Supplemental Article

IncobotulinumtoxinA (Xeomin): Background, Mechanism of Action, and Manufacturing

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Abstract

IncobotulinumtoxinA is the third botulinum neurotoxin type A (BoNTA) to be approved for aesthetic use in the United States. This article introduces the new product with an overview of clinical applications and a discussion of the neurotoxin's molecular structure. The role and clinical relevance of complexing proteins in BoNTA products are discussed. Finally, incobotulinumtoxinA's mechanism of action is described.

Keywords

facial aesthetics, glabellar lines, neuromodulators, neurotoxins, Botox, onabotulinumtoxinA, Dysport, abobotulinumtoxinA, Xeomin, incobotulinumtoxinA, botulinum neurotoxin type A

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IncobotulinumtoxinA (Xeomin; distributed by Merz Aesthetics Inc and Merz Pharmaceuticals LLC. Xeomin is also known by the tradename Bocouture for aesthetics use outside of the US), a new US formulation of botulinum neurotoxin type A (BoNTA), was approved by the US Food and Drug Administration in 2010 for the treatment of blepharospasm and cervical dystonia. A year later, in July 2011, it was approved for the correction of glabellar lines. IncobotulinumtoxinA has a long history of use outside of the United States. It was approved in Europe in 2005 and has been marketed in 20 countries, with more than 261 000 patients treated worldwide, according to the manufacturer. This article briefly examines clinical applications, the mechanism of action of BoNTA injection, unique properties of incobotulinumtoxinA, and the specific manufacturing process for incobotulinumtoxinA. Analysis of the safety and efficacy data is described in a separate article in this supplement.

CLINICAL APPLICATIONS OF INCOBOTULINUMTOXIN-A

BoNTA products are commonly used for therapeutic and aesthetic purposes, including elimination of facial wrinkles. Dynamic facial lines are caused by patterns of facial muscle contractions. Injection with BoNTA causes a temporary paralysis of the affected facial muscles, which leads to a flattening of facial skin and an improved facial appearance. Today, injection of BoNTA injection for facial rejuvenation is the most common cosmetic procedure in the United States[1,2]; in 2010, more than 2.4 million BoNTA

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procedures were performed, according to statistics from the American Society for Aesthetic Plastic Surgery.[3] IncobotulinumtoxinA is officially indicated for the treatment of adults with cervical dystonia, treatment of blepharospasm in adults previously treated with onabotulinumtoxinA, and the temporary correction of moderate to severe glabellar lines in adults.

MOLECULAR STRUCTURE OF INCOBOTULINUMTOXINA

There are 7 different serotypes of botulinum toxins: types A, B, C, D, E, F, and G. Each differs in its molecular structures and functions, and each is produced from a different strain of the *C. Botulinum* bacteria. Most toxins that are used clinically are made from the *Clostridium botulinum* type A (BoNTA), also known as the "Hall strain." They include the now familiar toxins onabotulinumtoxinA (Botox; Allergan, Irvine, California) and abobotulinumtoxinA (Dysport; Medicis Aesthetics, Scottsdale, Arizona), as well as incobotulinumtoxinA. There is currently only one form of BoNTB (made from the B strain), which is known as rimabotulinumtoxin (Myobloc; Solstice Neurosciences, LLC, Louisville, Kentucky).

BoNTA is a protein complex, comprising 2 parts— the "light chain" and the "heavy chain." The core botulinum toxin has a molecular weight of 150 kDa.[4] When the neurotoxin is secreted from the *C botulinum* bacteria, it is naturally part of a larger protein complex, approximately 900 kDa in size. Part of this complex is the neurotoxin itself (made up of the light chain and heavy chain previously mentioned). The remaining part of the BoNT complex consists of complexing proteins, which are approximately 750 kDa in total. The complexing proteins are made of hemagglutinin proteins and smaller nonhemagglutinin proteins.[5] Complexing proteins are sometimes referred to as accessory proteins, protective proteins, or neurotoxin-associated proteins.

Role of Complexing Proteins

The complexing proteins protect the toxin in their natural environment (in a pH range of 5-7) and dissociate at physiologic pH.[6-8] Figure 1 illustrates the dissociation of botulinum toxin complex under physiological conditions. Because of this rapid dissociation, there is no clear evidence that complexing proteins protect the active toxin when injected for therapeutic or aesthetic purposes. OnabotulinumtoxinA and abobotulinumtoxinA contain these complexing proteins, whereas incobotulinumtoxinA does not. (Complexing proteins are discussed in the review of the literature article later in this supplement.)

The amount of neurotoxin product, along with complexing proteins and residual proteins, defines the *foreign protein load*. The human immune system may recognize any part of this protein load as a foreign substance and possibly trigger an immune reaction after injection. Several studies, mostly in the therapeutic literature, have suggested that a higher total protein content might contribute

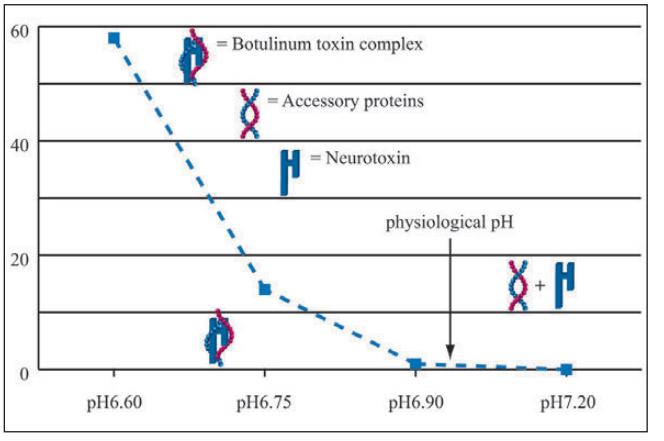

Figure 1. Dissociation of the botulinum toxin complex under physiological conditions. Adapted from Eisele KH. *Toxicon*. 2011;57(4):555-565.

to a greater chance of antibody formation.[9,10] As a result, the evolution of BoNTA products has corresponded to a reduction of the total protein content. The original onabotulinumtoxinA formulation contained 25 ng of neurotoxin complex protein per 100 units, whereas the current formulation contains only 5 ng of complex protein per 100 units.[11] Patients treated for therapeutic indications with the original formulation of onabotulinumtoxinA (used before 1998) were 6 times more likely to have blocking antibodies than those who received the newer formulation.[12] In a study of 130 patients treated for cervical dystonia with onabotulinumtoxinA, 4 of the 42 (9.5%) patients treated with original onabotulinumtoxinA, but none of the 119 patients treated exclusively with current onabotulinumtoxinA, had detectable blocking antibodies.[12] Müeller et al[13] reported on a study, also of 42 patients, who were treated with BoNTA for spasticity over a 2-year period, with a mean cumulative dose of 4610 units of onabotulinumtoxinA and 14 033 units of abobotulinumtoxinA. In that study, neutralizing antibodies were detected in 5 of 42 patients (12%). Three patients (6%) with neutralizing antibody titers were nonresponders to BoNTA treatment, most likely due to formation of neutralizing antibodies.

IncobotulinumtoxinA contains only 0.44 ng of neurotoxin complex protein per 100 units. Therefore, patients receiving incobotulinumtoxinA get the lowest amount of foreign proteins of the 3 currently US-available BoNTA products,[14] suggesting a possibly lower immunogenicity of incobotulinumtoxinA. The clinical relevance is unknown. In addition to concentrations of total protein content, antibody response is dose dependent; the higher the dose of the toxin, the higher the chance of antibody formation.[15] Nonresponse, in which patients do not respond at all to toxin treatment, is rare. However, some patients may experience secondary nonresponse, where, after 1 or more successful treatments, subsequent ones are ineffective because of antibody formation in response to the toxins. Because of the risk of antibody formation from either high-dosage one-time treatments or cumulative doses given

over time, the supplement authors recommend starting treatments at the minimal dose needed to achieve desired effects; they also recommend avoiding the possibility of "boostering" the immune system by not administering BoNTA more frequently than every 3 months, in accordance with product labeling. Clinicians have to be cognizant of this, since many patients today start BoNTA treatments at a relatively young age and are likely to receive a high cumulative dose over time.

DIFFUSION

It has been hypothesized that, because of the larger size of the toxin complex that contains complexing proteins, toxin diffusion from the injection site (and resulting adverse events) may be minimized.[16-19] It is thought that the smaller size of incobotulinumtoxinA might more easily diffuse away from target tissue and into adjacent tissues to produce an adverse event profile different from other BoNTA products. Clinical studies, however, do not support this hypothesis. Dodd et al[19] published a study of onabotulinumtoxinA, abobotulinumtoxinA, and a purified preparation of BoNTA (150 kDa), which showed that diffusion from the injection site did not differ among the 3 preparations. A study by Tang-Liu et al[20] showed no difference in the diffusion of the free or complexed form of BoNTA after injection into the muscle, even at high doses.

MECHANISM OF ACTION

With advances in new formulations of BoNTA has come improved understanding of its mechanism of action. In short, the clinical benefits of BoNTA are caused by the toxin's effects on intracellular targets and the prevention of acetylcholine release from synaptic vesicles at the presynaptic membrane in the neuromuscular junction (NMJ) of muscles, which produces chemodenervation. This action causes temporary muscular paralysis of the affected NMJ.[4]

Figure 2 depicts the BoNTA toxin molecule, and Figure 3 shows normal neurotransmission and the effect of BoNTA. In normal neurotransmitter function, the SNAP-25 protein is bound to the inside of the neuron cell membrane. Acetylcholine is transported inside the neuron by synaptic vesicles, and these synaptic vesicles attach to the neuron cell membrane by binding to SNAP-25 and forming

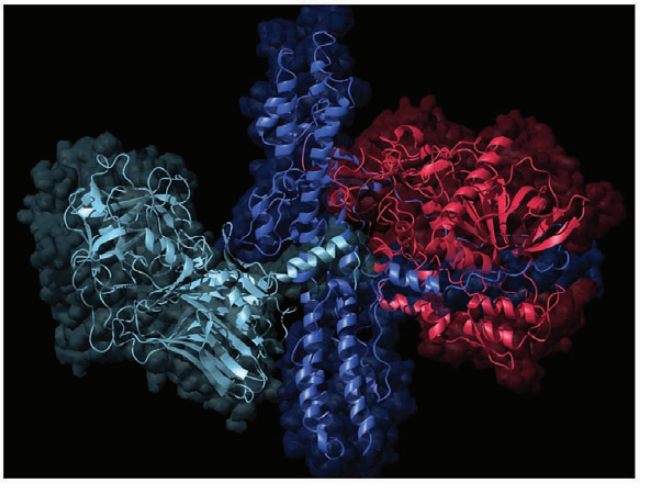

Figure 2. The type A botulinum toxin molecule. Image courtesy of Andy Pickett. Reprinted with permission.

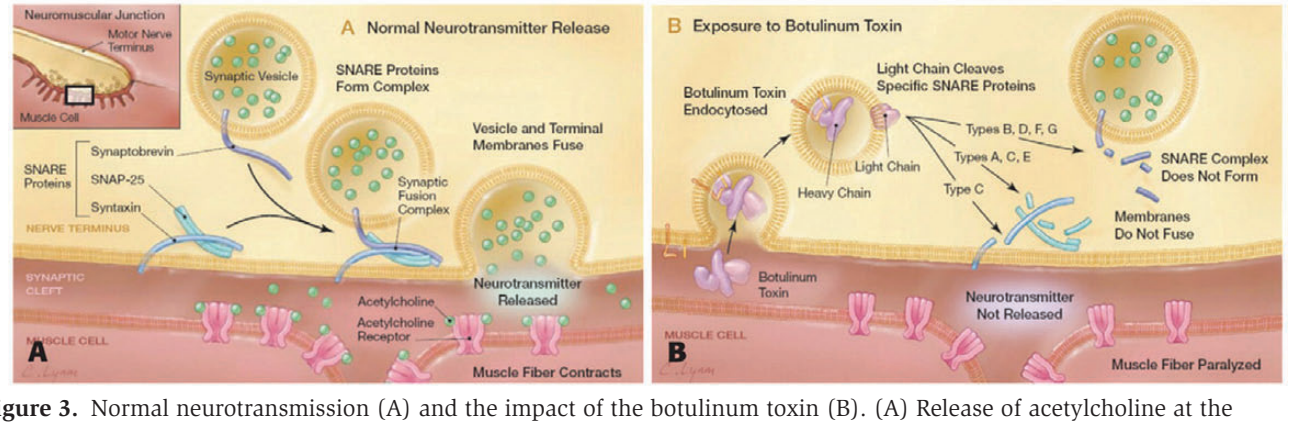

Figure 3. Normal neurotransmission (A) and the impact of the botulinum toxin (B). (A) Release of acetylcholine at the neuromuscular junction is mediated by the assembly of a synaptic fusion complex that allows the membrane of the synaptic vesicle containing acetylcholine to fuse with the neuronal cell membrane. The synaptic fusion complex is a set of SNARE proteins, which include synaptobrevin, SNAP-25, and syntaxin. After membrane fusion, acetylcholine is released into the synaptic cleft and then bound by the receptors on the muscle cell. (B) Botulinum toxin binds to the neuronal cell membrane at the nerve terminus and enters the neuron by endocytosis. The light chain of botulinum toxin cleaves specific sites on the SNARE proteins, preventing complete assembly of the synaptic fusion complex and thereby blocking acetylcholine release. Without acetylcholine release, the muscle is unable to contract. Reprinted with permission from Arnon SS, Schechter R, Inglesby TV, et al; Working Group on Civilian Biodefense. Botulinum toxin as a biological weapon: medical and public health management. *JAMA*. 2001;285:1059-1070.

a synaptic fusion complex. The synaptic vesicle then fuses with the cell membrane through exocytosis, a calcium-dependent process. Acetylcholine is released outside of the cell into the synaptic cleft (the area between the neuron and the muscle cell). Finally, acetylcholine binds to acetylcholine receptors on the muscle, causing muscular contraction.[21]

BoNTA inhibits the neurotransmitter function by blocking the transmission of acetylcholine from the neuron to the muscle. This process inhibits muscle contraction and causes muscle paresis or paralysis. Botulinum toxins specifically target the cholinergic neurons (those that use acetylcholine as a neurotransmitter). First, the heavy chain of the botulinum toxin binds to receptors outside of the neuron cell membrane. The cell then internalizes the toxin through endocytosis, forming a new vesicle, the endosome. It is through the endosome that the toxin enters the cell.[21]

Next, the light chain of BoNTA translocates from the endosome to the cystol of the neuron, where it cleaves the SNAP-25 protein, thus preventing vesicles carrying acetylcholine from forming the synaptic fusion complex. Acetylcholine transmission is, therefore, blocked because exocytosis can no longer occur due to the cleaved and rendered nonfunctional SNAP-25 protein. Without acetylcholine release, the muscle cannot move. New SNAP proteins are synthesized after approximately 3 months, which accounts for why the effects of BoNTA generally dissipate around this time.[21]

MANUFACTURING PROCESS

All BoNTA products are manufactured using a similar process. First, *C botulinum* bacteria are fermented in large batches in anaerobic conditions. The bacteria multiply and secrete the toxin. Bacteria are then removed from this mixture, leaving behind a mixture that contains only the toxin. This mixture is then separated and purified to isolate the toxin complex from residual components of the bacteria. Excipients, such as human serum albumin, are added to the toxin formulation. Depending on which of the 3 BoNTA are produced, NaCl, lactose, or sucrose may be used as excipients. IncobotulinumtoxinA undergoes a further purification to remove the complexing proteins. Finally, the solution undergoes lyophilization. Immediately before use, the product is reconstituted with sterile saline, preservative free in accordance with the label.

Formulations of protein biologics made from living organisms are difficult to manufacture and are purified and standardized before clinical use. The clinical pharmacology of each formulation can be thought of as the manufacturing process, each with its own intricate steps of isolation and purification that are carried out under highly specific and reproducible conditions. Although the same purified protein may be produced, differences in the manufacturing processes, known as biosimilars, can result in small differences in the final product.[22]

DISCUSSION: INCOBOTULINUMTOXINA IN CLINICAL PRACTICE

IncobotulinumtoxinA is supplied in 50- and 100-unit vials and is reconstituted using sterile, nonpreserved saline. The package insert contains detailed information about diluent volumes for reconstitution, but 0.25 mL of diluent results in 20 U per 0.1 mL in a 50-unit vial. Although the product labeling specifies nonpreserved saline, in the supplement authors' clinical experience, preserved saline is an acceptable alternative and may, in fact, minimize patient discomfort compared with nonpreserved saline. A 1:1 conversion ratio between onabotulinumtoxinA dosages and incobotulinumtoxinA dosages is used by the authors, who administer incobotulinumtoxinA in a similar fashion to onabotulinumtoxinA. Finally, incobotulinumtoxinA need not be refrigerated before reconstitution, unlike onabotulinumtoxinA and abobotulinumtoxinA. Reconstituted incobotulinumtoxinA solution should be administered within 24 hours after dilution. During this time period, reconstituted incobotulinumtoxinA should be stored in a refrigerator at 2°C to 8°C (36°C to 46°F). A box warning on incobotulinumtoxinA and the 2 other BoNTA products alerts users to the potential for distant spread of toxin beyond the injection site.

CONCLUSIONS

IncobotulinumtoxinA was recently introduced into the US market as the third BoNTA product for aesthetic uses. Its aesthetic indications include the temporary correction of moderate to severe glabellar lines. Its molecular structure and manufacturing process are similar to the other BoNTA products, with one notable exception: incobotulinumtoxinA is free of complexing proteins. The absence of complexing proteins has been associated with a decreased risk of the development of an antibody response in patients studied in clinical trials. The adverse event profile of incobotulinumtoxinA is similar to the 2 other BoNTA products. IncobotulinumtoxinA is supplied in 50- and 100-unit vials, similar to onabotulinumtoxinA, but does not require refrigeration before reconstitution.

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member for Medicis, Merz Aesthetics, Galderma, Compulink, and Allergan. Dr Sclafani is a paid consultant and grant recipient for Aesthetic Factors. Dr Sykes is an advisory board member for Mentor and Allergan and a member of the speakers bureau for Sanofi-Aventis and Medicis. Dr Waldorf is an advisory board member, paid consultant, and speakers bureau member for Merz Aesthetics, Medicis, Allergan, Valeant, Solta, Bropelle, P&G, Johnson & Johnson, Unilever, and Rhythera. Unless otherwise noted, the faculty and planners have nothing to disclose. Editorial and writing assistance for this manuscript was provided by Medical Education Advocates.

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