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Resistance to Botulinum Toxin in Aesthetics

Sebastián Torres Farr

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Abstract

Botulinum toxins are widely used worldwide for the treatment of medical and esthetic disorders. They are considered the gold standard for the treatment of muscular spasticity and facial dynamic wrinkles. Moreover, they are a valid alternative in the treatment of pain and hyperhidrosis. Several adverse events to their applications have been described, being the most frequent hematomas, migraines, palpebral ptosis, ectropion, or lack of response. Resistance to botulinum toxins in the medical field has been described, at higher dosages and short intervals. Nevertheless, resistance to botulinum toxin in esthetics has been considered traditionally anecdotic. Recent evidence suggests that resistance to botulinum toxins in esthetics may have higher prevalence than expected. A full analysis of the argument is given with up dated information regarding botulinum toxin resistance in medical and esthetic arenas; including elements for suspicion and diagnosis, valid alternatives for effective prevention, education and treatment of this misdiagnosed condition.

Keywords: resistance, neutralizing antibodies, botulinum toxin, proteins

1. Introduction

When botulinum toxin (BTX) was first introduced to esthetics, it was after an observation related to its side effects in the context of blepharospasms treatments [1]. This fact, as with many other drugs permitted the study, development and use of this tool into another area of medicine, such as cosmetic surgery.

The exponential growth of the technique, due to the medical community and patient's interests, increased the knowledge surrounding this molecule, and the safety concerns that called attention at the beginning of the century due to the use of a toxin in esthetics. Moreover, it permitted the study of the adverse events related to the applications [2, 3]. Among the former the most common include; hematomas, migraines, palpebral ptosis, ectropion, or lack of response. A complete list is shown in **Table 1**.

Any physician with some experience in the esthetic field understands the importance of patient satisfaction. Whenever we get a patient complaint after a BTX treatment, such as in cases of none or partial response, we face an immediate judge regarding trust, loyalty and professionalism. For these reasons, it is of prime importance to educate or patients concerning the possible causes of their complaint.

The causes of lack of response include: drug potency, toxin type, injection technique, unexpressive patients, cold chain alteration, toxin condition, dilution, insufficient dose, unreal expectations and resistance [4]. The causes are summarized in **Table 2**.

With the introduction to the market to new BTX type A, we have found empirically, that even though each toxin claims to have unique and non-interchangeable units, there are toxins that are more powerful than others in clinical response and lasting effect. This has called the attention to the **drug potency** parameter, especially when patients are switched from toxins between different treatments [5].

Clostridium botulinum bacteria produces seven serologically distinct types of botulinum neurotoxin (designated as types A, B, C1, D, E, F, and G). All subtypes of toxin act by preventing the release of acetylcholine at the neuromuscular junction. The most potent and used **toxin type** is A. It is clear that when changing the type, very rare condition, we can expect a lesser response to the latter.

BT treatments adverse events
Pain
Hematoma, bruising, inflammation
Headaches
Palpebral ptosis
Pseudo ptosis
Drug interaction
Diplopia
Asymmetries
Ectropion
Allergies
Unsatisfied patient
Muscular atrophy
Dysphagia
Resistance

Table 1. Botulinum toxin (BT) treatment adverse events.

BT lack of response
Drug potency
Toxin type
Injection technique
Unexpressive patients
Cold chain
Toxin condition
Dilution
Insufficient dose
Un real expectations
Resistance

Table 2. Possible causes of lack of action of botulinum toxin (BT).

Technical errors during the procedure such as intra dermal toxin injections or placement distant to the target muscle can manifest as lack or partial response in affected areas. Adequate touch ups should guarantee patient overall satisfaction.

Unexpressive patients are not good candidates for BTX treatments, because they seldom have problems with dynamic wrinkles, and therefore are difficult in perceiving post procedure improvements with the technique.

The **cold chain** can be a major concern for BTX that are thermo-sensitive. Viability of this type of BTX at room temperature is around 240 h. According to Allergan, during BTX production, a total of 120 h are consumed at room temperature for production, while the remaining 120 h enables logistics (shipping and delivery to destination). Whenever the cold chain is not respected, it is highly probable that part of the vial content is damaged, therefore affecting the performance of it.

To guarantee indemnity storage should be done frozen (−5 to 8°C) until used and after reconstitution refrigerated at 2–8°C. The use of non-thermo-sensitive BTX could be advisable if Cold chain rupture is an issue.

Toxin condition can be altered whenever one of the following is verified: reconstitution >6w (according to Dr. Doris Hexsel’s publications), alcohol contact with toxin during disinfection, application of ice packs after treatment (favors vasoconstriction and lesser absorption), freezing the toxin after reconstitution (thermic shock damages toxin), or energetic shake of the vial after reconstitution. Gentle handling and attention to this details should allow to prevent this event.

Dilution has been traditionally considered as a personal preference of the physician. Nevertheless, recent communications by this author have questioned this and open debate regarding the possibility of augmenting the potency of the drug by reducing the amount of solvent in the reconstitution, or better said, by concentrating the toxin. The effects obtained

through this are lesser side effects (such as migration) and prompt, more important and long lasting clinical benefits. The author recommends 0.5 ml as reconstitution for 125 Speywood Units (Azzalure-Galderma) or 50 Botox units (Vistabex-Allergan) or 1 ml for 100 Botox U (Botox-Allergan) or 2 ml for 300 Speywood Units (Dysport-Ipsen).

Doses are gender and individual specific. Most of the textbooks available regarding esthetic applications of BTX present recipes or treatment forms with practical advices about dose and target muscles. Nevertheless, all of this represent general recommendations to orient the clinician to target the specific dose for the specific patient. In this way, a correct clinical response is obtained without over or under treatment of the areas involved.

Patient with **unreal expectations** are poor candidates for BTX treatments. A correct clinical history, physical exam and discussion of the procedure with photographs, of previous treatments, will help to rule out them and avoid complications.

2. Resistance to chemodenervation definition

This condition is defined as the absence of benefic response and muscular atrophy after BTX treatment, due to an antibody response (of neutralizing antibodies) to the protein content within the product that neutralizes the effect.

The antibodies anti-BTX can be:

- i. Neutralizing
- ii. Non-neutralizing: they do not influence directly on the therapeutic effect, but augment foreign protein charge, increasing potentially the formation of neutralizing antibodies.

All above rank the protein content within a BTX vial as a potential trigger to develop resistance. Although there are areas within the progenitor toxin complex (PTC) that have been identified as more prone to develop neutralizing antibodies, potentially all proteins could develop an immune response that may interfere action and develop resistance. The proteins within a vial are classified in two groups:

- Structural proteins: they are present within the toxin complex and include peripheral proteins (hemagglutinin and nonhemagglutinin proteins) and core proteins of the neurotoxin itself. The diagram of the toxin is shown in **Figure 1**.
- The role of these peripheral proteins also known as Nontoxic neurotoxin associated proteins (NAPS) is related to the oral ingestion of the toxin. Non-toxic-nonhemagglutinin proteins (NTNHA) protect the progenitor toxin complex against digestive proteases and gastro intestinal acidic ambient, whereas hemagglutinin proteins (HA) enhance intestinal absorption of it [6].

The neurotoxin molecule is a 150 kDa protein structure made up of a 100 kDa heavy chain and 50 kDa light chain held together by a disulfide bond and associated with a zinc atom. The heavy chain has the C-terminus on it and is responsible for the high affinity docking on the presynaptic nerve membrane, being it rapid and irreversible. The light chain is responsible

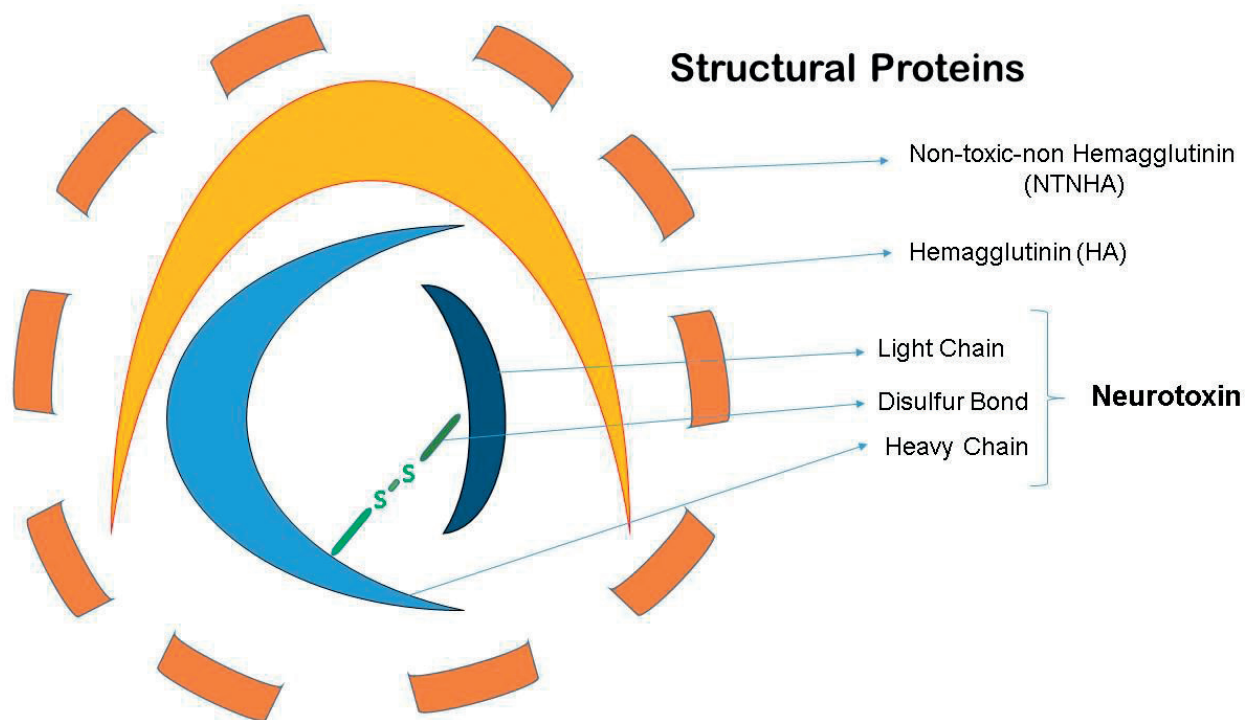


Figure 1. Diagram of progenitor toxin complex evidencing structural proteins (NTNHA, HA and neurotoxin).

Product	Toxin type	Lab	Formulation	Units/vial	Excipients	Storage
Azzalure	A	Galderma	Lyophilisate	125 Speywood	125 µg SA 2.5 mg Lactose	2–8°C
Dysport	A	Ipsen	Lyophilisate	300 Speywood	125 µg SA 2.5 mg Lactose	2–8°C
Vistabex	A	Allergan	Vaccum dried powder	50 Allergan	500 µg SA 0.9 mg NaCl	2–8°C
Botox	A	Allergan	Vaccum dried powder	100 Allergan	500 µg SA 0.9 mg NaCl	2–8°C
Bocouture	A	Merz	Lyophilisate	50 Merz	1000 µg SA Cane sugar 5 mg	RT
Xeomin	A	Merz	Lyophilisate	100 Merz	1000 µg SA 25 mg Sucrose	RT
CBTX-A*	A	Lanzho Institute of Biological Products	Lyophilisate	100	20 mg gelatin 25 mg dextran 25 mg SUCROSE	–5/–20°C
Myobloc/ Neurobloc	B	Solstice	Liquid	5000, 10,000, or 20,000	500 µg/ml SA 10 nM Na succinate 100 nM Na chloride, Na octanoate, pH = 5.6	2–8°C

*CBTX-A, Chinese BoNT/A complex; SA, serum albumin, RT, room temperature.

Table 3. Principal characteristics of most common BTX products available.

for the intracellular cleavage of proteins required for transmission of acetylcholine across the neuromuscular junction. Regions of the light chain have been recognized by human anti-toxin antibodies from cervical dystonia patient's immunoresistant to toxin treatment [7–11]. The antigenic structure of the active toxin recognized by human antibodies relies predominantly on three peptides within the light chain named L11, L14 and L18. These three antigenic regions reside in close proximity to the belt of the heavy chain. The regions L11 and L18 are accessible in both the free light chain and the holotoxin forms, while L14 appears to be less accessible in the holotoxin. Antibodies against these regions could prevent delivery of the L-chain into the neurons by inhibition of the translocation.

- Excipients proteins: they are present in most commercial products available in the market in the form of serum albumin, the only exception being the Chinese BTX-A, that uses gelatin as protein stabilizer. This plasmatic transport protein is regularly used in hemodynamic treatments as a volume stabilizer. Moreover, it is present in dairy and eggs and is susceptible of allergenic response. Nevertheless, most of the allergic reactions or intolerances are due to lactoglobulin and not serum albumin. The role of this protein in the vials serves especially as protein stabilizer to prevent the adsorption of the toxin to glass and plastic surfaces and probably to maintain the toxin in the target area after being injected. In this case, according to the author theory, would be the principal responsible of limiting micro diffusion. On the other hand, macro diffusion, associated with the most feared complication such as palpebral ptosis, depends on the solvent quantity (or dilution) for the vial preparation [12].

The other excipients are not important regarding resistance to BTX. Details of the principal characteristics of the most common BTX products available in the market is given in **Table 3**. Excipients within the vials respect a very strict proportion, and when altered could affect lasting effect.

3. BTX resistance risk factors

The principal aspects recognized are:

- Higher doses: this characteristic exposes medical patients to a major risk in comparison to esthetic applications, and wrongly induced physicians to believe that resistance was not possible in the esthetic field [13–15]. As recently demonstrated in the author's publication, resistance in esthetics is not only possible, but more frequent than expected.
- Short treatment intervals: according to this feature, treatments should only be done every 6 months if possible and touch ups should be limited within the first week and only once.
- Individual predisposition: some patients are more susceptible than others in resistance development. As with many autoimmune disorders, females seem more prone to be affected, although males usually are treated with higher doses of BTX. A correct clinical history may identify allergic or autoimmune disorders, which could put the patient at increased risk of

resistance and could constitute a relative contraindication for the treatment. The exact phenotype of these patients has not been fully understood or identified yet, and genetic tests could give some information about it in the future.

4. Diagnosis

BTX resistance is based in 2 pillars: clinical suspicion and laboratory tests.

The former is of vital importance as you can only diagnose what you have been trained to see or suspect. Patients present generally upset, after 2 or more BTX treatments in which they refer that the treatment did not give the expected result, did not work or the intensity or duration of the clinical response was less than expected or experimented in previous treatments.

The common physician action here is to raise the dose previously used or to change the commercial preparation employed (always staying in BTX-A), increasing both patient anxiety and resistance.

Laboratory test can be clinical, in vivo or in vitro [16].

Clinical tests are intramuscular measurements of compound potential action and are indirect and not specific.

In vivo tests are considered the gold standard for the diagnosis, since they represent the only FDA approved. The most popular is the bioassay or mouse lethality test. They give quantitative results about the presence of Antibodies in the patient's serum. The test consists in the injection of the suspected patient serum intravenously or intra peritoneal in mouse together with the inoculation of BTX-A. Toxicity or death of the animals is measured and is expected if no or low titers antibodies are present within the patient serum.

It has medium sensibility and specificity, takes 2–4 days, and is rather expensive (140 Euros). Moreover, is complicated (not available in many regions), and has obvious animal rights issues.

The test is available in Hannover Germany in Toxogen Lab (www.toxogen.de). The sample of patient blood (14 ml) or serum (5 ml) not frozen nor heparinized should be sent in a cold plastic container through express mail. The test form is presented in **Figure 2**.

In vitro tests include the mouse diaphragm assay (MDA), enzyme-linked immunosorbent assays (ELISA) or the immunoprecipitation assay (IPA). They are not specific and measure only one part of the reaction.

A new in vitro test known as Neuronal Cell-based Botulinum Neurotoxin Assay, based on histological preparations of mouse neural cord is available. It guarantees less animal's death and suffering (72 tests/animal) and is highly sensitive, specific and safe. For these reasons, it may be the test of the future [17].

A summary of diagnostic tests is given in **Table 4**.



toxogen GmbH
Feodor-Lynen-Straße 35
30625 Hannover

Order Form

Please test submitted sample(s) for antibodies directed against Botulinum toxin

	Type A	<input type="checkbox"/>	
	Type B	<input type="checkbox"/>	please mark box with a cross where applicable

Patient's particulars

Name:	
First name:	Date of birth:

Treatment

Indication:	
Dose:	Total dose:
Time between injections:	Duration of treatment:

Sender

Doctor:	Phone:
E-Mail:	Fax:
Hospital:	Ward:
Address:	Postal code:
	Country:

Recipient of invoice (if not identical with sender)

Name:	First name:
Address:	Postal code:
	Country:

Date: _____ Signature: _____

Feodor-Lynen-Strasse 35 30625 Hannover Tel 0511 642132-83 Fax 0511 642132-77 labor@toxogen.de www.toxogen.de	Bankverbindung: Sparkasse Hannover BLZ: 250 501 80 Konto: 250 032 78 BIC-/SWIFT: SPKHDE 2H IBAN: DE77 2505 0180 0025 0032 78	Eingetragen: Amtsgericht Hannover HRB-Nr. 56383 USt-ID Nr. DE 189154617	Geschäftsführer: Prof. Dr. H. Bigalke Tel.: 0511 642132-83
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Figure 2. Resistance test form.

Type	Name	Characteristics
Clinical	Compound Action Potential	Intramuscular measurements (Frontalis, Extensor Digitorum Brevis, SCM) Indirect ↓Sp
In vivo	Mouse Lethality Test	Gold Std, quantitative, FDA approved Medium Se/Sp, complicated Expensive 2–4 days Animal rights
In vitro	MDA ELISA IPA	↓Sp Single phase measurements
	Neuronal Cell-based Botulinum Neurotoxin Assay	Histologic sample of rat neural cord <N° (72 test/animal) & animal suffering ↑Se/Sp Safer, future

SCM, sternocleidomastoid muscle; ↑, high; ↓, low; Se, sensitivity; Sp, specificity; MDA, mouse diaphragm assay; ELISA, enzyme-linked immunosorbent assays; IPA, immunoprecipitation assay.

Table 4. Diagnostic test for BTX-A resistance.

5. Prevention

The prophylaxis for resistance development includes: adequate treatment intervals, personalized treatments, minimal effective dose, periodical tests execution, patient education, vast therapeutic arsenal, treatment onset >30 years (esthetic) and to avoid patients with strong allergic or autoimmune phenotype.

As previously mention, treatment intervals should be 6 months, with the minimum possible interval around 3 months, and touch should be done within the first week after the treatment and only once.

The treatments should be personalized, escaping from traditional formulas, using the minimal effective dose for the target area and avoiding treating areas with little or no mobility or wrinkles (example: procerus muscle in some patients). It is important to keep track on the individual treatment schema and lasting of the clinical effect, as the duration can be the first indicator that there is some resistance development.

In case of suspicion, serologic test should be promptly indicated.

Patients should be informed about this condition and about the other therapeutic alternatives, like bioplasties or laser, which can be combined to avoid the neurotoxin abuse, or “neurotoxin dependent syndrome”. In this way we will obtain natural results and maintain facial mobility without wrinkles. The idea is to make patients to consider both; skin quality and facial wrinkles.

Some congress communications in Latin America, have shown the use of BTX in 16 years old patients for wrinkles prevention. In this case, although it may be effective, it would expose the patient to a very long way on toxin treatments, and potential resistance [18]. For this reason,

in this group wrinkles prevention is advised through a correct cosmetic skin care, sun protection and nutrition. We recommend cosmetic BTX treatments not to be started before the 3rd decade of life.

Finally, within the clinical history attention should focus on the autoimmune and allergic phenotypes. Although it is not quite clear, both groups would have an important tendency to develop resistance and should be considered a partial contraindication for BTX applications.

Each case should be discussed and cost benefit decision should be taken with an informed patient.

6. Treatment

Treatment alternatives include botulinum toxin type B (BTX-B), medical electronic and infiltrative devices, peelings and cosmetic skin care. A special mention should be given to psychological support for the patient with BTX resistance.

BTX-B, also known as Myobloc (US) or Neurobloc (EC) (Solstice Laboratories) is a toxin of diverse type and although it has a quicker onset than BTX-A is not as potent and the effects will last less. Even though antibody titers tend to diminish in time, new treatments with BTX-A are not suggested. Other important fact to mention is that the drug is intended in Europe for Cervical Dystonia, and not for esthetic use, so it is very difficult to obtain the product in a private practice setting.

Medical electronic devices embrace non-ablative or ablative procedures. Non-ablative methodologies include infrared, radiofrequency and localized ultrasounds, only to name a few. Ablative procedures include lasers, like CO₂ or erbium.

All above improve collagen synthesis and skin reorganization and may increase dermic thickness reducing visible wrinkles.

Infiltrative devices group bioplasties (to improve skin hydration or metabolism), or needling through derma rollers or dermic pens.

Peelings may enhance skin renewal and tone. They may be superficial, intermediate or deep.

Cosmetic skin care should always be present in our esthetic practice; it will enhance and maintain our results and is considered to be the single most important measure in skin anti-aging. The regimen should consider adequate detergent, day and night time topical preparations and should be adjusted according to the skin type and patients' habits.

Finally, it is important to consider the physiological implications of the patient BTX resistant. For many of them, it is considered a major loss, especially if they were used to regular treatments and had experimented its benefits for a long time. It is suggested to listen to the patient fears and to provide alternative options, to avoid helpless or abandon feelings. Psychological support with specialist is advised if during controls, patients is perceived extremely anxious, hesitated or depressed.

7. Conclusion

Botulinum toxin is one of the best therapeutic weapons available for the treatment of facial dynamic wrinkles and we must, if possible, prevent, suspect, diagnose and treat the presence of resistance, to perpetuate a safe and efficient use of the product for our patients in the future.

Author details

Sebastián Torres Farr

Address all correspondence to: storres100@gmail.com

Torres Aesthetic Clinic, Las Condes, Santiago, Chile

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