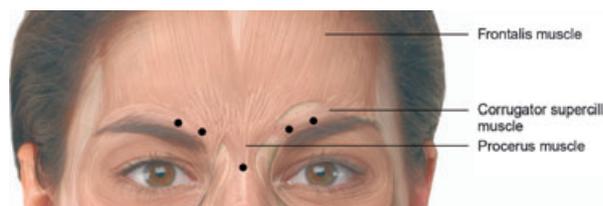


Figure 4 Typical injection sites to treat glabellar frown lines. Courtesy of Merz Pharmaceuticals.

In addition, more men are requesting esthetic treatment than previously.^{11,52}

The ongoing improvements in esthetic procedures with BoNT-A have led to greater acceptance and a widening clientele. Whereas the techniques involved in BoNT-A treatment in men are similar to those in women, there are a number of differences to bear in mind. For example, men require larger doses of BoNT-A than women, which is possibly related to their greater muscle mass.⁵¹ There are also differences in esthetic ideals in men; whereas women require an arched eyebrow, men prefer a lower, horizontal brow.^{51,53} Each patient's treatment should be individualized for optimum results. Taking careful medical notes and photography can assist the physician with personalizing treatment to meet patients' needs. Indeed, there has been a move away from a “blanket” approach, which uses the same doses and injection for all individuals, toward a more individualized approach.¹¹ Furthermore, simultaneous treatment of multiple sites on the face can help to achieve improved patient satisfaction.^{11,54} In a placebo-controlled study treating the entire upper face (16 sites) of 40 women, onabotulinumtoxinA (64 U) was associated with significant improvements in patient-reported outcomes.⁵³

New techniques aim to avoid the “frozen” look that was associated with early BoNT-A procedures. These newer approaches aim to provide a more natural, harmonious, balanced effect¹¹ and rely on an appreciation of the three-dimensional structure of the face. Indeed, there is now a greater appreciation that BoNT-A is one part of overall facial rejuvenation therapy, in which all aspects of aging are addressed in the individual: BoNT-A-induced muscular relaxation combined with lasers, light sources and retinoids for skin improvement, and filling agents to address age-associated volume loss and recontouring.^{11,54} For example, on the mid-face, BoNT-A injections can be given in conjunction with both semi-permanent fillers, such as poly-lactic acid and calcium hydroxylapatite, or temporary fillers, such as hyaluronic acid or collagen, which

are inserted under the skin in different layers to correct depressed folds, wrinkles, furrows, scars, angles, flaccidity, and volume depletion.^{55,56} Likewise, the combination of BoNT-A and hyaluronic acid filler is now the standard approach for the lower face.¹¹ In a glabellar frown line study, in which onabotulinumtoxinA plus hyaluronic acid filler was compared with filler alone ($n = 38$), combination therapy was associated with a significant improvement on the facial wrinkle scale at rest compared with filler alone, with even more marked differences at full contraction.⁵⁷ Another minimally invasive facial rejuvenation technique that has been shown to extend or improve results is the combination of BoNT-A with augmentation and facial sculpting using autologous adipose tissue.⁵⁶ Administration of lasers or light sources immediately following BoNT-A injection has also shown benefit by producing nonablative or fractional skin rejuvenation that results in superficial improvement, reduced pigmented lesions, vascular alterations, reduction in wrinkles, and skin tightening.⁵⁸ This concept aims to treat both the wrinkles and folds and to improve photodamage. Furthermore, BoNT-A preparations can also be administered in conjunction with other skin care techniques, such as chemical peels and dermabrasion, to improve superficial skin texture and pigmentary problems.⁵⁶ Finally, new esthetic indications are starting to emerge, including the treatment for scars, the horizontal upper lip line, musculus risorius and excessive gingival display (“gummy smile”), and masseter reduction to improve facial shape.¹¹ These new targets will in themselves drive the development of future treatment approaches using BoNT-A.

Safety of BoNT-A for esthetic use

Adverse event profiles may also differ between abobotulinumtoxinA and onabotulinumtoxinA, with a higher incidence of adverse events often reported with abobotulinumtoxinA than with onabotulinumtoxinA in clinical trials.¹² This may be a result, at least in part, of greater diffusion of abobotulinumtoxinA from the injection site.

The adverse event profile of incobotulinumtoxinA appears to be comparable to that of the other BoNT-A preparations. In the recent noninferiority study investigating incobotulinumtoxinA vs. onabotulinumtoxinA in the treatment for glabellar frown lines, the incidence of adverse events considered to be related to treatment was low in both treatment arms, occurring in 3.2% of patients receiving incobotulinumtoxinA and 5.2% receiving onabotulinumtoxinA.¹⁹ Data from a small trial investigating incobotulinumtoxinA

(20–23 U) for the treatment of glabellar frown lines in 10 women over a period of 3 months show that the agent was well tolerated, with only one case of headache reported.⁵⁰ Likewise, data from studies in therapeutic indications also show that incobotulinumtoxinA is well tolerated and associated with a low incidence of adverse events, which are typically mild to moderate in nature.⁵⁹

From a practical point of view, there have been differences between BoNT-A trials with regard to injection volume and dosing, as well as injection pattern.⁶⁰ As a result, inexperienced injectors are likely to have a steep learning curve when adjusting from one formulation to the other as minor differences in injection site may lead to substantially different treatment outcomes.¹¹ Similarly, inexperienced injectors may observe more adverse events in their patients. This is supported by a recent long-term study that investigated safety profiles after repeated dosing of abobotulinumtoxinA for the treatment for glabellar frown lines.⁶¹ Patients ($n = 1415$) were re-treated with 50 U or variable dosing, based on gender and muscle mass over 24 months. Findings indicate that the safety profile was comparable between fixed and variable dosing, and that incidence of AEs was constant or decreased over treatment cycles, possibly because physicians' technique improved with experience.⁶¹ There was also no evidence of cumulative safety issues following repeated abobotulinumtoxinA treatment for glabellar frown lines in an analysis of data from five phase III clinical trials.⁶²

The most common adverse events reported for esthetic use of BoNT-A formulations are headache, and pain and hematoma, which, along with ecchymosis and bruising, may occur on the upper and lower face and at extra-facial sites. Lid and brow ptosis can be important adverse events in the periocular region.¹⁵ Any adverse events that do occur can generally be managed with simple interventions (Table 2).⁶³

In a review of adverse events reported to the FDA following esthetic BoNT-A treatment over a 13.5-year

Table 2 Common adverse events during esthetic BoNT-A treatment and their management. Reproduced with permission from Wollina U, Konrad H. Managing adverse events associated with botulinum toxin type A: a focus on cosmetic procedures⁶³

Symptom	Management
Pain	Temporary, usually needs no treatment
Bruising	Pressure and cooling, resolves with time
Lid ptosis	Apraclonidine or phenylephrine eyedrops
Brow ptosis	BoNT-A treatment for a 'brow-lift', fillers

period, 1031 adverse event reports were identified, of which 955 (93%) were considered to be nonserious.⁶⁴ No deaths were reported, and of the 36 serious adverse events, 30 (which included headaches, focal facial paralysis, muscle weakness, dysphagia, flu-like syndromes, and allergic reactions) were included as possible complications of BoNT-A treatment in the FDA-approved label, whereas the remaining six did not appear to be causally related to BoNT-A. Of the nonserious adverse events, the most common were lack of effect (63%), injection-site reaction (19%), and ptosis (11%).

BoNT-A has been used clinically for more than 20 years, and it is considered to have a good safety profile, with few, mild, temporary adverse effects.^{32,63,65} To a large extent, adverse effects with BoNT-A can be avoided or minimized through knowledge of likely side-effects and good technique.⁶³ In addition, thorough knowledge of the target structures allows physicians to select the optimal dose, time, and technique for the individual patient.

Despite the established safety and tolerability of BoNT-A, in April 2009 the FDA issued a statement that BoNT-A products would carry a “black box” warning.⁶⁶ This relates to the diffusion of toxin beyond the site of injection and noted that incidences of such events were generally related to therapeutic uses (cervical dystonia in adults and off-label indications of cerebral palsy in children and spasticity in adults). The statement further noted that there have been reports of symptoms consistent with toxin diffusion after esthetic use, although there are no definitive serious adverse event reports related to toxin diffusion associated with onabotulinumtoxinA at the licensed dose of 20 U for glabellar frown lines. Importantly, the statement stresses that dosage strength (potency) of different BoNT-A preparations are not equivalent and are not interchangeable between products.

The future of BoNT-A in esthetics

Ongoing studies comparing the safety and efficacy of commercial preparations, including incobotulinumtoxinA and onabotulinumtoxinA in esthetic use, are in progress and may help to refine our use of BoNT-A preparations. One area for future investigation includes the potential use of topical BoNT-A. While topically applied BoNT has been studied in 12 patients for the treatment for primary axillary hyperhidrosis with favorable results,⁶⁷ further investigation is required. Other studies have suggested improvement in crow’s feet after topical BoNT-A has been applied.⁶⁸

Conclusions

The role of BoNT-A in facial esthetics and rejuvenation continues to develop, with advances in BoNT-A products and formulations, and advances in application techniques. The next generation of BoNT-A products, which aim to improve existing formulations, are now becoming available.

Acknowledgments

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