The Science and Manufacturing Behind Botulinum Neurotoxin Type A-ABO in Clinical Use

Mitchell S. Wortzman, PhD; and Andy Pickett, PhD

BACKGROUND: Since the first comprehensive description of the physiologic effects of botulism toxicity in the 1820s, specific formulations of botulinum neurotoxin type A (BoNT-A) have been developed. Now, a new botulinum neurotoxin type A formulation (BoNTA-ABO; Dysport [abobotulinumtoxinA]; Medicis Aesthetics, Scottsdale, AZ) has been made available in the United States and these same physiologic effects have become beneficial clinical targets. This formulation has been used successfully for nearly 20 years in Europe and other countries under the trade name Dysport (Clostridium botulinum type A toxin–hemagglutinin complex; Ipsen Biopharm, Wrexham, UK). BoNT-A injections are administered to achieve temporary local flaccid paralysis of targeted muscles. Injection of BoNT-A formulations for aesthetic purposes was by far the most common minimally-invasive (nonsurgical) cosmetic procedure performed in the United States in 2008.

OBJECTIVE: The objective of this review is to describe the latest data regarding the mechanism of action of BoNTA-ABO, the potential roles of neurotoxin-associated proteins (NAP), the manufacturing standards for these biologic products, and the specific manufacturing process and characteristics of BoNTA-ABO.

METHODS: A systematic search using the US National Library of Medicine PubMed database was performed and the relevant articles were reviewed. Direct input and data from the worldwide manufacturer of Dysport have been included.

RESULTS: The four sequential steps in the mechanism of action of BoNTA-ABO are binding, internalization, translocation, and intracellular proteolysis of the target protein. Although all BoNT-A products must meet standards for quality, potency, and safety, they should not be considered equivalent formulations because they have different production strains of the bacterium C botulinum, as well as different isolation and manufacturing processes that result in unique product characteristics. The production steps for Dysport—including a unique proprietary purification process using column chromatography and a unique proprietary finishing process—result in consistent and unique product features. Studies confirm that Dysport has a high degree of long-term batch-to-batch consistency for a range of specified properties, including specific potency, protein composition, toxin complex charge-density properties, and endopeptidase activity. In the native, natural form, NAP protect the endogenous neurotoxin from degradation in the acidic environment of the stomach; in biologic formulations, they may have effects on the structural stability, binding, uptake, and transcytosis of BoNT-A products in other areas of the body. NAP are most likely to stabilize the neurotoxin in a vial of clinical product.

CONCLUSIONS: A thorough understanding of the mechanism of action, product characteristics, and effects of NAP is important to ensure appropriate and safe clinical use of BoNT-A products. Now approved in the United States, Dysport is an important addition to the group of available BoNT-A formulations. (Aesthetic Surg J;2009;29:S34–S42.)

BoNT-A is a naturally-occurring zinc metalloprotease produced by the Gram-positive, spore-forming bacillus Clostridium botulinum, which is normally found in soil and water. BoNT is the most toxic naturally-occurring substance known. The precise lethal dose of BoNT type A (BoNT-A) in humans is not known. However, extrapolation of data from primate studies suggests that the lethal...
dose of crystalline BoNT-A given intravenously or intramuscularly in humans is about 40 units/kg.\textsuperscript{5–8}

After oral ingestion, BoNT passes through the stomach and is absorbed from intestinal epithelial cells into the bloodstream. BoNT is then distributed systemically and travels to the neuromuscular junctions, where acetylcholine release from somatic and autonomic nerve terminals is blocked, causing chemodenervation and the paralysis of striated muscle.\textsuperscript{3,9,10} BoNT is naturally produced as large complexes that include the core neurotoxin protein and auxiliary nontoxic proteins that appear to protect the toxin from being broken down in the acidic environment of the stomach, thereby facilitating the translocation of BoNT across the intestinal mucosal layer.\textsuperscript{3} Different strains of the *Clostridium botulinum* bacterium produce seven serologically distinct types of BoNT neurotoxins: A, B, C, D, E, F, and G.\textsuperscript{3,10} The toxin types are defined based on their absence of cross-neutralization (eg, anti-A antitoxin does not neutralize neurotoxin toxin types B through G).\textsuperscript{9} In addition, there are known to be multiple subtypes of each serotype, giving a total of some 17 BoNT subtypes currently identified. New subtypes are being identified and characterized routinely.\textsuperscript{11}

Justinius Kerner, a poet and medical officer in Germany, first described the symptoms, physical findings, and progression of botulism poisoning based on a review of 230 cases in two manuscripts published in 1820 and 1822.\textsuperscript{12} At that time, Kerner suggested the clinical use of BoNT to block abnormal motor movements for such conditions as chorea.\textsuperscript{13} However, no further progress on the science and certainly not on the consideration of BoNT for medical use was made until the late 1940s, when researchers at Fort Detrick, evaluating BoNT for military applications, developed concentration and crystallization techniques using acid precipitation. These techniques became the basis of manufacturing for clinical products in use today.\textsuperscript{5,13} The toxin was eventually introduced to the academic community and researchers at Johns Hopkins were the first to use small doses to paralyze the limbs of chicks.\textsuperscript{13}

**CLINICAL APPLICATIONS OF BoNT**

In 1973, Dr. Alan B. Scott, a senior scientist at the Smith Kettlewell Eye Research Institute in San Francisco, CA, published the first results of primate experiments in studies seeking a nonsurgical treatment for strabismus, which became the first data on potential medical uses of BoNT.\textsuperscript{14} Techniques for freeze-drying, stabilizing with human serum albumin, and assuring the sterility, potency, and safety of a formulation were achieved by Dr. Scott in close collaboration with Edward Schantz, originally based at Fort Detrick, Frederick, MD, then later at the Institute for Food Research at the University of Wisconsin, Madison, WI. This work culminated in 1977 in an application to the US Food and Drug Administration (FDA) for investigational treatment in human strabismus.\textsuperscript{13}

Since this early work, clinical uses of BoNT, particularly serotype A, have been evaluated extensively. BoNT-A is currently used successfully for numerous therapeutic and aesthetic applications and is supported by more than 20 years of clinical experience.\textsuperscript{12} According to a recent conversation with a major US healthcare insurer, there are approximately 200 documented therapeutic and aesthetic applications for BoNT (Pickett, personal communication, June 2009). Because of its unique pharmacology, efficacy, and wide range of applications, BoNT has become one of the most widely used biologic agents worldwide.\textsuperscript{12,13}

BoNT-A is commonly used for aesthetic purposes. Facial wrinkles are caused by repeated muscle contraction and can be of concern to patients. Glabellar lines, the wrinkles that form between the eyebrows, are a common problem. This application for BoNT-A was apparently discovered serendipitously when it was noticed that frown lines disappeared in patients who received injections around the eyes and upper face while being treated for blepharospasm.\textsuperscript{15–17} Focal injection of BoNT-A causes a temporary flaccid paralysis of the underlying facial muscles, leading to a flattening of the facial skin and improved appearance.\textsuperscript{12} Targeted injections of BoNT-A have revolutionized the approach to aesthetic rejuvenation of the aging face by providing a safe and effective method of improving facial appearance that can be targeted to individual goals. Currently, BoNT-A has many uses in facial rejuvenation, including the treatment of vertical glabellar frown and horizontal forehead lines, wrinkles from actinic damage, lateral canthal lines (crow’s feet), nasal flare, eyebrow elevation or shaping, facial asymmetry, upper lip creases, and chin dimpling.\textsuperscript{14,18} Aesthetic use is likely the single most common clinical application of BoNT-A.\textsuperscript{13}

Optimizing the efficacy of BoNT-A and minimizing the potential for adverse events (AE) requires knowledge of the toxin’s potency, precise injection techniques, and a clear understanding of how the toxin has the potential to cause disease if used inappropriately.\textsuperscript{19} After focal injection for aesthetic use, BoNT-A should not be present in the peripheral blood at measurable levels and studies have shown that administration of the recommended amounts at each treatment does not lead to systemic effects.\textsuperscript{6,17,20} Indeed, BoNT is considerably less toxic in the blood than when injected intramuscularly, having a relatively short half-life of approximately 230 to 260 minutes for native, nonmetabolized toxin in the blood.\textsuperscript{18,21} Healthcare professionals should be aware that botulism could result from iatrogenic overdose, misinjection, or use of unlicensed preparations of BoNT-A.\textsuperscript{5,18,22} Rare cases of generalized botulism-like syndrome have been reported after medical uses of BoNT-A at the higher clinical dosages employed for the treatment of adult and pediatric patients with multiple sclerosis, torticollis,\textsuperscript{23} focal hyperhydrosis,\textsuperscript{24} and muscle spasticity.\textsuperscript{25,26}

The use of unlicensed toxin preparations has caused serious harm to patients.\textsuperscript{6,27,28} No cases of systemic bot-
ulism with detectable serum toxin had been attributed to aesthetic botulinum toxin injections until November 2004, when four suspected botulism cases were reported to the Centers for Disease Control and Prevention; systemic botulism was confirmed with laboratory testing. All four patients had been injected with a highly concentrated, unlicensed preparation of BoNT-A and may have received doses that were about 3000 times the estimated human lethal dose by injection. An update on the incident issued by the FDA in August 2008 confirmed the extent to which such unlicensed BoNT-A had been used by clinicians and the depth to which the FDA had gone in its ongoing investigations, with concomitant prosecutions. Healthcare professionals should ensure that they use only approved formulations of BoNT-A. Unfortunately, numerous counterfeit or look-alike BoNT products exist and are readily available from Internet sites.

DIFFUSION VERSUS SPREAD
The subject of the “movement” of BoNT-A away from the site of injection has been extensively discussed. Many terms have been used to describe this movement and there is concern that adverse effects may result from it. However, only two terms are applicable in context. The term “spread” refers to the process that occurs as a result of the injection itself. This process is fast and active, and is governed by the injection skills and practices of the administering clinician. The term “diffusion” refers to the subsequent slow and passive process that occurs as unbound toxin moves away from the injection site, down the concentration gradient. BoNT-A must move away from the injection site until all of it is bound, a process that takes several days to complete. Like any other high local concentration of molecules, BoNT-A will move to attain a concentration equilibrium; unlike other molecules, it will be actively taken up and bound to toxin receptors during this process. Clinicians can affect the spread stage by injecting more or less volume. Changing the concentration of BoNT-A in a given injection will also affect diffusion to a greater or lesser degree; the higher the concentration injected, the more BoNT-A molecules will be present and taken up over a larger area of receptors.

Data from rodent studies recently raised the concern that BoNT-A may migrate to remote areas of the central nervous system (CNS). In one study, BoNT-A that had been injected into the whisker muscles of rodents was found in remote areas of the CNS. However, the study has been criticized and contains a number of aspects that are not applicable to the clinical use of BoNT-A, especially in the low doses used for aesthetic applications. Most importantly, the doses used in the rodent studies were too large to extrapolate the results to use in humans and could therefore be considered to have overloaded the systems under examination. In addition, there is no evidence from long-term clinical use in humans that BoNT-A injected into peripheral muscles, skin, or other tissues causes clinically detectable effects on the CNS. In patients who have received repeated injections of high doses over a long period of time, such as those with dystonia, there is no evidence from muscle biopsy to suggest any permanent muscle degeneration or atrophy.

Despite the safety of BoNT products shown in these studies, after a comprehensive safety review of marketed BoNT formulations, the FDA recently required all manufacturers of approved and marketed BoNT products to update their labeling to include a boxed warning describing postmarketing safety data on distant spread of toxin effects. The warning applies equally to therapeutic and aesthetic uses of BoNT products, despite the FDA’s acknowledgement that the risk has not been identified in any reports for aesthetic treatments when products are used as recommended (see www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm175013.htm). The FDA explained that there have been serious AE reported in association with the unapproved use of such products for aesthetic purposes. Patients treated with BoNT have shown electrical effects remote from the site of injection and changes to brain activity have been identified in several studies. However, these effects are more likely to be related to CNS plasticity and adjustment of the body to the limited paralysis induced by the local doses.

The next issue to address is local diffusion of BoNT-A after injection at therapeutic doses. Concerns have been raised in the literature regarding enhanced diffusion of BoNT-A formulations with so-called “smaller complex size,” leading to AE. It is important to clarify the terminology that has been used in the literature to describe this event.

First, reports from studies showing greater diffusion with Dysport injections have been linked by the reporters to smaller toxin complex particle size; however, information on complex particle size has never been published because there are not sufficient or robust scientific data to publish at this time. These data are exceptionally difficult to obtain for a BoNT-A complex under physiologically relevant conditions. Reports of greater diffusion arise from studies where the doses of Dysport administered have been higher in comparison to the other agent studied. These reports of greater diffusion show that it is a dose-driven phenomenon. Next, despite publications that suggest otherwise, there is no link or relationship between BoNT-A complex size, dissociation, and diffusion because very soon after injection, the toxin complex rapidly dissociates under physiologic conditions, releasing the neurotoxin core to act. This dissociation happens for all BoNT-A complex products. The complex size, having no relationship to diffusion, cannot therefore have a role in any toxin migration to distal sites after injection.

Therefore, claims of differences among products in complex size and diffusion or dissociation characteristics...
are flawed. Pickett wrote a review of these topics that was published in the journal *Toxicon* in 2009.32

It is important to note that all three botulinum toxin products are now required to carry a boxed warning about possible spread of toxin effect. The FDA mandated this step because of reports that effects of BoNT may spread from the area of injection to other areas of the body and cause symptoms such as unexpected loss of muscle strength or muscle weakness, blurred vision, and eyelid ptosis.46 Again, no definitive reports of serious AE have been associated with the approved use of treating glabellar lines when BoNT products are used in accordance with labeling. Symptoms have been reported mostly in children with cerebral palsy treated for muscle spasticity, an unapproved use of BoNT in the United States. The doses used in this unapproved indication are often much higher than those used for FDA-approved indications.45

**OVERVIEW OF THE SAFETY OF BoNT-A-ABO FOR AESTHETIC USES**

Numerous studies have shown that focal injections of BoNT-A formulations for aesthetic indications are safe and are associated with only minimal side effects when appropriate dosing and intramuscular injection techniques are followed.3,4 Moy et al47 recently reported the results of a study evaluating the safety of Reloxin (ie, BoNTA-ABO; Dysport [abobotulinumtoxinA]; Reloxin was the proposed trade name during the product’s clinical development program) for the treatment of glabellar lines in an open-label assessment of 1200 patients who each received up to five treatments over 13 months.37 Dysport was diluted in 2.5 mL of sterile preservative-free physiologic saline solution (0.9%) to a concentration of 50 units (U) per 0.25 mL. For each treatment, 0.05 mL was injected into each of five injection sites (10 units each) in the glabellar area on day zero of each treatment cycle. There was a minimum 85-day interval between treatments. Most (72%) treatment-emergent AE were considered to be unlikely or not related to study treatment. Overall, 36% of patients experienced AE that were probably or possibly related to treatment, with the most frequently occurring being injection site events (18%), nervous system disorders (14% overall and 12% headache), and ocular events (9% overall and 4% ptosis). Ptosis occurred in fewer than 2% of patients in any treatment cycle. AE around the injection site or eyes were usually reported by day seven and then resolved. A total of 45 patients had a total of 55 instances of ptosis across all cycles; most episodes lasted less than three weeks and decreased in incidence during successive cycles from 2.4% in cycle 1 to 0.6% in cycle 5.46 In a placebo-controlled pivotal study of Dysport (20, 50, or 75 units of Dysport, or placebo injected across the glabellar area), the most common AE were mild headache and nasopharyngitis, with a similar incidence observed in all groups.58

**LICENSED BONT PRODUCTS**

Several licensed commercial preparations of BoNT are available for clinical use with specified indications. The first BoNT-A formulation, Botox (originally called Oculinum), was marketed in the United States for therapeutic use in 1989 by Allergan (Irvine, CA). However, issues with the inactive toxin protein content of the original Botox, which caused significant levels of antibody formation in patients,49 led Allergan to change the product in 1998.50 The new formulation was called New Botox. In 2002, the same new formulation was also launched as Botox Cosmetic for aesthetic use (glabellar lines).9,17 In 2000, a BoNT type B, Myobloc, was approved by the FDA for therapeutic use and marketed by Elan Pharmaceuticals (Dublin, Ireland).9,16 PurTox (Mentor, Santa Barbara, CA), a “purified BoNT” formulation that does not contain NAP, is undergoing phase III testing.51 In addition, a new formulation of botulinum toxin type A (Xeomin [BTX-A, NT 201]; Merz, Frankfurt, Germany) that is also free of complexing proteins is licensed in a number of countries, including Europe, South America, and Canada.51

In 1991, a BoNT-A formulation called Dysport (C botulinum type A toxin–hemagglutinin complex) was first marketed in the United Kingdom and then in Europe by Ipsen (Wrexham, UK). Since that time, Dysport has been developed for use with a variety of indications in the therapeutic areas of neurology, rehabilitation medicine, ophthalmology, and dermatology, including cervical dystonia, adult and pediatric spasticity, blepharospasm, and hemifacial spasm.9 The FDA recently approved Dysport (abobotulinumtoxinA; Medicis Aesthetics, Scottsdale, AZ) for use in the United States for the treatment of cervical dystonia and glabellar lines.52

**MECHANISM OF ACTION OF BoNT-A INJECTION: REVERSIBLE FLACCID PARALYSIS OF TARGETED MUSCLES**

The BoNT-A complex typically contains a core neurotoxin protein, a nontoxic nonhemagglutinin (NTNH) protein, and several hemagglutinin (HA) proteins.53 The core neurotoxin protein has a mass of approximately 150 kDa.52 These large BoNT complexes are most stable in the pH range of 5 to 7 because the protein subunits dissociate at higher pH levels.4,54 BoNT-A complexes may contain other NAP derived from the NTNH protein.52 Figure 1 depicts the structure of the BoNT-A molecule.

The 150-kDa neurotoxin protein is a single chain protein and is the component that has the pharmacologic activity, but only when cleaved by a proteolytic enzyme to form two polypeptide fragments—a 100-kDa heavy chain (Hc) and a 50-kDa light chain (Lc)—after production by the bacteria; these are linked by a disulfide bond. The Hc has two functional units: a receptor-binding domain with high affinity for a specific polysialogated membrane ganglioside “receptor” and, separately, a protein named SV2 in the presynaptic membrane of motor nerves and a translocation domain that binds to transmembrane vesicle proteins after internalization of the toxin molecule, leading to a conformational change and
the formation of ion channels. The Lc is a zinc-dependent endopeptidase.5,55

With the advances in clinical formulations of BoNT has come an enhanced understanding of its molecular mechanism of action.52 The clinical benefits of BoNT, as far as is known in detail to date, are mainly caused by the effects of the toxin on intracellular targets and prevention of acetylcholine release from synaptic vesicles at the presynaptic membrane in the neuromuscular junction (NMJ) of muscles, producing chemodenervation. This action effectively destroys the activity of the affected neuromuscular junction, causing muscular paralysis.3 More recent data indicate that a maximum paralysis of an NMJ in the order of 80% of the original activity56 can be achieved; the reason for the residual activity and why it cannot be entirely eliminated are not yet fully known.

Figure 2, A illustrates the processes of normal neurotransmitter release.3,10 At the NMJ, presynaptic motor nerve endings containing acetylcholine-filled synaptic vesicles are in close contact with the postsynaptic NMJ and muscle fibers. In normal neurotransmission, the synaptic vesicles fuse with the presynaptic neural cell membrane and release their contents into the synaptic cleft (space) through exocytosis, a calcium-dependent process. The “docking” of synaptic vesicles with the neural cell membrane is facilitated by the formation of a complex of proteins known as soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) proteins. The SNARE proteins include a 25-kDa synaptosomal-associated protein (SNAP-25), a vesicle-associated membrane protein (VAMP), synaptobrevin, and syntaxin. These proteins anchor and bind the vesicle membrane to the presynaptic neuronal cell membrane by linking in a synaptic fusion complex.57 The exact mechanisms involved in the SNARE complex formation are only just becoming clear; detailed data have now become available on both the timing of the SNARE protein interactions with each other and the molecular forces involved.58 After docking and fusion, a pore in the vesicle and membrane opens and the acetylcholine released traverses the synaptic cleft, binds to receptors on muscle cells, and initiates muscle contraction postsynaptically through a series of further complex reactions.3

BoNT induces reversible cholinergic blockade at the NMJ by preventing vesicle fusion with the presynaptic membrane through the enzymatic cleavage of one or more of the SNARE complex proteins, reducing acetylcholine release into the synapse, and decreasing muscle contraction.3 BoNT does not affect the synthesis or storage of acetylcholine or the conduction of electrical signals along the nerve fiber.3 The mechanism of action of BoNT is shown in Figure 2, B.3,10 There are four sequential steps: binding, internalization, translocation, and intracellular proteolysis.3,52,54,59

**Step 1: High affinity binding.** BoNT has a high affinity for receptors in the neural cell membrane, which increases the targeting of BoNT. The Hc is responsible for this highly selective binding mechanism, which involves interaction with the acceptor protein SV2 (for BoNT-A) and polysialated membrane gangliosides. This binding may also be facilitated by the NAP in the complex.58 The concept that the binding takes place in fast and slower reactions with each component is now well established.54,60,61

**Step 2: Internalization.** The neural cell plasma membrane may invaginate around the toxin-receptor complex and form a vesicle within the neural cell that contains the toxin. However, the greater likelihood is that the vesicle may be a recycling synaptic vesicle that has just become emptied of acetylcholine.54,60

**Step 3: Translocation.** The endosome releases the Lc, a zinc-dependent endopeptidase (ie, metalloprotease) by transmembrane crossing as a result of a pH gradient between the vesicle and the cytoplasm of the NMJ.60 During this step, the Hc acts as a “chaperone” for the translocation of the Lc.54,59 Specifically, the Lc unfolds on the inside of the endosome, is transported through a pore opened in the neural cell membrane with the assistance of the Hc, and then refolds on the
other side, within the presynaptic NMJ. The translocation process is pH-dependent.\textsuperscript{54,58–60}

\textbf{Step 4: Intracellular proteolysis.} Once located at the cytosolic surface of the NMJ presynaptic membrane, the Lc cleaves one of the SNARE proteins, depending upon the BoNT serotype. This cleavage of one of the SNARE proteins results in fundamental damage to one or more of the proteins involved in SNARE complex formation and therefore interferes with vesicle fusion and neurotransmitter exocytosis.\textsuperscript{3,54,59} The specific target of BoNT-A is the SNAP-25 protein, which is one of the protein components essential for docking and fusion of the synaptic vesicle and hence for the exocytic release of neurotransmitters. The BoNT-A Lc only cleaves the critical and highly specific Gln197–Arg198 peptide bond on the inner surface of the presynaptic membrane. Therefore, acetylcholine cannot be released when the nerve is stimulated, resulting in flaccid muscle paralysis. The interaction of the SNAP-25 protein with the Lc is based on critical molecular geometry and is very sensitive to minor changes.\textsuperscript{54,61} Also, minor changes to the molecular structure of the catalytic Lc site lead to inactivity.\textsuperscript{54,61} The enzymatic activity of the Lc also depends on the BoNT serotype; serotype A cleaves SNAP-25 and serotype B cleaves VAMP.

\textbf{A POSSIBLE ROLE FOR NEUROTOXIN-ASSOCIATED PROTEINS}

Whether found in nature or developed in culture media, BoNT formulations are associated with nontoxic NAP during their production by the bacteria.\textsuperscript{50,58} In addition to the core neurotoxin protein, \textit{C botulinum} type A also secretes five other NAP, which are expressed from the BoNT-A gene cluster.\textsuperscript{56,63} These NAP have also been called NTNH proteins.\textsuperscript{64} In most cases, the function of these NAP is unknown. Early work revealed the hemagglutination ability of some NAP and hence distinguished these by calling them hemagglutinins (abbreviated HA or Hn).\textsuperscript{62} There is some evidence that the BoNT-A complex also has small amounts of other proteins such as flagellin, which are not expressed directly as part of the BoNT-A gene cluster, but whose genes are present in the bacterial genome in nearby locations.\textsuperscript{52,65}

One of these NAP, hemagglutinin-33 (Hn33), has been studied in detail and has been shown to increase the endopeptidase activity of the neurotoxin core of BoNT-A by 21-fold in \textit{in vitro} studies. Hn33 has been reported to increase the endopeptidase activity against SNAP-25 by 13-fold in rat synaptosomes.\textsuperscript{66} The mechanism may be through enhancing the binding of BoNT-A toxin complex to glycolipids and glycoproteins found in cell membranes. NAP also may serve as bridging units for higher-order structures of the toxin complexes.\textsuperscript{58} In addition, NAP may play an important role in influencing the structural stability of BoNT and facilitating internalization and translocation.\textsuperscript{58} The content of NAP in BoNT-A formulations may be an important factor for maintaining the activity of these agents. NAP may even be developed for use as activators for BoNT-A formulations for therapeutic use.\textsuperscript{58,65}

There is evidence to suggest that NAP are useful in preserving the stability of BoNT-A when it is in the product vial.\textsuperscript{67,68} In fact, after removal of NAP, significant additional quantities of human serum albumin are required to stabilize the BoNT-A component.\textsuperscript{59} High quantities of human serum albumin are unnecessary for the complex-based BoNT-A products, with \textit{Dysport} having the lowest content of all.

\textbf{THE MANUFACTURING PROCESS FOR DYSPORT: PRODUCT CONSISTENCY}

The manufacturing process of clinical-grade BoNTA-ABO takes place in two stages. The first stage is the production of the highly potent BoNTA-ABO bulk toxin, the drug substance (DS). Because of the quantities of
BoNTA-ABO being produced and handled, this can only be made in exceptional facilities that meet the highest safety standards. In addition, the DS must be made under current good manufacturing procedures in order to meet the requirements for a licensed biologic product. These two critical aspects of DS manufacturing can be at opposition with each other because of their very nature, but this is resolved by manufacturing in high-grade, clean room facilities, with the actual manufacturing procedures carried out in containment systems to keep the BoNTA-ABO and the technical staff separated. These clean rooms have special air filtration systems to meet the required standards of pharmaceutical cleanliness. Also, the containment systems have separate and independent environmental systems with fail-safe mechanisms to ensure that both the staff and the environment are fully protected against DS release at all times. The entire operations provide the most stringent pharmaceutical standards in the industry, exceeding those required for even cytotoxic drug handling.

The second stage is the formulation, filling, and freeze-drying of the final dosage form, the Dysport drug product (DP). With the exception of the formulation stages, which are also carried out in containment systems to provide additional biosecurity, the formulation, filling, and freeze-drying of the DP are performed in standard, highest-grade clean room conditions—even though only a small amount of DS is being handled. Typical equipment used in the manufacturing of injectable DP is employed.

Clinical formulations of protein biologics produced by living organisms are difficult to manufacture and must be purified and highly standardized before clinical use. The clinical pharmacology of each formulation can be described as a function of the manufacturing process, which usually requires an intricate series of isolation and purification steps that must be carried out under very specific and reproducible conditions. Although the same purified protein may be produced, any differences in the manufacturing processes may result in differences in the final product.

In the manufacturing process for clinical BoNT-A formulations, the 150-kDa protein associated with HA and NTNH proteins is isolated and purified after bacterial fermentation. The distinct bacterial strains and processes used for fermentation and purification of BoNT formulations result in important differences in the multiprotein complex surrounding the core neurotoxin protein. Therefore, BoNT-A formulations are not interchangeable and have specific dosing and administration requirements (ie, injection volumes, techniques, and patterns).

The recommended doses for glabellar lines for the two formulations in the United States are as follows: (1) for Dysport, a total dose of 50 units divided in five equal aliquots of 10 units each (single-use, sterile 300-unit vial for reconstitution with 2.5 or 1.5 mL of 0.9% NaCl injection USP); (2) for Botox Cosmetic, a total treatment dose of 20 units in 0.5 mL (concentration of four units/0.1 mL of 0.9% sterile, nonpreserved saline).

In addition, the methods of product potency assay are different between the BoNT-A products, resulting in one LD50 unit of one product not being the same as an LD 50 unit of another. Despite differences in the final products, the manufacturing process for biologic products must ensure that every batch of the resulting product meets detailed specifications and standards for protein composition and has consistent biochemical characteristics and biologic activity.

After fermentation from a proprietary Ipsen C botulinum Hall strain, the unique proprietary manufacturing process for the BoNT-A formulation of Dysport includes the purification steps of acid precipitation followed by column chromatography to yield the highly potent DS. Following dilution, formulation, and filling, the result is a sterile, freeze-dried BoNTA-ABO product.

Interbatch reproducibility and comparability of the toxin–hemagglutinin complex in the final formulation ensure the consistency of clinical material. Full consistency data for batches of Dysport bulk toxin produced over a 14-year period were recently reported. The results showed that the formulation contains all protein products of the C botulinum type A neurotoxin gene cluster, including the neurotoxin core protein (Hc and Lc components), NTNH, and HA70, HA34, and HA17.

The Dysport formulation of BoNTA-ABO has a consistent batch-to-batch toxin protein load per vial of product (the amount of foreign protein per vial) with a mean toxin protein content of 4.35 ng per 500 LD 50-unit vial, based on data from multiple Dysport bulk toxin and clinical product batches across the manufacturing history. No similar data on the toxin protein content of the DP have been published for the other BoNT-A products, so no comparisons can be made based upon scientific information and batch reproducibility over time. The US package insert for Botox notes that the product contains approximately 5 ng of toxin protein and provides no additional supporting information. However, the Botox product was changed in 1998 because of concerns about immunogenicity and other issues. The protein load per clinical dose is important to clinicians because it bears directly on the toxin protein load administered and therefore the potential for the development of an immune response and subsequent formation of anti-BoNT neutralizing antibodies, which may lead to secondary nonresponse.

Endopeptidase activity assays are used to measure the specific cleavage of SNAP-25 by the BoNT-A Lc component in the bulk toxin, preformulation. The cleavage of SNAP-25 is the most crucial step before prevention of acetylcholine release by BoNT-A. SNAP-25 endopeptidase activity, the key biologic characteristic of an active BoNT-A clinical formulation, was shown to be similar across three tested Dysport batches. This activity was shown by looking at the half-maximal SNAP-25 cleavage; for the three
batches, the toxin protein concentrations were within a narrow response range (0.2–0.3 ng/mL)\textsuperscript{52}.

**CONCLUSIONS**

The use of BoNT-A formulations for the aesthetic treatment of facial lines is both safe and effective if the unique characteristics of each formulation are considered and specific dosing and administration recommendations are followed.\textsuperscript{4,70} Aesthetic use has become routine and many doses have been administered in clinical practice with no major complications.\textsuperscript{4,12} However, the available biologic preparations of BoNT-A are not interchangeable. Based on analysis of long-term manufacturing data, the Dysport formulation has been shown to have a high degree of consistency in protein load over the long-term, and similar consistency in biochemical and functional characteristics (such as endopeptidase activity).\textsuperscript{52} For clinicians, these results provide reassurance that this formulation is reliable for routine clinical use.\textsuperscript{52} Now approved in the United States, Dysport is an important addition to the group of available BoNT-A formulations.

**DISCLOSURES**

The authors were compensated for their contributions to this manuscript and as investigators for the study. The writing of this manuscript was funded by Medicis Aesthetics (Scottsdale, AZ). Dr. Pickett is employed by Ipsen Biopharm, the manufacturer of Dysport. Dr. Wortzman is employed by Medicis Pharmaceutical Corp., the US distributor of Dysport.

**REFERENCES**


44. Eisele KH, Taylor HV. Dissociation of the 900 kDa neurotoxin complex from *Clostridium botulinum* under physiological conditions. Presented in poster form at Basic and Therapeutic Aspects of Botulinum and Tetanus Toxins, Baveno, Italy, June 12–15, 2008 [P164 published as an abstract in *Toxicon* 2008;51(suppl 1):10.


72. Straughan DW. Progress in applying the three Rs to the potency testing of botulinum toxin type A. *Altern Lab Anim* 2006;34:305–313.


80. MRI pages/news_releases.html?d=H11005

81. Scottsdale, AZ 85256. E-mail: mwortzman@medicis.com. Reprint requests: Mitchell S. Wortzman, PhD, 7720 N. Dobson Rd., Scottsdale, AZ 85256. E-mail: mwortzman@medicis.com. Accepted for publication September 8, 2009.