Formulation Composition of Botulinum Toxins in Clinical Use

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ABSTRACT

The use of botulinum toxin (BoNT) has now become the treatment of choice for a range of debilitating neuromuscular diseases and for aesthetic medicine. BoNT products are licensed as prescription-only medicines by the health authorities of each country in which they are approved. The authors describe here the current status with regard to BoNT formulations and detail more precisely what is in each product. These data have been presented previously, in a fragmented way, in papers that discuss characteristics of the BoNT products but not the formulations in detail. This information is essential for clinicians in order to enable informed decisions about which products they wish to use clinically. The authors have also examined developments that are either in progress or likely to occur, based on currently available information.

INTRODUCTION

Botulinum type A toxin (BoNT-A) is a well-established treatment for a number of clinical conditions involving muscle hyperactivity, including focal neurologic disorders. Since first approved by the U.S. Food and Drug Administration (FDA) 21 years ago to treat two eye-muscle disorders, blepharospasm (uncontrollable blinking) and strabismus (crossed eyes), BoNT-A products have been developed for use in a variety of indications within neurology, rehabilitation medicine, ophthalmology and dermatology, including cervical dystonia, adult and pediatric spasticity, blepharospasm, hemifacial spasm and for the treatment of aesthetic indications such as glabellar lines and hyperhidrosis.

Clostridium botulinum strains produce type A neurotoxin naturally as a complex that typically contains a core neurotoxin protein, a non-toxin non-hemagglutinin (NTNH) protein and several toxin-associated hemagglutinin (HA) proteins. The final product formulations used clinically are produced by the appropriate dilution of purified, highly potent bulk BoNT-A.

A growing understanding of the molecular mechanism of action of botulinum toxins has paralleled advances in their clinical use. Recent studies have led to the understanding that the 150-kDa core protein of BoNT-A toxin dissociates from the toxin complex under physiological conditions and is internalized into vesicular compartments, a process which requires binding to the SV2 synaptic vesicle protein and interaction of the heavy chain (Hc) component with polysialylated membrane gangliosides. From the vesicle, the light chain (Lc) component of the neurotoxin translocates into the neuronal cytosol with the aid of the Hc, as a result of the pH differences between the interior and exterior of the vesicle. The Lc then attaches to the inner surface of the cell membrane, once located in the region of the cytosolic surface of the membrane, the BoNT-A Lc specifically binds and proteolytically cleaves a member of the SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) family the 25-kDa synaptosomal-associated protein (SNAP-25). SNAP-25 is a SNARE component necessary for the exocytic release of neurotransmitters. Thus, BoNT-A blocks the release of the neurotransmitter acetylcholine; the other BoNTs also prevent acetylcholine release by cleavage of similar SNARE family member proteins. The elucidation of this knowledge to an increasing level of detail has made biochemical, functional and potency characteristics of products available to clinical researchers and practitioners.

There are numerous BoNT-A products available; not all of them are legal or licensed by any recognized regulatory authority. All the products however, have been formulated from high-potency, concentrated BoNT-A before filling into the vials to give the finished product. What is in these vials is important, as they are the legal or licensed products. Excipients in some protein products can also serve to stabilize the product and to provide a suitable environment within the vial for long-term preservation. Similar excipients are also included in type B toxin products.

Excipients

All current BoNT-A products are small-volume parenteral (injectable) products that contain excipients in the product vials. An excipient is an intentionally added substance (i.e., not a manufacturing impurity) that does not exert a therapeutic effect, but may act to improve product delivery. Excipients are often necessary to provide bulk to a product and may also fulfill roles such as wetting agent, solvent, emulsifier or preservative. Not all excipients are inert substances and some are macromolecular, such as human serum albumin (HSA), amino acids and sugars. Excipients in some protein products can also serve to aid in the availability of the active component post injection, although there is no evidence available that this is the case with any excipients present in BoNT products.
Licensed Botulinum Toxin Products

There are several licensed products containing BoNT. The two commercially licensed BoNT-A product is Xeomin® (Merz Pharma, Germany), available since 2005 in Germany and now subsequently in 20 countries, including European countries, South American countries and Canada. Xeomin is reported to comprise a preparation of the naked BoNT-A, purified free from the complexing proteins, but only one paper has been published from the manufacturer about this product in terms of either the biochemical characteristics or the purity, and this is a short review with no supporting data. Limited product data have been provided by other workers.

Overall, these three products are all presented in a dried, powder format, either lyophilized (Dysport and Xeomin) or vacuum dried (Botox). There are no substantial differences in these drying processes, although freeze-drying is more common for the long-term preservation of proteins and initially gave Dysport the advantage of refrigerator storage (2–8°C) instead of frozen at -5°C (Botox; now with amended storage conditions for also 2–8°C). Freeze-dried products tend to produce a typical cake, visible in the vial as a powder before reconstitution (mainly due to the bulking agent present), as opposed to vacuum drying, where only a vague haze is seen in the final product vial.

Alternatively available is Neurobloc/Myobloc (Eisai, Hertfordshire, UK), containing BoNT-B as the active component; this is offered as a solution with no requirement for reconstitution. The main characteristics of these products are described in Table 1.

There are a number of other BoNT-A products that are licensed in specific countries (BTX®, Lanzhou, China; Neuronox®, Mexico; Lantox®, Russia; Prosione®, Brazil), though several of these are from a common source. Generally, these are local products but also have limited distribution to other territories. Details of these are presented in Table 2.

Formulation of BoNT-A Products

BoNT is extremely potent, with BoNT-A being the most toxic protein known, having a lethal dose in humans of just 1 ng/kg. Therefore, products containing BoNT have an exceptionally low amount of the toxin protein in each product vial. For example, the Dysport product vial containing 500 units of BoNT-A contains just 4.35 ng of the BoNT-A protein complex, as shown in Table 1.

The exceptional potency means that only minuscule amounts of the bulk toxin are required for batch formulation. To accurately dispense the correct amount of toxin into each vial would be impossible, so the toxin must be initially diluted in order to dispense with precision. This is the method for the licensed BoNT products that are all manufactured by filling vials with a solution of the diluted, formulated active ingredient. Of note is that the units of each product, regardless of source, are specific to that product alone and are not interchangeable. This is due to the assay methods employed, while all being the same LD₅₀ method in principle, differ in their format and analysis.

The core BoNT protein is known to be unstable in water, especially when diluted. When produced by the bacteria, the toxin is manufactured in conjunction with other complexing proteins known to stabilise the core active neurotoxin. Therefore, one of the roles of the excipients added is to aid in the stabilization of the toxin protein in solution by controlling such parameters as

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**Table 1. Overview of Main Licensed BoNT Product Characteristics Containing Botulinum Toxin**

<table>
<thead>
<tr>
<th>Product</th>
<th>Dysport</th>
<th>Botox</th>
<th>Xeomin</th>
<th>Neurobloc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Company</td>
<td>Ipsen</td>
<td>Allergan</td>
<td>Merz</td>
<td>Solstice/Eisai</td>
</tr>
<tr>
<td>Toxin Type</td>
<td>A (+complex)</td>
<td>A (+complex)</td>
<td>A</td>
<td>B (+complex)</td>
</tr>
<tr>
<td>Units vial</td>
<td>500</td>
<td>100</td>
<td>100</td>
<td>2500</td>
</tr>
<tr>
<td>Toxin protein (ng)</td>
<td>4.35a</td>
<td>5</td>
<td>0.6b</td>
<td>55</td>
</tr>
<tr>
<td>Excipients</td>
<td>HSA 125 µgᵃ</td>
<td>HSA 500 µgᵇ</td>
<td>HSA 1000 µgᶜ</td>
<td>HSA 500 µg/ml</td>
</tr>
<tr>
<td></td>
<td>Lactose 2.5 mg</td>
<td>NaCl 0.9 mg</td>
<td>Sucrose 4.7 mg</td>
<td>NaCl 0.1 M</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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the pH and salt concentration and the steric conformation of the active. The pH of a solution can be the most critical formulation variable, affecting the active protein's conformational and colloidal stability when in solution, together with the aggregation rate/state of the protein (active or excipients) and the rate of degradation pathways, such as deamidation.

Also, for those products processed to finally remove the liquid component by drying, excipients are necessary to provide stability in the desiccated form, and ideally to provide a format, usually a powder, that can be seen with the naked eye, aiding the clinician in product reconstitution. Visualization of material in the container, although not necessary for activity, is reassuring to the consumer to provide visible confirmation of the presence of product and is valuable for the analysts in product testing, also enabling confirmation of the presence of product more readily.

Unfortunately, there is the added complication of mis-reporting of product composition in journals, for example the publication by Wenzel and colleagues comparing two botulinum toxin type A formulations using manufacturers product summaries, showing the need to check information is relevant and current, as highlighted in Pickett and Caird. Clinicians need to have access to accurate and up-to-date information on the products they are using in order to treat patients with a full understanding of product composition and effects and in order to and fully inform patients on the expected outcome, safety and also ethical aspects of their treatment.

**Excipients of Botulinum Toxin Products**

The four main licensed botulinum toxin products all contain HSA (Table 1), a highly-purified protein from human plasma. The type and grade of HSA used is identical to that used clinically as a product administered for the treatment of, for example, burns. Albumin is present in the blood of all mammals and many examples are available commercially (e.g., bovine, ovine, equine and porcine albumin). However, the albumin for the BoNT products is solely derived from human plasma. This is primarily because the BoNT-A products are parenteral drugs and therefore the risk of adverse reaction to any foreign protein is significantly lower with a human derived component than with any other species. Using HSA means that only a native human protein is being administered with the BoNT.

The use of HSA in BoNT products has been well established for over 20 years, since the original BoNT-A products were licensed. Both Dysport and Botox contain HSA, supplied as licensed pharmaceutical products in their own right by one of the worldwide plasma fractionation companies before being employed as an excipient. This ensures the highest standard of HSA available, meeting all United States (U.S.) and European pharmacopoeial requirements, in particular the mandatory safety requirements that ensure the product is free from viruses and related contaminants. Despite being a human plasma-derived product, HSA has an exceptional safety record. There have been no known viral transmissions due to HSA therapeutic uses. Equally, no known viral transmissions have been reported from use of licensed BoNT products containing HSA.

The role of HSA is clearly as a stabilizer for the BoNT products, both BoNT-A and BoNT-B. Normally, the other proteins of the BoNT complex, produced at the same time as the neurotoxin by the bacteria, act to stabilise the BoNT. However, under physiological conditions, the complex disintegrates to free the naked neurotoxin molecules to bind and act. The BoNT cannot act unless it has been freed and significant data exist to demonstrate this dissociation. The pH within the BoNT product vials is physiological after reconstitution with Saline for Injection (European Pharmacopoeia, U.S. Pharmacopoeia) and there is therefore a clear possibility, even a likelihood, that the complex present for several BoNT-A products is already dissociated in the vial prior to injection, as the formulation solution at physiological pH leads to dissociation of the complex. The excess of HSA (Table 1) will therefore stabilize the neurotoxin in the absence of the complexing proteins and within the vial to provide a protein-rich environment for the BoNT. No details are yet known about the binding of HSA to BoNT, if at all, but the molecular size of HSA is approximately 66 kDa as compared to the BoNT of 150 kDa. The possibility that several molecules of HSA become bound to BoNT exists, surrounding and protecting the active. The pH of a solution can be the most critical formulation variable, affecting the active protein's conformational and colloidal stability when in solution, together with the aggregation rate/state of the protein (active or excipients) and the rate of degradation pathways, such as deamidation.
BoNT as a substitute for the original complexing proteins. Further work on this area needs to be performed.

The HSA component may also act to decrease the adsorption of the toxin onto the glass walls of the container and subsequently the syringe, and decrease the aggregation of the toxin molecules. There are, however, no scientific data yet published on the adsorption of the toxin to surfaces and therefore no conclusions about this aspect can be reached; equally likely is an inactivation of the toxin, for as yet unidentified reasons, instead of adsorption. In the Xeomin preparation, the product consists of the BoNT-A neurotoxin, devoid of the usually associated complexing proteins, and so the HSA concentration is much higher than in Dysport and Botox (Table 1).

The lactose component of the Dysport preparation protects the steric conformation of the neurotoxin during the drying procedure, as does the sodium chloride in the Botox formulation.

Unlicensed Products
Today, numerous BoNT-A products are available. Apart from the licensed products (Table 1) that are only available through their authorized distribution networks, the Internet has provided a wide selection of low cost, illegal alternatives. However, most of these products are manufactured by unlicensed companies in unlicensed premises with unknown standards of quality. What has also become clear is that many of these illegal and unlicensed BoNT-A products contain animal-derived components such as gelatin.

The alternative products have a variety of compositions. In addition to the apparent BoNT content (which is not always there) excipients such as gelatin (a tonicity adjusting agent), and dextran, mannitol and sucrose (all bulking agents and cryoprotectants) have been used.

The emergence of a dynamic and fast moving counterfeit BoNT market brings unknown substances to the forefront. Counterfeits have been shown to contain some, all or none of the expected ingredients of the genuine products, and most worryingly, the active toxin product not necessarily at the strength stated, and of unknown provenance.

From the information available, China appears to have the highest number of unlicensed BoNT-A products available. An early Chinese specification for BoNT-A products, no longer available, indicated that these were to be formulated using dextran and gelatin as stabilizers. Gelatin is an animal-derived product and must be manufactured in strict accordance with both European and U.S. guidance for use in human pharmaceuticals. Of greater concern is that gelatin has been used as a stabilizer in certain licensed vaccine products, although this stabilizer is being phased out, and limited cases of anaphylactic shock to the gelatin component in these vaccines on first injection have been reported. Gelatin is clearly used as capsules for oral drugs, with long experience. However, there seems to be no knowledge on long-term effects from repeated injections of products containing gelatin, which could potentially occur with the BoNT-A products, as their effects are only temporary and re-treatment is necessary. Until more is known about long-term effects of products containing gelatin (and, indeed dextran), caution in their use should be exercised.

Safety Profile in Relation to Composition
Injection of a drug containing a foreign protein to a patient can result in the formation of antibodies by the host defence systems. These can be raised against any of the protein components of the formulation including the active component itself (in this case BoNT-A or -B), or indeed, to impurities of the excipients.

Antibodies to the active component can elicit an immune response when the drug is administered to the patient that have a neutralizing effect, rendering the treatment ineffective. Antibodies formed to other components may have no effect on the efficacy of the treatment—that is, the potency of the active ingredient—but may have other effects, for example the formation of self-antigens.

In BoNT products, the common, major protein component of the widely available products is the excipient, HSA. This is very well tolerated in the body, as it is not recognized as a foreign protein, being derived from human plasma. In the past, animal derived homologues of human proteins, for example porcine and bovine insulin, administered to patients in the treatment of diabetes, were highly immunogenic, leading to serious anaphylactic reactions. Recombinant versions of human proteins, e.g., insulin, growth factors and hormones have also been shown to elicit an immune response in the formation of antibodies. Although undoubtedly some antibody formation was due to trace impurities of the recombinant production organisms usually derived from bacteria, in general this cannot be explained by the classical reaction to a foreign protein, and is thought to occur by a second mechanism, namely the breaking of immune tolerance. This may be triggered by presenting protein in a repetitive way, for example by multi-dosage treatments that happen over a prolonged period of time. Aggregation of protein is also thought to be a contributing factor, as the immune system may confuse these aggregates with viruses activating B cells to proliferate and produce auto-reactive binding antibodies. This breaking of immune tolerance occurs in a minority of patients and tends to be after a prolonged treatment regime.

Immune tolerance to the active BoNT component could also occur at a very low incidence. This manifests as what has been termed as primary non-response, when no efficacy of BoNT
is found after the initial injection, or secondary non-response (SNR), which occurs often after multiple treatments. Recent work has shown that only about one half of patients with SNR actually have antibodies against BoNT, indicating that other causes of treatment failure may be prevalent. Overall, the number of patients developing antibodies after long-term BoNT treatment depends on the condition being treated and is in the order of 2% for cervical dystonia, or at undetectable levels in the case of use for aesthetic treatments. Antibody response at such low levels may be because the neurotoxin is administered in such low doses (nanograms), at an infrequent period and also because the neurotoxin is only transiently present, as it is rapidly internalized into its target cells, avoiding detection from the circulating immune cells.

**New Formulations For the Future?**

Although the current BoNT formulations are well established and have provided convenience, stability and appropriate dosage forms for over 20 years, new formulations might provide enhanced benefits—longer duration stability in solution, for example.

To identify what new directions might be taken for formulating BoNT in the future, a search of patents, both pending and issued, is useful. Because of their ability to protect technologies and careful consideration should be made of this aspect.

Table 3 lists: Patents Granted* and Patent Applications** associated with BoNT technologies.

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**TABLE 3.**

**Summary of the Latest Patent Technology Described for Future Use With BoNT**

<table>
<thead>
<tr>
<th>Number</th>
<th>Year</th>
<th>Title</th>
<th>Assignee</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patents Granted*</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7579010</td>
<td>2009</td>
<td>Botulinum toxin pharmaceutical compositions with a recombinant human serum albumin</td>
<td>Hunt, T.J.</td>
<td>Allergan patent concerning use of recombinant albumin and zinc in formulations</td>
</tr>
<tr>
<td>7491403</td>
<td>2009</td>
<td>Pharmaceutical botulinum toxin compositions</td>
<td>Borodic, G.</td>
<td>Inclusion of a sequestration agent. Consider increasing the albumin content</td>
</tr>
<tr>
<td>7211261</td>
<td>2007</td>
<td>Stable liquid formulations of botulinum neurotoxin</td>
<td>Moyer, E.</td>
<td>Applied to BoNT-B only</td>
</tr>
<tr>
<td>Patent Applications**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20090163412*</td>
<td>2009</td>
<td>Compositions of methods of topical application and transdermal delivery of botulinum toxins with reduced non-toxic proteins</td>
<td>Waugh, J.M.</td>
<td>Revance Inc. patent now in clinical trials</td>
</tr>
<tr>
<td>MX20080006750</td>
<td>2008</td>
<td>Botulinum nanoemulsions</td>
<td>Edelson, J.</td>
<td>For administration of toxin by injection, cream or transdermal patch</td>
</tr>
</tbody>
</table>

*US Patent and Trademark Office online search.  
**European Patent Office online search.
required, making a change to a recombinant version, with the associated negative aspects, a redundant prospect.

**CONCLUSION**

The formulation of the currently available, licensed BoNT products has been well established for over 20 years, in the case of Dysport and Botox. These formulations have been determined as robust and appropriate for the product uses, where only a minimum of foreign (BoNT) protein is injected at administration.

New BoNT products, mainly illegal and unlicensed, have quite different formulations and excipients. The long-term safety and suitability for repeat dosing in patients has yet to be determined. These formulations cannot be considered as optimal for a human-use product.

The potential for the introduction of new product formulations is now a real possibility, with work already being pursued in clinical studies and considerable technological interest identified. If these new formulations are introduced, they will need to show distinct, clear advantages over the currently well-established product formulations. This can only be demonstrated by extensive non-clinical and clinical studies in direct comparison.

**DISCLOSURES**

Andy Pickett and Karen Perrow are employed by Ipsen, which is the manufacturer of a type A botulinum toxin product.

**REFERENCES**


48. Jones T. Allergan are spending $30m to remove HSA from Botox and replace with rHA. *PharmaNet Symposium*, London. 2007.