BJDOR
BRAZILIAN JOURNAL OF DENTISTRY AND ORAL RADIOLOGY

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PUBLICAÇÕES CIENTÍFICAS DE ACESSO ABERTO

Review

The Role of Antibodies in the Failure of Botulinum Toxin Treatment: A Literature Review

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Abstract: Surgery for the removal of third molars is a commonly performed surgical procedure in Dentistry. It is associated with inflammatory events and possible complications, being more evident after the removal of lower molars, as in most cases, there is a need for more invasive approaches. It is essential for the dentist to minimize such effects, providing a more peaceful postoperative period with less morbidity for the patient. In this context, platelet-rich fibrin (PRF) was developed, which is considered a platelet aggregate obtained by centrifugation of the patient's own blood and aims to minimize inflammatory effects, accelerate healing and repair processes, and reduce postoperative complications. A literature review on the effect of L-PRF in sockets after the removal of mandibular third molars was conducted, evaluating its efficacy on pain, swelling, trismus, and healing. A bibliographic survey was performed on the PubMed, Web of Science, and Elsevier databases, using the following descriptors: 'platelet rich fibrin', 'third molar', and 'surgery', limiting to articles written in English published from 2013 to 2023. Thus, we have a current scientific overview of the real effect of using this platelet aggregate in the extraction of lower third molars. The studies reviewed, for the most part, showed positive results in improving pain, swelling, trismus, and healing.

Keywords: Botulinum Toxins; Botulinum Toxins Type A; Vaccine Immunogenicity.

Citation: Peixoto AL, Sales JC, Medeiros JR, Silva BFA, Viana FAC. The Role of Antibodies in the Failure of Botulinum Toxin Treatment: A Literature Review. Brazilian Journal of Dentistry and Oral Radiology. 2024 Jan-Dec;3: bjd44.

doi: https://doi.org/10.52600/2965-8837.bjdor.2024.3.bjd44

Received: 2 April 2024
Accepted: 7 May 2024
Published: 14 May 2024

1. Introduction

Botulism first appeared in historical records around the 18th century, with reports of an outbreak in Germany that led to six deaths after consuming sausage. In 1895, following another outbreak in Belgium where 34 musicians were intoxicated after consuming smoked preserved ham, university professor Émile van Ermengem conducted an extensive investigation, isolating the bacterium and naming it Bacillus botulinus, later known as Clostridium botulinum [1]. Considered one of the toxins with the highest mortality rate, its lethal dose is between 1 ng and 3 ng of toxin per kilogram (kg) of body mass. The toxin from the bacterium Clostridium botulinum, found in soil, can contaminate vegetables, meats, and fish during storage. It is characterized as gram-positive, large rod-shaped, with peritrichous flagella, and strictly anaerobic, meaning it cannot develop in the presence of oxygen gas [2].

Botulinum toxins exert specific pharmacological action by blocking transmission in cholinergic nerve fibers and preventing the release of acetylcholine, resulting in flaccid paralysis [2]. Inhibition of motor cholinergic junctions can lead to clinical manifestations such as muscle weakness, blurred vision, dysphagia, and in more severe cases, respiratory muscle paralysis causing respiratory failure. Currently, the potent effects of botulinum toxin are utilized for clinical use in treating certain conditions that may also be seen as side effects in clinical toxin contamination cases, such as dystonias, eyelid spasms, and expression wrinkles.

Modern use of botulinum toxin treatment began in the 1970s with ophthalmologist Alan B. Scott, who sought alternatives for strabismus. After experiments on healthy volunteers and high success rates, it was approved by the Food and Drug Administration (FDA) as a medication named "Oculinum" [3]. It gained wide acceptance for treating excessive muscle spasms [4]. Patients reported rejuvenating effects in the treated area, seeking the treatment again even without specific indication. It wasn't until after 1992 that research began on its use for facial wrinkles. Since then, it has proven to be a safe, effective, and minimally invasive treatment for movement disorder-related diseases, dermatological indications like hyperhidrosis, and aesthetic purposes [4].

The desired effects of Botox last for several months, but after repeated injections, some patients stop achieving the expected results [4]. Botulinum toxin is frequently chosen for aesthetic treatments, being a well-known and effective protocol. However, the high demand and early use have led to reports of neutralizing antibody development, resulting in unmet patient expectations.

The immunogenicity of Botox use depends on various factors, including formulation, antigenic protein content, toxin dosage, frequency, and early exposure to the toxin. As with any non-human protein, commercial Botox preparations can trigger an immune reaction, especially with frequent use.

2. Methodology

The present work is designated as an integrative literature review (IR), composed of the following steps: formulation of the review question, search and selection of primary studies, data extraction from the studies, critical analysis of the primary studies included in the review, synthesis of the review results, and presentation of the method. The guiding question that directed this study was: *Is botulinum toxin capable of triggering a vaccine-like effect?*.

Data collection began in September 2022, using the following databases: PubMed/MEDLINE, Elsevier, and BVS (Virtual Health Library) by combining terms using the Boolean operator "AND." The controlled descriptors used were found in the Health Sciences Descriptors (DeCS) database and identified by the following terms: "Botox" AND "Immunogenicity," "Botox Toxin Treatment," AND "Immunological botulinum," "Immunoresistant" AND "Botulinum." The inclusion criteria for selecting the articles were: original articles, available in full text, published within the last 10 years, and written in English. Studies related to the use of botox in areas of the body other than the face, as well as theses, dissertations, or monographs, were excluded. For data analysis, a critical reading of the titles and abstracts of each found article was conducted to verify if they were appropriate to the guiding question and strictly adhered to all presented inclusion and exclusion criteria.

3. Results and discussion

A final sample of 12 articles was obtained (Table 1), distributed across the cited databases, after applying the previously established inclusion and exclusion criteria. Among the studies presented in this review, the highest number of publications was in the year 2020 (n=4, 48%), raising questions about the potential influence of the COVID-19 pandemic on the increased number of publications during that year (Figure 1).

Table 1: Analysis of Selected Studies According to the Year of Publication.

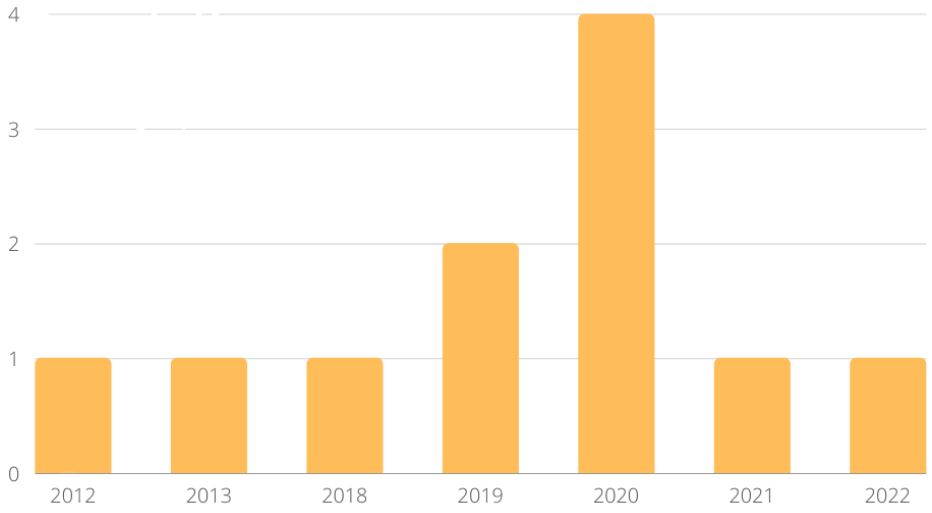
Reference	Main Results
[5]	For a low rate of antigen presence, a product with low antigenicity risk should be selected. To reduce the risk of neutralizing antibody formation, it is recommended to use the product at the minimum dose and avoid booster injections.

[6]	Some newer formulations with purified core neurotoxin without accessory proteins may have overall lower immunogenicity. Various assays are available for antibody detection, including structural assays like ELISA and mouse-based bioassays, but there is no consistent correlation between these antibodies and clinical response.
[7]	The likelihood of developing neutralizing antibodies increased with the single and cumulative treatment dose and was influenced by the formulation of botulinum toxin type A, while all other factors analyzed, including disease etiology and treatment duration, had no additional influence.
[8]	Since botulinum toxin antibodies can have serious consequences for affected patients, a careful analysis of risk factors is necessary to reduce them whenever possible.
[9]	Both ELISA and immunoblot results implicated that Hn-33 is primarily responsible for eliciting the antibody response in the complex.
[4]	The development of neutralizing antibodies is a possibility even with the use of low-dose botulinum toxin type A in aesthetic practice and can result in secondary treatment failure.
[10]	IncobotulinumtoxinA demonstrates strong clinical efficacy and safety, as well as low immunogenicity in a variety of indications.
[11]	Repetitive and long-term treatment with botulinum toxin can induce the immune system to develop antibodies against the toxin.
[12]	Injections of rimabotulinumtoxinB offer only short-term improvement on average, with no long-term prospect due to the high risk of developing a secondary immune response.
[13]	Patients unresponsive to onabotulinumtoxinA produced high levels of antibodies against complexing proteins that could respond to incobotulinumtoxinA.
[14]	Adherence to botulinum toxin therapy showed a trend towards higher single doses, shorter injection intervals, larger cumulative doses, and longer treatment durations.
[15]	The development of neutralizing antibodies against botulinum neurotoxin is often the reason for secondary treatment failure.

Potentially known for its characteristics of paralyzing or weakening the target muscle for a determined period of time, botulinum toxin has been known for over 25 years as a therapeutic source for disease interventions or as an aesthetic choice for wrinkle treatment. The bacterium Clostridium Botulinum, characterized as anaerobic, spore-forming, and rod-shaped, is the source from which the protein known as botulinum toxin is derived. It is characterized as an inactive precursor protein with a high molecular weight of 150 kD, consisting of a 100 kD heavy chain and a 50 kD light chain, along with central neurotoxins and 900 kD of complexing proteins, identified as proteins linked to neurotoxins [5]. Classified into eight distinct serotypes (A, B, C1, C2, D, E, F, and G), only types A and B are available for clinical use. Commercially, botulinum toxin type A is

frequently chosen among three products: onabotulinumtoxin (Botox), abobotulinumtoxin (Dysport), and incobotulinumtoxin A (Xeomin). Their similarities lie in the mechanism of action, but they differ in manufacturing processes and the weight of complexing proteins [5].

Figure 1: Analysis of selected studies according to the year of publication.



The mechanism of action of botulinum toxin is divided into three phases. The first phase is called binding, as after the toxin is injected into the muscle, the protective accessory proteins dissociate, and the active chain is released. Next, the active molecule of botulinum toxin binds to the receptor. In the second phase, the active part of the botulinum toxin is internalized into the nerve cell through receptor-mediated endocytosis, with the light chain being released into the cytoplasm. The third phase, characterized as blocking, involves the cleavage of Snap-25. This blockage of acetylcholine exocytosis affects sympathetic neural transmission to muscle structures and other exocrine glands of the striated muscles, preventing contractions from occurring [4].

Immunogenicity is the ability of a given molecule, including foreign proteins or biological drugs, to elicit an immune response in the host. Regardless of the biological drug, such as a recombinant therapeutic protein, gene therapy vector, or vaccine, it can become a target of the immune system, especially if administered repeatedly or in high doses. Protein-based vaccines, developed to trigger adaptive immunity and the formation of specific antibodies against potential pathogens, perform this function [8].

Immunogenicity is unfavorable when the production of anti-drug antibodies or other immune processes leads to the failure of the therapeutic efficacy of a biological drug. This can occur through direct neutralization or modification of pharmacokinetics [7]. Accessory proteins present in botulinum toxin formulations have a non-therapeutic role, quickly detaching the central neurotoxin at neutral pH. Therefore, the total protein load in the formulation of the three most used brands can influence the immunogenicity of each [10]. Studies indicate that clostridial accessory proteins (HA-1) act as adjuvants to the immune response, potentially contributing to the development of therapeutically relevant neutralizing antibodies against botulinum neurotoxin. Preclinical data suggest that formulations with minimal clostridial protein content are most desirable, as they may delay host immune response stimulation, preventing neutralizing antibody formation and clinical non-responsiveness. Therapeutic products differ in the amount of accessory proteins they contain. Formulations lacking accessory proteins, such as incoBoNT-A, show no efficacy loss, and daxiBoNT-A is still in experimental phases, with no documented side effects yet [15].

Primary non-response implies a lack of response to the treatment from the initial application. This may be due to low dosages, injection into an inappropriate muscle, or

the presence of contractures. Treatment failure may also occur in patients with reduced sensitivity to the toxin or dystonia [15]. Proper storage of botulinum toxin, according to manufacturer recommendations, is essential to avoid potency degradation. Clinical analysis of each case is crucial to prevent incorrect diagnoses. Secondary non-response occurs when a patient who previously had effective treatment no longer responds to subsequent injections. Causes can vary among patients, including lifestyle factors, stress, dose intervals, or placebo effects [6]. Studies suggest that incobotulinumtoxinA shows lower immunogenicity rates compared to other commercially available botulinum toxin formulations. Even with increased toxin doses, success rates were not achieved [6].

Neutralizing antibodies target the central neurotoxin, particularly the heavy chain binding site, while non-neutralizing antibodies target accessory proteins. Some antibodies were also observed in regions where the light chain of the toxin is located [11]. These antibodies can decrease during a prolonged period of toxin disuse (approximately 30 months), with detection dropping by about 60% after six years. However, this immune response can reactivate with recurrent treatments. Patients with elevated antibody levels may require up to four times the usual dosage to achieve the desired result [4].

Plasmapheresis or intravenous immunoglobulin can help remove antibodies but are costly and carry risks. Switching between serum types in treatment is not an initial approach due to the higher risk of resistance to the alternative serum. Switching to a formulation with lower complexing protein concentration may yield positive results due to potentially lower immunogenicity [8]. After ruling out other treatment failure causes, neutralizing antibodies can be suspected, and laboratory assays can detect these antibodies. Structural assays like IPA (immunoprecipitation assay) and ELISA (enzyme-linked immunosorbent assay) are sensitive for antibody detection but cannot distinguish between neutralizing and non-neutralizing antibodies [9]. MPA (mouse protection assay) and MHDA (mouse hemidiaphragm assay) are bioassays using animal tests to identify antibodies affecting toxin efficacy. MPA is the gold standard for detection and quantitative measurement but has relatively low sensitivity. In contrast, MHDA uses fewer animals, reducing cost and time but has a high rate of false positives due to its high sensitivity, which may predict treatment failure inaccurately [13].

4. Conclusion

In clinical practice, it is crucial to pay attention to the intervals between botulinum toxin applications and measure the doses to ensure that the desired final effect meets treatment expectations over time. Proper storage of the product and a thorough understanding of the anatomy for injections also play fundamental roles in the success of the procedure. Even with these precautions, the development of neutralizing antibodies can still occur. In such cases, it is important to explore the causes through a detailed anamnesis, clinical examinations, and laboratory tests before modifying the previously used botulinum toxin formulation.

Funding: None.

Research Ethics Committee Approval: None.

Acknowledgments: None.

Conflicts of Interest: None.

Supplementary Materials: None.

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