Review

Controversies about botulinum toxin type A duration effect

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Abstract. *Purpose:* Botulinum toxin is a commonly used treatment in aesthetic medicine. However, physicians have expressed concerns about the brief duration of the toxin's effect on patients. Nonetheless, the manufacturers and distributors of the toxin deny any manufacturer-dependent cause for the shortened efficacy of the toxin. The aim of this paper is to analyse the possible causes that may influence the duration of action of botulinum toxin through an extensive review of published articles on the subject. *Results:* The causes of the condition may be linked to the patients' immune response or non-immune factors. These factors include the association of toxins with accompanying proteins, which can affect the neuromuscular receptors, as well as the patient's emotional expression through gesticulation, toxin reconstitution, or the injection technique performed. *Conclusions:* In conclusion, to achieve positive outcomes, it is essential to consider potential factors that may adversely affect the duration of the effect, conduct a thorough patient assessment, administer treatments at safe intervals, and avoid using questionable toxins.

Key words: Botulinum toxin, botulinum toxin A, duration of toxin effect, immunogenic response, toxin reconstitution, toxin injection technique

Introduction

For some time now, there has been a growing and widespread concern among doctors who use botulinum toxin type A (BT-A) for aesthetic purposes, regarding the short duration of its effects. It is not uncommon to hear that, upon repeating treatment with the same brand of BT-A, the duration of the relaxing effect will be shorter. BT-A is the most widely used treatment in aesthetic medicine worldwide[1,2], hence the importance of elucidating possible causes that may explain a temporary shortening of its effect.

BT-A is one of the serotypes produced by the bacterium *Clostridium botulinum*, a complex mixture of neurotoxic and non-neurotoxic proteins. The neurotoxic effect is mediated through the inhibition of the release of neurotransmitters.

The approved type A botulinum toxins act in the same way through the 150 kDa active moiety (Table 1). BT-A penetrates the neuron through glycoprotein 2 (SV2) synaptic vesicles as demonstrated by electron immunomicroscopy[3,4]. Each vesicle may contain one or two TB-A molecules[5,6], depending on the specificity of each neurotoxin subtype in relation to SV2 isoforms. In the case of BT-A, it interacts mainly with the SV2C isoform, although it needs to be glycosylated[7].

Muscle weakness is due to the specific action on the acetylcholine receptor of the 150 kDa molecular weight protein, of which 100 kDa corresponds to the heavy chain and 50 kDa to the light chain. The latter cleaves SNARE (soluble N-ethylmaleimide sensitive attachment protein receptor) proteins, preventing the release of acetylcholine into the synaptic cleft[8], resulting in temporary and reversible muscle paralysis[9-11]. The relaxing effect of BT-A usually starts within 2-5 days, peaks in 10-12 days and lasts five to six weeks before wearing off in two to three months[12-14].

Table 1. Shows the Type A TBs that have been approved by the FDA and/or EC

Serotype	**OnaBT-A**	**AboBT-A**	**IncoBT-A**	**PraboBT-A**	**DaxiBT-A**	**LetyBT-A**
Manufacturer	AbbVie Allergan	Ipsen Galderma	Merz	Evolus	Revance	Croma-Pharma
Brand name	Vistabel	Azzalure	Bocouture	Jeuveau/ Nabota	Daxx	Letybo
Subtype	A1	A1	A1	A1	A1	A1
Weight	900 kDa	500 kDa	150 kDa	900 kDa	900 kDa	900 kDa
Complexing proteins	Yes	Yes	No	Yes	No	Yes
Active fraction	150 kDa	150 kDa	150 kDa	150 kDa	150 kDa	150 kDa
Excipients	Saline solution Serum albumin	Gelatin ph. Lactose Serum albumin	Sucrose Serum albumin	Saline solution Serum albumin	Saline solution Polysorbate 20 Lactose	Saline solution Serum albumin

Immunogenicity

BT-A has an immunogenic capacity, as protein can naturally develop primary or secondary immunoresistance. The immunogenic response interferes with both the onset and duration of the effect[8,9]. An analysis of the immunogenic causes should not be performed without considering non-immunogenic causes, as they also influence the duration of the BT-A relaxation effect[15-17].

Other factors depend on the injecting physician and the injection method. Several authors have studied that intradermal injections of TB-A at high doses are more immunogenic than intramuscular injections at the same concentration[18], related to the presence of numerous dermal dendritic cells[19-21]. This should dissuade the physician from practicing so-called mesobotox (intradermal TB mesotherapy)[20].

Immunogenic causes can be prevented, but not avoided. In contrast, non-immunogenic causes are preventable. The aim of this study is to identify immunogenic and non-immunogenic causes to increase the efficacy and duration of the effect of BT-A in patients. Causes attributable to BT-A patient idiosyncrasies, possible external or environmental causes, and toxin reconstitution and injection must be analyzed.

Primary non-responders

They are all patients who derive only partial or no benefit from BT treatment, from the first and subsequent injections in case of further attempts[21]. However, it is also not clear that this concept is not due to malpractice, either by injecting low doses of BT or by not injecting adequately into the appropriate muscles[22]. Genetic patterns linked to the presence of antibodies (Ab) have been found in patients with various muscle dystonia or spasticity conditions, but this is hardly exportable to the general population seeking aesthetic improvement without neurological conditions.

The action to be taken in the case of a patient who does not respond primarily may be as indicated in the algorithm (Figure 1).

Secondary non-responders

Initially there is a good response to the BT treatment, however there is a lack of response following two treatments in a row. Seeing as it is a protein, it is always possible that repeated injections may induce the formation of neutralizing Ab[23]. It is worth noting that the prevalence of neutralising Ab, in BT treatment, for aesthetic or neurological reasons, is very low (3.5%), with patients responding with the presence of Ab and patients not responding, even if no Ab are detected in them[24-25]. On the other hand, there are concurrent studies indicating that the production of Ab is linked to the use of high doses of BT, which are usually those used in clinical pictures with spasticity or dystonia capable of affecting large muscles in large territories; unrelated to the doses used in aesthetic medicine[8]. To deal with this possible situation, it is advisable to

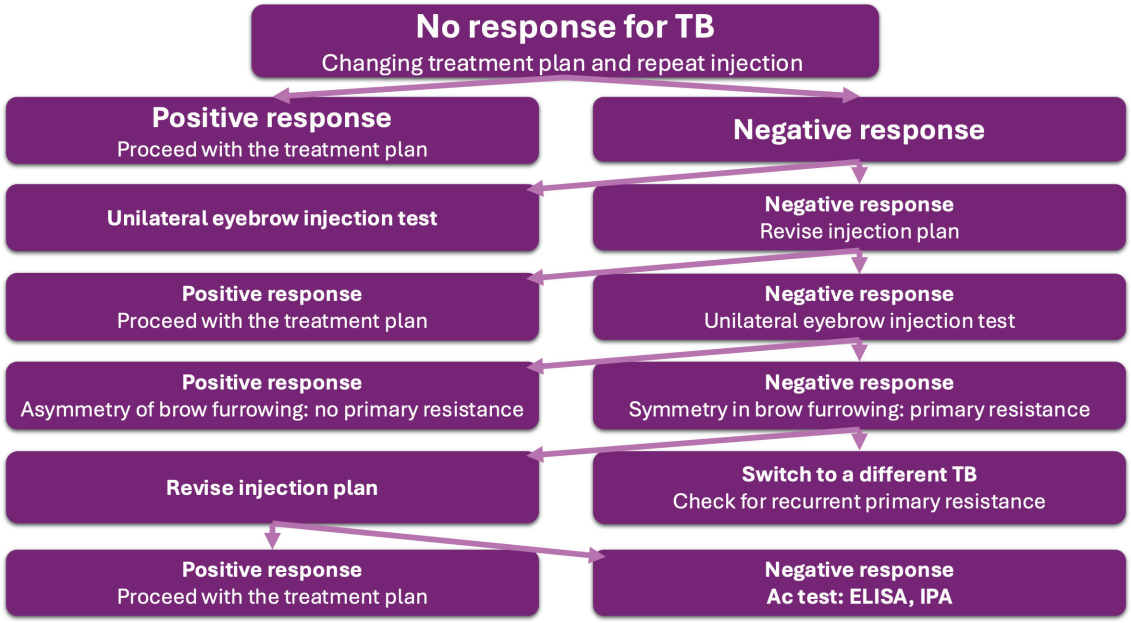

Figure 1. Algorithm for patients with primary resistance. Modified from Bellows and Jankivic (2019).

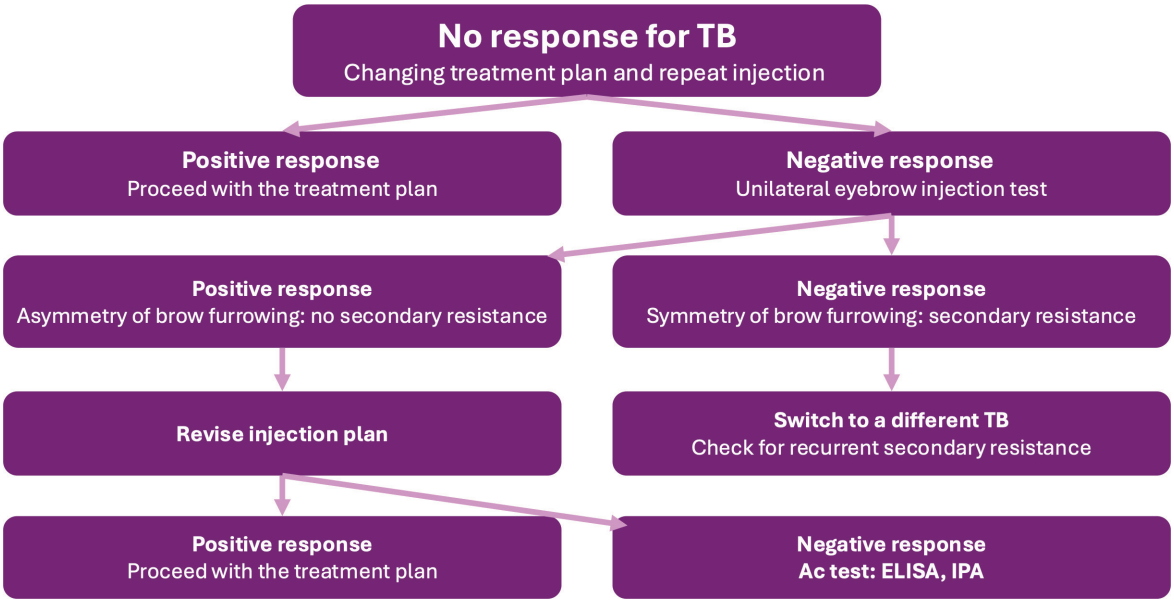

Figure 2. Algorithm for patients with secondary resistance. Modified from Bellows and Jankovic (2019).

follow the guidelines of the corresponding algorithm (Figure 2).

The search for antibodies against BT began with bioassays that distinguish between neutralising and non-neutralising antibodies.

The Mouse Protection Assay (MPA) is regarded as the gold standard, despite the drawback that many mice do not survive the test[26]. For this reason, the Mouse Hemidiaphragm Assay (MHDA) is often preferred, as it requires fewer animals and offers higher

sensitivity, although it comes with a higher risk of false negatives[27].

Although highly sensitive, structural assays such as ELISA (Enzyme-Linked Immunosorbent Assay) and IPA (Immunoprecipitation Assay) do not distinguish between the various forms of Ab[26,28].

To avoid the inconvenience of testing, clinical immunoresistance tests are used. One of them consists of injecting 20 U of Ona or IncobotulinumtoxinA or 50 U of AbobotulinumtoxinA into the right corrugator and assessing the frowning of the eyebrows between 1 and 3 weeks. If the result is positive, corrugator paralysis is induced, resulting in asymmetric furrowing, thus ruling out patients who are assumed to be non-responders[25].

There are different neurotoxins, and they are not interchangeable with each other[29-32]. Therefore, the determination of the total content of each cannot be taken lightly due to the accompanying proteins in them, as in the case of AbobotulinumtoxinA and OnabotulinumtoxinA[10]. To establish a comparison, the neurotoxin content as such, i.e., the 150 kD fraction, which is the active part, must be analyzed. However, results vary depending on the funding of the studies.

This could be due to the use of vials with higher concentrations intended for different therapeutic purposes than those used in aesthetic treatments, or because no distinction is made between the two types of treatments. It is also unclear whether the toxin approved for aesthetic use, sourced from the same manufacturer as the one used in hospitals, is exactly the same, even though the manufacturing serial numbers for Botox® and Vistabel® are identical, for example[14].

A recent meta-analysis, combining different indications for BT, reports that 0.5% (27 patients) out of a sample of 5,876 had neutralizing Ac at the end of their treatment. However, at the final evaluation of the study only 0.3% (16 patients) remained seropositive[24]. It is noteworthy that of the 10 treatments performed only 2 were aesthetic, with the particularity that there were patients who had undergone more than 15 treatment sessions, indicating that a low incidence was recorded.

The rise in reported immunogenic reactions may be attributed to the introduction of new toxins, many of which originate from South-East Asia[33]. Some studies have suggested that certain toxins contain impurities of a protein nature, which could contribute to increased immunogenicity rates. Additionally, the increasing use of BT in both on-label and off-label indications may also be a relevant factor. It is important to note that not all substances classified as neurotoxins are active, which can complicate the interpretation of the results[33,34].

Performing multiple aesthetic treatments on the same patient in a single session may not be advisable, even if the indications are correct. The cumulative quantities of units used in treating upper third wrinkles, masseter muscles in the presence of bruxism, or axillary, palmar, or plantar hyperhidrosis, will be similar to those used in treating dystonia and/or spasticity in neurological conditions. Ideally, treatments should be separated by an interval of more than three months. As mentioned prior, using larger amounts can lead to the development of immunoresistance[28,29].

Finally, the practice of offering cosmetic treatments for BT in separate areas, such as glabellar, frontal, and crow's feet wrinkles, may encourage patients to undergo one treatment per area each month based on their financial availability. However, there is no published data on whether this approach could lead to patients developing immunoresistance due to the frequency of injection. The lack of data is due, among other reasons, to the fact that BT is administered by non-physicians. In addition to a technical fault, facial expressions should be considered as an interplay between agonist and antagonist muscles.

Non-immunogenic causes

Possible non-immunogenic causes of adverse reactions must be thoroughly analyzed. This includes examining the different toxins on the market, the patient, and the method of treatment.

Attributed to BT

There is a discrepancy in the potencies of toxins, but there is no well-conducted research that clearly differentiates this aspect. According to Nestor et al (2017), some toxins differ from others when considering the association of the neurotoxin with accompanying protein complexes, although this may not be

reflected in the clinical practice[35-37]. The application of this treatment is also dependent on the distribution and density of neuromuscular receptors, which can vary according to gender, age, and muscle mass. Therefore, a thorough assessment of each treatment area is necessary[8,38].

It is important to distinguish between the diffusion and dispersion of BT. Diffusion is a passive phenomenon that is independent of the injection site or technique, while dispersion is an active process that depends on the site and depth of injection, the extrusion force exerted during injection, the volume injected, and the needle gauge used[22,39]. The term 'migration' should only be used to indicate the effect of BT beyond the original location being the muscle targeted during treatment. A common example is the drooping of the upper eyelid caused by the migration of injected BT into a corrugator muscle, which ultimately affects the levator muscle of the upper eyelid.

Most marketed BTs, except for LetybotulinumtoxinA, are approved for use in individuals under 65 years of age, as strength and muscle mass typically decline with age. However, creating a personalized evaluation based on these criteria for each patient is challenging as there is no established scale that can validate these postulates beyond doubt[40,41].

Attributed to patient

In general, the duration of the relaxing effect of BT is longer in women than in men. It is important to ensure that the number of BT units applied is proportional to the degree of muscle development, which is usually accompanied by a higher number of neuromuscular junctions. This is particularly important for men, who tend to have more developed muscles than women, and may be undertreated if the dosage is not adjusted accordingly. Similarly, young patients require more BT units due to their powerful muscles being triggered more frequently[16].

De Maio's (2008) classic study found that hypertonic patients, whose gesticulation is not linked to emotional expression, were poor candidates for treatment with BT due to the notably shorter duration of the effect (only 2 to 3 months). However, in hyperkinetic patients, whose facial mimicry corresponds to the expression of emotions, the effect of BT injection was longer lasting[42]. According to a study, stressful situations in a patient's life may lead to a shorter duration of the BT effect. This is attributed to a longer than usual contraction of the facial muscles[43].

External factors

Environmental temperature may influence BT behavior. When heat or cold are prominent, they may shorten the duration time of treatment. However, this is difficult to document as prolonged exposure time would be needed to influence this. Heat and the cold have opposite influences on thermoregulation, and the local influence of the chemical mediators involved also plays a role[44-45]. The documented benefits of BT on Raynaud's phenomenon are related to the action of nitric oxide on the vasodilator mechanism[44-46]. Some authors prefer to use preparations containing only 150 kD BT without complexing proteins in warmer climates and for longer durations. However, it is still not recommended to generalize this approach due to insufficient evidence[47].

Reconstitution of Botulinum Toxin

The best way to preserve potency and prolong the length of action is through the correct reconstitution of the toxin by the physician. To achieve this, introduce the saline into the vial with little pressure and without vigorous shaking or aspiration with reinjections into the vial itself. Additionally, use a fine needle for the manipulation or reconstitution of the BT. While one study concludes that shaking the vial does not affect the result or shorten the duration of the effect[48], a more recent study reports that rough handling results in a loss of BT efficacy of up to 42%, as well as a delayed onset of its relaxing effect. However, it is important to note that this study was conducted in mice and not in humans[22].

If the reconstituted BT is not used immediately due to any cancellation or delay of the patient to whom it is to be administered, it must be kept in the vial and refrigerated at a temperature of 4 to 8°C to prevent possible degradation[12].

Technique

The efficacy and duration of BT are influenced by various factors, including the injection technique. To ensure optimal results, it is recommended to use a 30G needle and inject BT slowly, avoiding excessive pressure on the plunger and using a larger gauge to prevent the dispersion of BT into unwanted muscles[35,36]. The amount of TB that reaches the intramuscular receptors is also affected by the volume injected and the angle of the needle on the skin[16].

The use of applying cold locally can alleviate any discomfort from the injection[49]. However, it is important to avoid intense cold as it may reduce the absorption of the toxin into the underlying muscle, leading to a delay in the onset or a shortened duration of effect[50]. Therefore, ethyl chloride can be applied for a few seconds to reduce pain without limiting the uptake of BT into the muscle[51]. There has been no reduction in the effect of BT when anesthetic creams have been applied, as they have a vasodilator effect[52]. However, it is important to properly clean the area before injecting and change the needle to reduce pain[53].

The injection must be administered intramuscularly to achieve a paralyzing effect. Subcutaneous injections are only limited to providing a relaxing effect. It is important to avoid injecting too deeply into shallow muscles such as the orbicularis or frontalis. This may cause the BT to be deposited in a retromuscular position, giving an effect like that of a subcutaneous injection. To ensure greater efficacy, the BT injection should be administered in the thickest part of the muscle where there are more neuromuscular junctions than in peripheral areas. It is important to use the appropriate dose for the muscle being treated to avoid side effects, such as possible palpebral ptosis[54]. To achieve a faster onset of action and longer duration of effect, it is recommended to administer injections with smaller volumes and higher concentrations, particularly in the frontal area. This is because they can reach more neuromuscular receptors[37].

The physician should be responsible for cleaning the treated area after the end of the treatment. It is important to avoid pressing or massaging the treated areas as this could cause unwanted effects by distributing the BT to nearby muscles. The patient should gesticulate immediately after treatment to diffuse and fix the active fraction of BT, reaching more neuromuscular terminals and contributing to greater relaxation[55].

A progressive photographic comparison is the most efficient way of determining the duration of the treatment's effect. Therefore, new photographs, both static and dynamic, should be taken before and after the treatment to detect subtle changes. This is essential for both the clinician and the patient[56]. Furthermore, photographs or videos that capture dynamic gesticulations may reveal asymmetries and changes that are not noticeable to the patient. This can help prevent potential complaints after treatment[57].

Conclusions

The patient should be thoroughly examined both statically and dynamically using photography and/or video to document any changes before and after treatment.

The success of BT treatment depends on considering both immunogenic and non-immunogenic causes. To minimize non-immunogenic causes, it is important to correctly reconstitute the BT, refine the injection technique, and apply the effective dose to achieve the desired effect while considering interactions with neighboring muscles.

To avoid treatment failure, it is recommended not to use high doses, aim for longer treatment periods, and avoid repeating treatments at short intervals to satisfy patients. It is also not advisable to give too many treatments to the same patient, as this may increase doses and lead to neutralising antibodies.

BT is a valuable tool in our medical practice. It is essential to avoid using toxins of uncertain origin. Attending to the details is a way of showing care for our patients.

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Received: 2 August 2024
Accepted: 27 February 2025
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