

Clinical Relevance of Botulinum Toxin Immunogenicity

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Abstract

Botulinum toxin type A is a 150 kD protein produced by *Clostridium botulinum*, which exists in a complex with up to six additional proteins. The ability of botulinum toxin to inhibit acetylcholine release at the neuromuscular junction has been exploited for use in medical conditions characterized by muscle hyperactivity. As such, botulinum toxin is widely recommended by international treatment guidelines for movement disorders and it has a plethora of other clinical and cosmetic indications. The chronic nature of these conditions requires repeated injections of botulinum toxin, usually every few months. Multiple injections can lead to secondary treatment failure in some patients that may be associated with the production of neutralizing antibodies directed specifically against the neurotoxin. However, the presence of such antibodies does not always render patients non-responsive. The reported prevalence of immunoresistance varies greatly, depending on factors such as study design and treated indication. This review presents what is currently known about the immunogenicity of botulinum toxin and how this impacts upon patient non-response to treatment. The complexing proteins may act as adjuvants and stimulate the immune response. Their role and that of neutralizing and non-neutralizing antibodies in the response to botulinum toxin is discussed, together with an assessment of current neutralizing antibody measurement techniques. Botulinum toxin preparations with different compositions and excipients have been developed. The major commercially available preparations of botulinum toxin are Botox[®] (onabotulinumtoxinA; Allergan, Inc., Ireland), Dysport[®] (abobotulinumtoxinA; Ipsen Ltd, UK), and Xeomin[®] (incobotulinumtoxinA; botulinum toxin type A [150 kD], free from complexing proteins; NT 201; Merz Pharmaceuticals GmbH, Germany). The new preparations of botulinum toxin aim to minimize the risk of immunoresistance in patients being treated for chronic clinical conditions.

1. Introduction

The anaerobic, spore-forming, rod-shaped bacterium, *Clostridium botulinum*, produces the botulinum toxin, which causes botulism after ingestion. The botulinum toxin complex contains a 150 kD neurotoxin together with a complex of up to six additional proteins. The neurotoxin is cleaved by clostridial proteases into a heavy chain (~100 kD), which binds to gangliosides and a protein receptor on pre-synaptic nerve endings, and a light chain (~50 kD), which blocks the release of the neurotransmitter acetylcholine, causing dose-dependent weakening of the target muscle.

There are seven immunologically distinct serotypes of botulinum toxin (A to G), of which types A and B are used clinically. The latest commercially available botulinum neurotoxin

type A is Xeomin[®] (incobotulinumtoxinA; botulinum toxin type A [150 kD], free from complexing proteins; NT 201; Merz Pharmaceuticals GmbH, Germany). Botulinum toxin type A is also marketed as Botox[®] (onabotulinumtoxinA; Allergan, Inc., Ireland), Dysport[®] (abobotulinumtoxinA; Ipsen Ltd, UK) and there are also Chinese formulations not described here. There are differences between the botulinum toxin drug substances, such as the bacterial strains from which they are produced, the manufacturing processes, and the type or quantity of excipients contained in each formulation. Currently, the only botulinum toxin type B product available is Myobloc[®] (rimabotulinumtoxinB; Solstice Neurosciences Inc., USA; also called Neurobloc[®]; Eisai Europe Ltd, UK in some countries).

Botulinum toxin was first used clinically in the late 1970s in ophthalmology to treat strabismus,^[1] and over the last 20 years

has gained widespread use in conditions requiring inhibition of excessive muscle spasm. The broad range of medical indications for botulinum toxin include treatment of movement disorders (e.g. spasticity, cervical dystonia), urological disorders (e.g. overactive bladder), dermatological conditions (e.g. axillary hyperhidrosis), as well as cosmetic applications. As the indicated clinical conditions are chronic in nature and the botulinum toxin effects typically last no more than several months, patients have to be injected repeatedly to maintain symptom control.

The European Federation of Neurological Societies (EFNS) dystonia guidelines recommend botulinum toxin type A as first-line treatment for primary cranial (excluding oromandibular) or cervical dystonia, and state that it is also effective for writing dystonia.^[2] Botulinum toxin type B can be used if there is resistance to type A.^[3] An evidence-based systematic review by the American Academy of Neurology (AAN) states that botulinum toxin injections should be offered as a treatment option for cervical dystonia and may be offered for blepharospasm, focal upper extremity dystonia, adductor laryngeal dystonia, and upper extremity tremor.^[4] A lower level of evidence was detected for hemifacial spasm, focal lower limb dystonia, and motor tics. Another AAN evidence-based systematic review recommends botulinum toxin for the treatment of spasticity in adults and children with the strongest evidence (level A).^[5]

Botulinum toxin is regarded as foreign by the host and therefore has the potential to induce an immune response, particularly with repeated administration, which can lead to secondary treatment failure. This review will discuss the response to botulinum toxin over time, including current understanding of the immunogenicity of botulinum toxin and its clinical implications.

2. Patient Non-Responsiveness to Treatment

Many patients respond well to treatment with botulinum toxin but, for a variety of reasons, some patients do not.^[6] Non-response can be defined as a lack of patient (subjective) or physician (objective) satisfaction with the therapeutic outcome of a drug. It may be transient, in which one application of the treatment is not efficacious but subsequent applications are, or permanent, such that neither the first nor subsequent applications produce the desired clinical effect. Similarly, there may be only a partial lack of response (some therapeutic aspects are not achieved) or a complete lack of response (no therapeutic or side effects can be detected).

Primary non-response refers to a lack of response to treatment from the first application. It may occur in patients with pseudo-dystonia, or those with a clinical subtype that has reduced sensitivity to botulinum toxin, such as anterocollis in

cervical dystonia, and sensory blepharospasm.^[6] Misdiagnosis is another important cause of primary non-response. For example, myasthenia gravis is not treated by botulinum toxin, but the first noticeable symptom is often weakness of the eye muscles,^[7] and so it may be mistaken for blepharospasm (for which botulinum toxin treatment is indicated).^[6] Inadequate dosing, handling errors during drug storage or preparation, or problems with drug administration (e.g. an inaccessible muscle or injection into the wrong muscle) can also cause either a primary or secondary non-response.^[6]

Secondary non-response occurs during the course of therapy when a previously effective treatment no longer produces clinical benefit. This is often subjectively described and influenced by a patient's expectation. Sometimes there may be a placebo effect of the first series of treatment, or the patients return for a re-injection before they have returned to baseline. Other patient factors also have an effect, as some disorders worsen over time and can be exacerbated by stress. In addition, the despondency caused by a chronic debilitating condition can result in symptom deterioration such that a previously efficacious dose induces a subclinical response.^[6]

An important reason for secondary treatment failure of any therapeutic protein is its neutralization by antibodies; even proteins with an identical sequence to the human protein can be immunogenic.^[8] The clinical effect may wane gradually, eventually leading to complete treatment failure. In one study of 27 patients with complete treatment failure due to neutralizing antibodies, the majority (81%) of patients had previously experienced partial antibody-induced treatment failure.^[9] Most patients in this study developed complete treatment failure within 40 months of starting botulinum toxin treatment (figure 1).

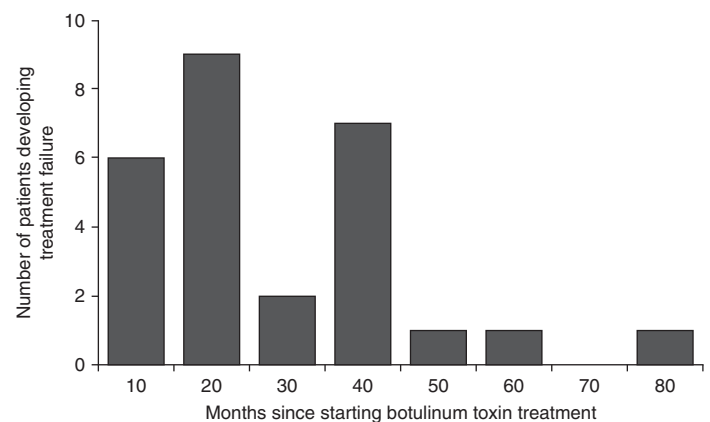


Fig. 1. Histogram showing latencies between the initiation of botulinum toxin therapy and the occurrence of complete antibody-induced therapy failure in a study of 27 patients.^[9]

3. Neutralizing and Non-neutralizing Antibodies

Native botulinum toxin is a complex of the 150 kD neurotoxin and other non-toxic clostridial proteins, including hemagglutinins. All of these foreign proteins are antigens and have the potential to induce an immune response, but only the neurotoxin component has a therapeutic effect. Antibodies that block its pharmacological effects are termed neutralizing or blocking antibodies. The non-toxic clostridial proteins, collectively referred to as complexing proteins or neurotoxin-associated proteins (NAPs), protect the neurotoxin against degradation in the acidic conditions of the host gastrointestinal tract (particularly in the stomach).^[10] Under physiological pH conditions, the complexing proteins dissociate almost completely from the neurotoxin following constitution with saline and even before injection into the target tissue.^[11] Therefore, complexing proteins are not expected to improve clinical outcome in therapeutic use of botulinum toxin, and specific antibodies generated against the complexing proteins are termed non-neutralizing. However, complexing proteins increase the bacterial protein load and can potentially increase the immunogenic risk of neutralizing antibody formation.

In a mouse study, the production of antibodies was increased with botulinum toxin type B in a large toxoid complex compared with the neurotoxoid alone.^[12] The presence of the complexing proteins was associated with adjuvant activity via the interleukin-6 cytokine pathway. Another preclinical study showed that the botulinum neurotoxoid type A with complexing proteins triggered a stronger immune response than the purified 150 kD neurotoxoid alone.^[13] This study reported that hemagglutinin Hn-33 exhibited stronger immunogenicity than the purified neurotoxoid type A. As Hn-33 is the largest component of the complexing proteins, this result indicated that Hn-33 is the most immunogenic component of the botulinum toxin type A complex. These studies utilized formaldehyde-inactivated toxoids rather than native proteins, which will have affected their immunogenicity,^[13,14] and it has been noted that some of the methods used in the mouse study^[12] may differ from the use of botulinum toxin in therapeutic applications.^[14] Although further investigations are needed, these studies revealed that the toxoid complex is more immunogenic than the purified neurotoxoid. The immunogenicity of toxoid components is of relevance since toxoid components (i.e. inactive neurotoxin – albeit not through formaldehyde inactivation) are contained in some commercial botulinum toxin products.^[15] It has therefore been proposed that reducing the foreign protein load of botulinum toxin by removal of the complexing proteins would help to reduce the formation of neutralizing antibodies, without

affecting therapeutic efficacy. The use of purified neurotoxin preparations could potentially reduce the rate of secondary treatment failure.

The role of complexing proteins has been further investigated in a recent *in vitro* study of the binding properties of the type A neurotoxin complex. The neurotoxin alone did not interact with non-neuronal cell types, but the complexing proteins (both alone and in complex with the neurotoxin) were shown to bind to neuroblasts, lymphoblasts, skeletal muscle cells, and skin fibroblasts.^[16] These findings raise the possibility that other, as yet unconfirmed, detrimental effects of complexing proteins may occur in host tissues, which may not directly influence the neurotoxin mechanism of action, but instead have an indirect effect similar to the adjuvant stimulation of neutralizing antibodies that block the clinical effects of the neurotoxin.

4. Attempts to Overcome Antibody-Induced Treatment Failure

Attempts to overcome the effects of antibody-induced treatment failure have had limited success. Massively increased botulinum toxin doses cannot overcome complete antibody-induced treatment failure, although it has been suggested that perhaps this may be effective in partial treatment failure.^[6] However, this strategy is simply expected to exacerbate the problem because the antibody response will increase when the antigen dose is increased.^[17]

The use of different botulinum toxin serotypes has also been investigated; an initial study of botulinum toxin type B demonstrated efficacy for the treatment of patients with type A-resistant cervical dystonia.^[18] However, in a later study, botulinum toxin type B appeared to be only temporarily effective in most patients with type A resistance.^[19] Patients may respond to the alternate serotype but eventually experience treatment failure due to the development of antibodies against the second botulinum toxin. These patients may also be primed to develop antibodies to the second serotype due to cross-reactivity between the first and second toxins.^[17] Therefore, once a patient has produced neutralizing antibodies against one serotype, switching to another is unlikely to produce a clinical response because immunoresistance to the second serotype will develop swiftly. Prior resistance to botulinum toxin type A has been shown to be an important risk factor for the development of anti-botulinum toxin type B antibodies.^[20] Clinical evidence suggests that botulinum toxin type B has low specific biological activity and is more immunogenic than botulinum toxin type A, inducing secondary treatment failure after only a few injections,^[20-22] although it should be noted that botulinum toxin

type B is applied in a markedly higher dose (higher protein load), which might be the reason for antibody production. The preferred approach is to prevent the formation of neutralizing antibodies in the first place.

5. Clinical Relevance of Antibodies

Neutralizing antibodies against the botulinum toxin may lead to issues with efficacy, and ultimately treatment failure as described above. Antigenicity is generally proportional to protein load, and a higher protein load per dose of botulinum toxin has been identified as a risk factor for increased antibody titer.^[20,23-25] This has been illustrated by the 5-fold reduction in protein load within the current onabotulinumtoxinA type compared with the original preparation, which has corresponded with a reduced incidence of neutralizing antibodies. Previous studies reported that up to 17% of patients with cervical dystonia treated with the original onabotulinumtoxinA preparation had neutralizing antibodies.^[26,27] A database review of patients with cervical dystonia from a single clinic reported that neutralizing antibodies were detected in four (9.5%) out of 42 patients who received the original preparation (100 U/25 ng protein) versus none of 119 patients using the newer product (100 U/5 ng protein).^[26] The authors concluded that this was related to the lower protein load.^[26]

IncobotulinumtoxinA is a botulinum neurotoxin product that does not contain complexing proteins.^[15,28-30] Initial experiments indicate that the minimized total protein load results in reduced immunogenic potential. Repeated intramuscular administration of high-dose incobotulinumtoxinA (up to 16 U/kg bodyweight) to cynomolgus monkeys did not induce detectable levels of neutralizing antibodies even when administered every 4 weeks for 4 months.^[31] Similarly, in a rabbit model, the development of neutralizing antibodies was observed with abobotulinumtoxinA and onabotulinumtoxinA, but not with incobotulinumtoxinA, even when administered at doses up to five times greater than those recommended for clinical use.^[32] IncobotulinumtoxinA has a low protein content, but a high specific activity, and retains clinical efficacy and safety profiles equivalent to conventional botulinum toxin type A formulations.^[15,33] In contrast to conventional formulations, incobotulinumtoxinA contains the pure 150 kD neurotoxin, free from complexing protein, and thus has a low foreign protein content. The amount of foreign protein content administered is considered as one of the factors for secondary treatment failure.^[34]

Recent peer-reviewed publications and presentations of current botulinum toxin formulations containing complexing proteins in long-term studies in cervical dystonia or spasticity have

reported neutralizing antibodies in up to 6.6% of patients.^[35-40] It should be noted that no controlled, long-term studies have compared the immunogenicity of different botulinum toxin products. Variability in the reported prevalence of neutralizing antibodies could be related to factors such as indication, administered dosages, assay methods, timing of serum sample testing, whether or not patients had previously received botulinum toxin therapy, and duration of treatment.^[36]

Studies of botulinum toxin type A containing complexing proteins suggest that, in addition to an increased protein load, a higher dosing frequency and greater number of injections (and the use of booster injections 2–3 weeks after original injections) may also increase the risk of neutralizing antibodies.^[6,41,42] Higher rates of neutralizing antibodies have also been reported in pediatric patients (approximately 30% of patients) who were prescribed current botulinum toxin type A formulations.^[42,43] This may be an age-related feature of the immune response^[44] or a dose-related effect, as suggested by the authors of one of these pediatric studies, due to the higher dose per kilogram body weight in this age group.^[42] A study of botulinum toxin type A in patients with urologic disorders (hyperactive detrusor or sphincter dysfunction) has reported the presence of antibodies against botulinum toxin in a higher proportion of patients (4/25 patients; 16%) than observed in cervical dystonia.^[45] The authors speculate that this may be because the urinary bladder (with its urothelium) is an immunoreactive organ that responds, for example, to urinary tract infections, which may also sensitize bladders to other antigens.

A waning clinical response to botulinum toxin over several injection sessions is generally the trigger to investigate whether neutralizing antibodies are being produced. However, immune responses can differ between patients, and the reduced clinical response could also be caused by either acute or delayed reactions such as hypersensitivity or the formation of immune complexes.^[44] Although it is important to detect the presence of neutralizing antibodies, their clinical impact should also be determined as they do not always lead to treatment failure, and thus antibody titers may occur at a subclinical level.^[20,40,46,47] Determining the true clinical impact of neutralizing antibodies in patients treated with botulinum toxin is complicated by the limitations of antibody measurement techniques and inter-clinic variability regarding the definition of secondary non-responsiveness.^[22]

6. Neutralizing Antibody Measurement Techniques

If neutralizing antibody production is suspected because the clinical effects of botulinum toxin decline and other causes of

Table 1. Laboratory and clinical tests used to measure botulinum toxin antibodies

Test	Description	Advantages	Disadvantages	References
Laboratory screening tests				
ELISA; FIA	96-well plate coated with botulinum toxin, and incubated with patient serum sample. Anti-botulinum toxin antibodies in serum are visualized with antibody linked to an enzyme that produces a color change on addition of substrate; FIA is similar but uses fluorescently labeled antibody instead of enzyme-linked antibody for detection	No animals required; useful initial screening tool; results available within hours; comparable specificity to MPA	Does not differentiate between neutralizing and non-neutralizing botulinum toxin antibodies	48
IPA	Radioactively labeled botulinum toxin is incubated with patient serum sample, and then a secondary anti-human antibody is added in excess. The resulting precipitate is centrifuged to collect the radioactively labeled botulinum toxin bound to any antibodies present in the sample	No animals required; quantitative; results available within hours; moderate costs	Does not differentiate between neutralizing and non-neutralizing botulinum toxin antibodies; less specific than MPA	49-51
Laboratory functional tests for neutralizing antibodies				
HDA; MDA	<i>In vitro</i> test using innervated murine diaphragm muscle and measuring time to paresis after addition of botulinum toxin with and without patient serum; prolongation of time to paresis confirms the presence of neutralizing antibody	Distinguishes between neutralizing and non-neutralizing antibodies; greater sensitivity than MPA; <i>in vitro</i> testing; more rapid than MPA	Complexity of equipment, technically challenging dissection, and low throughput	24,49,52,53
MPA	Patient serum mixed with botulinum toxin type A is administered to mice and the survival rate of the murine population is measured. Lack of toxicity confirms the presence of neutralizing antibody	Distinguishes between neutralizing and non-neutralizing antibodies; high specificity (low rate of false positives)	<i>In vivo</i> test; large quantities of test animals are required; slow turnaround of results	54
Clinical functional tests for neutralizing antibodies				
EDB test	Botulinum toxin is injected into the EDB. If strong stimulation of the peroneal nerve induces a response in the EDB muscle, this confirms the presence of neutralizing antibodies (i.e. no paresis)	High sensitivity and specificity; functional test; immediate results	Not standardized	55
SCM test	Botulinum toxin is injected into the SCM muscle and the reduction in electromyographical amplitude of the mean maximal voluntary activation is measured	Simple and reproducible; functional test; quantitative; equivalent sensitivity and specificity to MDA and MPA		56
UBI	Botulinum toxin is injected into one side of the corrugator muscle above the nose and the patient's ability to frown symmetrically is observed; reduced/no effect on frowning indicates neutralizing antibodies are present	Functional test	Non-quantitative; not tested for sensitivity or specificity	35
Sudomotor sweat test	Botulinum toxin is injected under the skin and if the area continues to sweat normally then neutralizing antibodies are present	Functional test	Non-quantitative; not yet standardized	6,57

EDB = extensor digitorum brevis; **ELISA** = enzyme-linked immunosorbent assay; **FIA** = fluorescence immunoassay; **HDA** = hemidiaphragm assay; **IPA** = immunoprecipitation assay; **MDA** = mouse diaphragm assay; **MPA** = mouse protection assay; **SCM** = sternocleidomastoid; **UBI** = unilateral brow injection.

treatment failure have been excluded, biochemical and/or functional tests can be employed to detect antibodies against botulinum toxin (table I).

Enzyme-linked immunosorbent assay (ELISA) and fluorescence immunoassay (FIA) tests are useful first screening tools, and the immunoprecipitation assay (IPA) is also used. However, these laboratory screening tests are not able to distinguish between neutralizing and non-neutralizing antibodies. The hemidiaphragm assay (HDA) and the mouse protection assay (MPA) are so far the only available laboratory functional assays for the detection of neutralizing antibodies, and provide good sensitivity. However, a key disadvantage of the MPA is that *in vivo* animal testing is needed. The HDA test has higher sensitivity than MPA and this may result in detection of sub-clinical antibody titers that do not result in treatment failure. Therefore, in clinical practice, a clinical test should also be included to evaluate cases of antibody-induced treatment failure. Functional assessments performed directly on the individual patient in the clinic yield results within days or weeks. They include the extensor digitorum brevis (EDB) test, the unilateral brow injection (UBI) test, the sternocleidomastoid (SCM) test, and the sudomotor sweat test.^[6,35,55,57]

7. Botulinum Toxin Products

Botulinum toxin products vary considerably in pharmaceutical composition (table II). These different physicochemical properties, coupled with the lack of standardization for measuring their potency (i.e. biological activity; expressed in U/ng protein, in which 1 U is the lethal dose in 50% of mice tested [LD₅₀]),^[15] means that the dosage cannot simply be interchanged between brands.^[5] Reports of the effective botulinum toxin type A clinical conversion ratios (i.e. U of Product X producing a similar clinical effect to U of Product Y) vary considerably in the literature, ranging up to 11:1 U for abobotulinumtoxinA: onabotulinumtoxinA,^[65,66] although it is generally agreed that there is a 1:1 U dose conversion rate between onabotulinumtoxinA and incobotulinumtoxinA.^[32,67-69]

Recently, Frevert^[15] sought to compare the neurotoxin content of each agent with a new and more sensitive ELISA, and found that 100 U of onabotulinumtoxinA, abobotulinumtoxinA, and incobotulinumtoxinA contained 0.73 ng, 0.65 ng, and 0.44 ng of neurotoxin, respectively (table II; figure 2). Calculations based on these data showed that the highest specific neurotoxin activity was found in incobotulinumtoxinA (227 U/ng), followed by abobotulinumtoxinA (154 U/ng) and onabotulinumtoxinA (137 U/ng). As incobotulinumtoxinA and onabotulinumtoxinA have demonstrated similar clinical activity,

Table II. Comparison of botulinum toxin products and formulations

Manufacturer	OnabotulinumtoxinA ^[65]	AbobotulinumtoxinA ^[69]	Chinese botulinum toxin type A ^[60]	IncobotulinumtoxinA ^[94,61,62]	RimabotulinumtoxinB ^[63]
Clostridial strain	Allergan, Inc., Ireland	Ipsen Ltd, UK	Lanzhou Institute of Biologic Products, China	Merz Pharmaceuticals GmbH, Germany	Solstice Neurosciences, Inc., USA
Toxin serotype	Hall A	Hall A	Hall A	Hall A	'Bean' strain
Dose (U per vial)	A 100 or 200	A 500	A 50 or 100	A 50 or 100	B 2500, 5000 or 10000
Presentation	Vacuum-dried powder for reconstitution	Freeze-dried lyophilized powder for reconstitution	Lyophilized powder for reconstitution	Freeze-dried lyophilized powder for reconstitution	Liquid
Molecular composition	150 kD neurotoxin plus complexing proteins	150 kD neurotoxin plus complexing proteins	150 kD neurotoxin plus complexing proteins	150 kD neurotoxin	150 kD neurotoxin plus complexing proteins
Total protein per 100 U	5.0 ng	0.87 ng	Not known	0.44 ng	Not known
Total neurotoxin per 100 U (proportion of total protein) ^[15]	0.73 ng (15%)	0.65 ng (75%)	Not known	0.44 ng (100%)	Not known
Specific neurotoxin potency ^[15]	137 U per ng	154 U per ng	Not known	227 U per ng	Not known
Clinical conversion ratio to onabotulinumtoxinA	–	3:1 ^[64]	Not known	1:1 ^[32]	Not known
Protein stabilizer	0.5 mg HSA ^a	0.125 mg HSA	5 mg gelatin ^b	1 mg HSA ^b	0.5 mg/mL HSA ^c
Other excipients	0.9 mg NaCl ^a	2.5 mg lactose	25 mg dextran; 25 mg sucrose ^b	4.7 mg sucrose ^b	0.01M disodium succinate, 0.1M NaCl, 0.01M HCl (to adjust pH), Water for injection ^e

a In 100 U vial; 1.0 mg HSA and 1.8 mg NaCl in 200 U vial.

b In both 50 and 100 U vials.

c In 5000 U vial.

HCl = hydrochloric acid; HSA = human serum albumin; NaCl = sodium chloride.

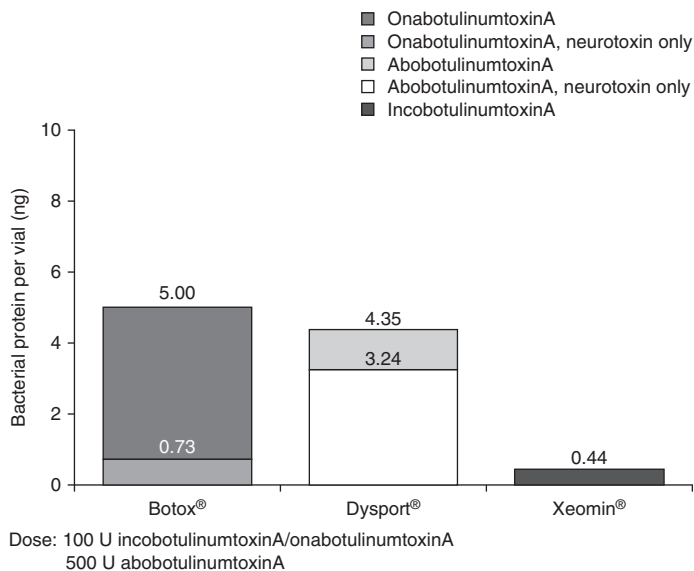


Fig. 2. Total bacterial protein and neurotoxin content of botulinum toxin type A products. Mean concentrations of botulinum neurotoxin type A in onabotulinumtoxinA, incobotulinumtoxinA, and abobotulinumtoxinA were analyzed by a sensitive sandwich ELISA.^[15]

these findings suggest that 0.44 ng of incobotulinumtoxinA neurotoxin has the same biological activity as 0.73 ng of onabotulinumtoxinA neurotoxin, and the authors therefore concluded that some of the 150 kD neurotoxin in onabotulinumtoxinA is likely to be inactive (possibly denatured), adding further protein load in addition to the complexing proteins to the injected dose.^[15] OnabotulinumtoxinA is unique among the botulinum toxin type A formulations in that it is vacuum dried by a process involving sodium chloride, which may have a detrimental effect on neurotoxin activity^[28] and may be responsible for its higher toxoid (inactive neurotoxin) content.^[15] This additional amount of inactive/denatured protein could increase the production of neutralizing antibodies against the onabotulinumtoxinA neurotoxin, leading to a greater risk of therapeutic failure. In contrast, incobotulinumtoxinA has a low neurotoxin (and overall protein) content but retains a high biological potency, so a comparatively small amount of protein confers clinical benefit while potentially carrying a lower risk of stimulating the patient's immune system.^[15] There has been discussion that the units of each botulinum product are not interchangeable due to differences in in-house LD₅₀ protocols, which result in differences in the potencies reported, and hence bias in the specific activities that were calculated.^[70,71] However, the potency assay demonstrates the same number of units for onabotulinumtoxinA and incobotulinumtoxinA, as reported in the product label.^[34,71] Moreover, several clinical studies have reported clear equipotency for these agents.^[67,68,72]

8. Conclusions

Development of neutralizing antibodies against botulinum neurotoxin is often the reason for secondary treatment failure. The presence of complexing proteins within botulinum neurotoxin agents increases the protein load and may exacerbate an immune response. Further studies investigating the long-term immunological effects of different botulinum toxin products are required, and it may be helpful to develop a consensus approach to schedules and techniques for antibody testing, as well as a more precise definition of what constitutes treatment failure. Better understanding of the possible immunological influence of the manufacturing techniques and constituents used in different botulinum toxin formulations is also needed. In the interim, physicians must use their clinical experience to decide how and when to treat their patients in order to minimize the risk of immunoresistance.

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