Usefulness of the Lymphocyte Transformation Test in Botulinum Toxin type A allergy

Giraldo Tugores M[1*], Martínez Rodríguez ME[2,3*], Fernández Lozano C[1,4*], Martínez Botas J[4], De la Hoz Caballer B[1], González de Olano D[1**], Terrados Cepeda S[1**]

[1]Allergology Department, Hospital Universitario Ramón y Cajal, IRYCS, Madrid, Spain
[2]Physical Medicine and Rehabilitation Department, Hospital Ramón y Cajal, IRICYS, Madrid, Spain
[3]Department of Medicine and Medical Specialities, University of Alcalá. Alcalá de Henares, Madrid, Spain
[4]Biochemistry-Research Department, Hospital Universitario Ramón y Cajal, IRYCIS and CIBEROBN, Madrid, Spain
*These authors should be considered as first author
**These authors should be considered as last authors and Corresponding author

Corresponding authors:
David González de Olano
Allergology Department, Hospital Universitario Ramón y Cajal, IRYCS, Madrid, Spain
E-mail: dgolano@yahoo.es

Soledad Terrados Cepeda
Allergology Department, Hospital Universitario Ramón y Cajal, IRYCS, Madrid, Spain
E-mail: soledadterrados@gmail.com

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.18176/jiaci.0924

Key words: Botulinum toxin. Lymphocyte transformation test. Allergy. Delayed Hypersensitivity. In-vitro test

Palabras clave: Toxina botulínica. Test de transformación linfocitaria. Allergy. Hipersensibilidad tardía. Test in-vitro

Botulinum toxin is the most potent neurotoxin known. Its main effect is the blockade of neuromuscular transmission. Three main Botulinum Toxin A (BoNT-A) have usually been employed for medical and aesthetic purposes: onabotulinumtoxinA, abobotulinumtoxinA, and incobotulinumtoxinA. BoNT-A injection is widely used as an option for the clinical treatment of spasticity after stroke or traumatic brain injury, being effective and well tolerated. [1] Currently, BoNT-A is the most extensively used agent for therapeutic uses and minimally invasive cosmetic procedures, mainly to diminish facial wrinkles. [1]

Immunological complications of BoNT-A administration are the formation of blocking antibodies leading, ocasionaly, to clinical nonresponse and hypersensitivity reactions, prompting local edema and erythema at the injection site. [2] In children, delayed hypersensitivity reactions following BoNT-A administration has not been reported.

CASE REPORT

We report a 14-year-old girl with a medical history of Moyamoya disease. She presented a lacunar stroke in 2014 with spasticity in the left upper and lower limbs since then. In May of 2019 she was prescribed intramuscular injections of Botox®

doi: 10.18176/jiaci.0924

(OnabotulinumtoxinA, Allergan Pharmaceuticals©, USA) in different muscle groups of the forearm, hand and left leg (doses of 20 to 50 IU in each muscle group) every three months to improve spasticity, in order to enhance motor function and quality of life. Antisepsis was performed with Chlorhexidine 10mg/ml and no local anesthetics were used. The injections were guided by ultrasonography with coverslips. In March 2021 she presented an erythematous and pruritic plaque at each of the Botox® injection sites 48 hours later (a total of four plaques). On examination, plaques measured 2x3 cm and were well defined. They lasted up to two weeks with hyperpigmentation and superficial fine desquamation (Supplementary figure 1) with spontaneous resolution. The same lesions appeared with the next administration of Botox®. Histopathology study was not performed. After two delayed reactions with Botox® administration she was referred to the Allergy Department. We indicated topical Betamethasone dipropionate twice daily at the injection site, starting 24 hours after the application of the toxin. The lesions did not appear with this treatment.

Patch tests (PT) with Botox®, Dysport® (AbobotulinumtoxinA, Ipsen Pharmaceuticals©, UK) and Xeomin® (IncobotulinumtoxinA, Merz Pharmaceuticals©, Germany) toxins were done at 1:10 dilution. They were all negative at 48- and 96-hours readings. Prick and intradermal testing with the same dilution of the toxins were negative at immediate and 48 hours readings. A negative prick test and an open test results with Chlorhexidine were obtained. To find out the underlying mechanism, a lymphocyte transformation test (LTT) against Botox®, Dysport® and Xeomin® was equally performed six months after the appearance of skin lesions, as described elsewhere. [3] Mononuclear cells were isolated from heparinized venous blood samples (30 mL) using LymphoPrep (STEMCELL Technologies, Vancouver, BC) gradient centrifugation. The

doi: 10.18176/jiaci.0924

cells were cultured in 96-well U-bottomed plates (200 μL/well) containing the following stimuli: Dynabeads Human T-activator CD3/CD28 (1 μL/well) (Gibco) as positive control: medium AIM V as control negative (unstimulated condition), Botox®, Dysport® and Xeomin® (50 IU, 25 IU, 12.5 IU, 6.25 IU, 3.25 IU and 1.5625 IU). Cultures were performed in triplicate and incubated for 4 days in a humidified incubator (37C, 5% CO2 in air). On day 4, the culture plates were centrifuged and 100 μL of each well was replaced with fresh AIM-V medium containing 10 μCi of 3H-thymidine. On day 6, cells were harvested with a vacuum manifold and radioactivity incorporation into DNA was measured using a liquid scintillation counter. The stimulation index (SI) was calculated as the ratio between the mean of triplicate disintegrations per minute of the drug-stimulated cultures and the mean of triplicate disintegrations per minute of the negative controls. A SI value ≥ 2 (or ≥3 for beta-Lactams) is considered positive. [4] TTL against Botox® and Dysport® had a SI ≥3 and negative to Xeomin® (Figure 1). LTTs in four exposed controls to Botox® and Dysport® showed no response (data not shown). Three months later, the patient was treated with Xeomin® (doses to 35 to 70 IU, in the same muscles as previously) with no reaction.

DISCUSSION

Allergic reactions to BoNT-A are uncommon. BoNT-A hypersensitivity has been mainly described as IgE-mediated reactions, caused by the toxin proteins themselves or by a common component such as gelatin used as solvent. The more complex proteins contained in the drug, the higher the probability of causing allergies. [5] Xeomin® has a purer formulation, in which haemagglutinins of clostridial origin are removed by a biological manufacturing process that reduces the risk of sensitization. [6] Few cases of local delayed hypersensitivity to BoNT-A confirmed by positive intradermal or PT have

doi: 10.18176/jiaci.0924

been described. [2,7] Two cases have reported reactions to different BoNT-A (Botox® and Dysport®) with tolerance to Xeomin®. [8]

LTT is an *in-vitro* test highly useful in the diagnosis of delayed hypersensitivity reactions, [4] especially in the context of drug allergy. [9] It has been proven that DRESS/DIHS (drug reaction with eosinophilia and systemic symptoms/drug-induced hypersensitivity syndrome), beta-lactam antibiotics, and a testing period of two weeks to two months after the allergic event are especially suitable for a well-performing LTT. [4] This technique complements PT, mainly in detection of cell proliferation to certain drugs with a higher probability of a positive result than PT. [4,9]

We report a case of local delayed cutaneous reactions to Botox® with negative *in-vivo* tests (intradermal and PT) to all three BoTN-A tested, positive LTT to Botox® and Dysport® and negative to Xeomin®. Highlighting, LTT was useful either to confirm sensitization to Botox® and Dysport® (probable cross-reactivity with the former) and to identify Xeomin® as a safe alternative. Although it seems to be a promising tool, more studies are needed to validate this technique in this type of reactions. The performance of an appropriate confirmatory work-up after an adverse reaction is mandatory in order to identify the causes and to be able to offer therapeutic alternatives.

Funding sources
The article has no funding source.

Conflicts of interest
The authors have no conflict of interest to declare.

doi: 10.18176/jiaci.0924

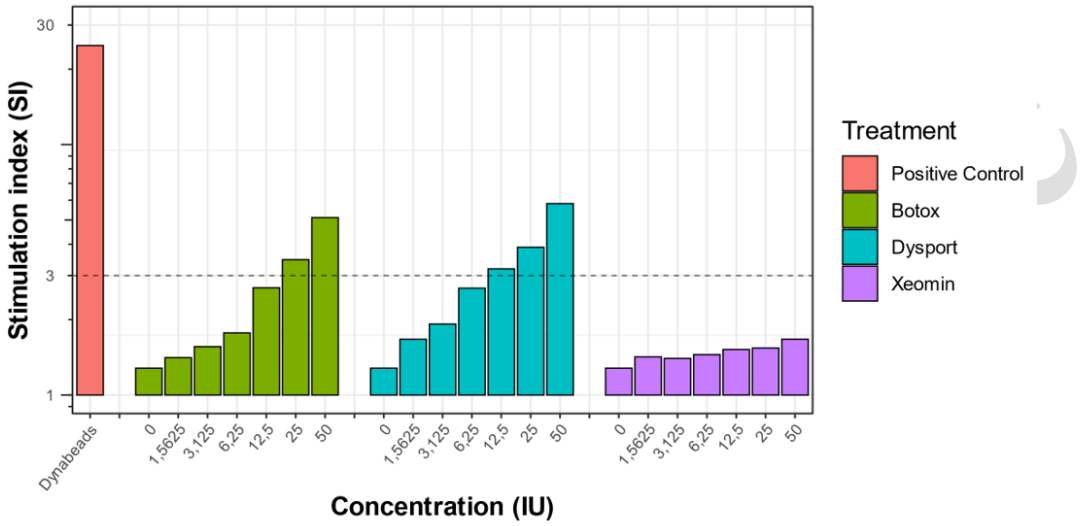
References

1. Hok P, Veverka T, Hluštík P, Nevrlý M, Kaňovský P. The Central Effects of Botulinum Toxin in Dystonia and Spasticity. Toxins (Basel). 2021;13(2):155.
2. Tamura BM, Cucé LC, Rodrigues CJ. Allergic reaction to botulinum toxin: positive intradermal test. Dermatol Surg. 2008;34(8):1117-9.
3. Giraldo-Tugores M, Fernández-Lozano C, Carrón-Herrero A, Gajate P, Martinez-Botas J, Pueyo-López C, et al. Successful rapid desensitization to atezolizumab in delayed hypersensitivity confirmed with lymphocyte transformation test. The journal of allergy and clinical immunology. In practice. 2022; 10(6), 1649-51.
4. Glässner A, Dubrall D, Weinhold L, Schmid M, Sachs B. Lymphocyte transformation test for drug allergy detection: When does it work?. Ann Allergy Asthma Immunol. 2022;129(4):497-506.e3.
5. Wang C, Sun T, Li H, Li Z, Wang X. Hypersensitivity Caused by Cosmetic Injection: Systematic Review and Case Report. Aesthetic Plast Surg. 2021;45(1):263-72.
6. Careta MF, Delgado L, Patriota R. Report of Allergic Reaction After Application of Botulinum Toxin. Aesthet Surg J. 2015 Jul;35(5):NP102-5.
7. Rosenfield LK, Kardassakis DG, Tsia KA, Stayner G. The First Case Report of a Systemic Allergy to OnabotulinumtoxinA (Botox) in a Healthy Patient. Aesthet Surg J. 2014 Jul;34(5):766-8.
8. Ricciardi L, Bove F, Fasano A. Xeomin(®) use in patients with systemic immune reactions to other botulinum toxins type A. Eur J Neurol. 2013;20(3):e45-6.
9. Sachs B, Fatangare A, Sickmann A, Glässner A. Lymphocyte transformation test: History and current approaches. J Immunol Methods. 2021;493:113036.

doi: 10.18176/jiaci.0924

Figure. Lymphocyte transformation test with BoNT-A.

IU, International Units.



doi: 10.18176/jiaci.0924