

Causes of Botulinum Toxin Treatment Failure

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Purpose: The purpose of this article is to review the cause of botulinum toxin (BT) failure and determine the ways to minimize the risks of its occurrence.

Methods: A PubMed and Google Scholar literature search was conducted with the search terms botulinum toxin, treatment, failure, causes, and prevention. Fifteen relevant articles were found and used as the scientific base for this article.

Results: The failure of BT therapy is associated with immunogenic and non-immunogenic causes and the formation of neutralizing antibodies toward the active components of BT or the complexing proteins. Enzyme-linked immunosorbent assay (ELISA) testing and mouse hemidiaphragm assay (MHA) can diagnose the failure. The risk of developing treatment failure can be minimized by using complexing protein-free formulations, selecting a treatment regimen with the least immunogenicity, proper injection technique, and gentle product handling.

Conclusion: The treatment failure can compromise the success of BT treatment. Current medical literature shows controversial evidence for and against BT immunogenicity. Therefore, the cause of BT failure is likely to be multifactorial.

Keywords: botulinum toxin, treatment failure, causes, immunogenicity

Introduction

Botulinum toxin (BT) has become the Penicillin of the twenty-first century due to its excellent safety and applicability across the medical fields. Its first application in medicine was in 1980 by Dr. Scott for the treatment of strabismus. It is still used in modern ophthalmology for disorders like blepharospasms, corneal astigmatism, nystagmus, and oscillopsia.¹ Probably BT has the most variety of clinical applications in neurology. It is used to treat torticollis, dystonic tics, spastic dystonia, post-stroke spasticity, essential tremor, Bell's palsy, deformities related to cerebral palsy, cervical dystonia, chronic migraine, neuropathic pains, Parkinson disease, myofascial pain syndrome, and occupational cramping.² In dermatology, BT is used for the treatment of hyperhidrosis, rosacea/flushing, and surgical scar prevention. It is used for chronic anal fissures, esophageal dysmotility, dystonias, bruxism, and stuttering in gastroenterology. BT is a treatment for overactive bladder, benign prostatic hyperplasia, bladder pain syndrome, pelvic floor spasms, and hot flashes in urology and gynecology. It is even used in psychiatry for the treatment of major depressive disorder and Tourette syndrome.²

Perhaps the most popular use of BT is to treat facial wrinkles. It is the most frequently performed non-surgical aesthetic procedure in the United States. According to the Aesthetic Society 2019 report, there were 1,712,994 BT procedures performed for aesthetic reasons, resulting in \$649,512,686 revenue for the year 2018.³ In the field of aesthetics, BT is most commonly used for the reduction of the frontalis, glabella, and lateral canthal lines. Other indications include chemical brow lift, correction of the "gummy smile," reduction of the masseter hypertrophy for the facial slimming, Nefertiti neck lift, body contouring (gastrocnemius injections), nasals "bunny" lines, perioral rhytids, mentalis muscle for reduction of chin "dimpling," and lip corner lift (depressor anguli oris injections).²

Resistance to the product can harm the success of BT therapy for medical and aesthetic applications. In recent years, there have been several reports of treatment failure of BT and the various formulations from many countries. The formation of neutralizing antibodies (nABs) is believed to be the main reason for the treatment failure. However, this notion is controversial and is not universally supported in the medical literature. Some scientists believe that the

resistance is also caused by non-immunogenic causes, such as improper product handling and inappropriate technique and dosing. Understanding the immunogenic and non-immunogenic causes of BT failure is essential for developing proper protocols for treatment failure prevention.

Discussion

It is important to understand the chemistry of the drug, differences in the formulations, and why the effects may subside to determine the causes of BT treatment failure.

Chemistry

Botulinum toxin is produced by the anaerobic, Gram-positive, sporulating bacterium *Clostridium botulinum*. It is one of the strongest biological poisons.¹ The toxin produced by the bacteria is a complex mixture of neurotoxic and non-neurotoxic proteins.⁴ There are seven serotypes, named A, B, C, D, E, F, and G. Each of them also has several subtypes based on the slight difference in amino acid sequence. That difference is responsible for different immunologic and biological properties.⁵ Only serotypes A and B are widely applied for therapeutic use due to the longevity of their effect.

The inhibition of neurotransmitter release produces the neurotoxic effect. The BT cleaves one to two out of three core proteins of the neuroexocytosis apparatus in the peripheral nerve terminal. This results in a temporary and reversible paresis of the muscles. After the introduction of the BT, the paresis starts in two to five days, it reaches its full strength in five to six weeks and starts to dissipate in two to three months.⁴

It is important to understand the muscle recovery process as it may play a role in developing non-immunogenic resistance. Recovery of the neuromuscular transmission is accompanied by the motor axon sprouts forming new synaptic contacts. After two to three months, the original terminal recovers, and the terminal sprouts withdraw.⁶ In addition, during the recovery, the voltage-gated calcium channels that are not normally expressed in mammals become active. Although it does not seem to affect the function, exposure and repeated exposure to BT cause significant physiological changes of the terminal and preterminal motor axon.⁶

Serotype A is the most commonly used type for therapeutic reasons. The Food and Drug Administration (FDA) approved commonly used Botulinum toxin type A formulations in the United States (US) are onabotulinumtoxin A (Botox, Vistabel, Vistabex; Allergan Inc, California, US), abobotulinumtoxin A (Dysport, Azzalure; Ipsen Biopharm Ltd., Berkshire, United Kingdom), and incobotulinumtoxin A (Xeomin, Bocouture; Merz Pharmaceuticals GmbH, Frankfurt Germany). Newer FDA-approved formulations are prabotulinumtoxin A (Jeuveau, Nabota, Nucevia; Daewoong Pharmaceutical, Seoul, Korea) and Daxibotulinumtoxin A (DAXI; Revance Therapeutics, Silicon Valley, US). Other BT types A approved for use outside the US are Relatox (Microgen, Moscow, Russia), Letibotulinumtoxin A (Regenox, Botulax, Zenox; Hugel Pharma, Seoul, South Korea), Neuronox (Meditoxin, Botulift, Cunox, Siax; Medytox Inc., Ochang, South Korea), and CBTX-A (Prosigne, Lantox; Lanzhou Institute of Biological Products, China).^{6,7} All of the products contain the same BT serotype. However, different manufacturers produce different compositions, concentrations, toxin complex sizes, and accessory proteins.⁷ The Botulinum toxin type B used in medicine is rimabotulinumtoxin B (Myobloc; Solstice Neurosciences, US). The use of BT types A and B is popular due to the high potency of those serotypes. Type E has been recently introduced for cosmetic and therapeutic use due to its fast onset of action. Bonti Inc, in California, US, developed the EB-001A and EB-001T for aesthetic and therapeutic uses.⁶

Treatment Failure

Millions of BT injections are delivered each year for medical and aesthetic reasons. Because the effect of the BT is temporary, the treatments are repeated to maintain the results. However, in some patients, the subsequent retreatments with exact dosing and techniques produce lessened results. The retreatment with a higher dose follows the diminishing of the result. For some people, at a certain point, even after retreatments, the effect of BT stops altogether. This phenomenon is a secondary nonresponse.⁷ Bellows and Jankovic defined the primary nonresponse as a situation in which patients treated with BT do not improve from the first or all the subsequent treatments.⁸ The secondary nonresponse is defined as a situation when patient had responded to at least one BT treatment, but have lost that response with subsequent treatments.⁸

Immunogenic Causes

The current theory for BT treatment failure is the immunogenicity of the product. Immunogenicity has been extensively researched in the last few years. The BT is composed of foreign active and inactive (complexing) proteins. Either one of them may act as an antigen, triggering the immune system to produce neutralizing antibodies (nAB).⁹ The presence of the nABs can be detected by ELISA. The mouse protection assay and mouse phrenic nerve hemidiaphragm assay can also be used to detect the presence of the nAB that shield the toxin effect in the mice.

Srinoulprasert et al conducted a study to detect the presence and type of nAB in the subjects who were BT naive, BT responders, and BT non-responders.⁹ In this prospective cohort study, the blood samples were tested by ELISA two weeks after the BT injection for 22 responders, 28 non-responders, and blood samples from 35 BT naive patients.⁹ The test results showed the presence of basal level the BT antibodies against the whole BT molecule in BT naive subjects. Exposed subjects had a mixture of AB, including the AB specific to BT three active sites and AB specific to the BT non-active sites. Only BT tolerant patients had a high level of the AB to three active sites, distinguishing them from the responders and BT naive patients. The presence of the AB to the whole BT molecule was not surprising because the BT is similar to the tetanus toxoid. This can explain the primary BT resistance. In secondary non-responders, the repeated exposure to BT stimulates the production of the more specific AB, such as the active sites AB, that is capable of neutralizing the effect of the BT toxin, leading to treatment failure.⁹

Two factors affect immunogenicity: the chemical content of a particular formulation of BT and the administration regime. The high dose per treatment as well as a high cumulative dose were both associated with the secondary treatment failure in the study described above.⁹ Another recent study by Albrecht et al has confirmed the same findings.¹⁰ In that study, 596 patients treated for neurological conditions received BT treatments every 12 to 13 weeks for several years. At the end of the follow-up period, 13.9% of all patients had developed neutralizing antibodies. Patients receiving the largest treatment doses were patients with cerebral palsy. That group of patients had 0.5–2.0% of nAB after one to two years of therapy. At an average of 5.6 years, that percentage went up to 15.7%.¹⁰

The formulation of the BT plays a vital role in immunogenicity. Wanitphakdeedecha et al demonstrated that the presence of a high (over 90.5%) amount of the anti-complexing proteins AB by absorption ELISA would result in treatment failure for the formulations containing complexing proteins.¹¹ The amount of the inactive components such as complexing proteins varies across different formulations. Currently commercially available in the United States, BTs are Botox, Dysport, Jeuveau, and Xeomin. Original Botox formula contained larger quantities of complexing proteins and caused up to 17% resistance for cervical dystonia patients.¹² The formula was changed in 1997 to increase specific potency and, therefore, reduce immunogenicity. However, the immunogenicity of the formulation still occurs.⁷ Updated Botox formulation was found to have 5.8–12.6 pg per vial of clostridial DNA and nontoxic non-hemagglutinin and hemagglutinin HA 34 DNAs.⁷ The presence of bacterial DNA can be an additional reason for Botox immunogenicity. Dysport manufacturing is different from that of Botox. It utilizes chromatography and dialysis for the product purification, which leaves the final product with partially degraded complexing proteins, and contaminants including flagellin, an immunogenic agent.⁷ Dysport, however, contains a larger amount of the active neurotoxin as compared to Botox and Xeomin.¹³ The latest FDA-approved BT Jeuveau, however, shows no impurities in testing and has an amount of complexing proteins, which is undisclosed by the manufacturer.⁷ Xeomin has a unique product composition because it consists of only the active component and no complexing proteins.⁷ In the study by Wanitphakdeedecha et al, 30% of patients non-responsive to onabotulinumtoxin A (Botox) responded to treatment of incobotulinumtoxin A (Xeomin).¹¹ In another study by Dressler et al, patients that had developed complete resistance to several formulations were successfully treated with Xeomin after a treatment break.¹⁴ In that study, eight patients with cervical dystonia were secondary BT non-responders with maximal nAB titers following Botox and Dysport therapy. The nAB titers dropped to insignificant levels after 3881.5 ± 2468.3 days. The BT therapy was resumed with Xeomin with no rise in nAB.¹⁴

Non-Immunogenic Resistance

The disagreements over BT immunogenicity exist because not all patients with BT nAB are resistant, and not all resistant patients have nAB.⁷ Meta-analysis by Lacroix-Desmazes et al analyzed 31 publications with a total of 5811 subjects with various medical and cosmetic indications treated with Botox, Dysport, and Xeomin.¹² The results showed that only 2.1% of those patients had neutralizing antibodies to BT.¹² Furthermore, an earlier meta-analysis by Fabbri et al demonstrated

that only 53.6% of secondary non-responders had nAB to BT.¹⁵ These findings suggest that immunogenicity is not the only reason for the BT treatment failure. Other factors can include disease progression, underdosing, inappropriate muscle injection, expiration of placebo effect, and changes in the pattern of muscle hyperactivity.^{12,15} Some other causes, such as the synaptic changes mentioned earlier, can be responsible for the failure.

Beyond the known factors and factors not related to immunogenicity, the reconstitution technique affects the efficacy of BT treatment. The BT drug is stored in a powder form that requires reconstitution prior to injection. The crystals of BT may be damaged by aggressive handling such as shaking, aspiration, or injection of the solvent with high pressure when using a small gauge needle. In a study by Dressler and Bigalke, the effectiveness of BT was compared by the mouse hemidiaphragm assay after both gentle and aggressive reconstitution.¹⁶ The aggressive reconstitution was done with a small gauge needle, with the solution injected and aspirated multiple times and a vial shaken up for 30 seconds. Gentle reconstitution was performed with a large gauge needle, with a single aspiration injection and two gentle shakes. A 42% loss of potency was noted when the solution was aggressively reconstituted compared to when the solution was reconstituted gently.¹⁶

Conclusion

Immunogenic and non-immunogenic causes result in botulinum toxin treatment failure. Non-immunogenic failure can be minimized by proper injection techniques, dosing, and product handling. A treatment regimen and products with the least risks of forming neutralizing antibodies can reduce immunogenic failure. Larger doses per treatment, higher overall doses, and a high frequency of treatments were all associated with an increase in nAB.^{9,10} To avoid immunogenic resistance, formulations with no impurities or complexing protein should be preferred. The treatments should be performed by specialized and trained medical professionals proficient in muscle selection and injection techniques. Proper reconstitution and storage must also be used for optimal product efficacy.

Disclosure

The authors report no conflicts of interest in this work.

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