


Usefulness of the Lymphocyte Transformation Test in Allergy to Botulinum Toxin Type A

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Botulinum toxin is the most potent neurotoxin known. Its main effect is to block neuromuscular transmission. The 3 main types of botulinum toxin A (BoNT-A) used for medical and cosmetic purposes are 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 and is both effective and well tolerated [1]. Currently, BoNT-A is the most extensively used agent in minimally invasive cosmetic procedures, mainly to soften facial wrinkles [1].

The main immunological complications of BoNT-A are formation of blocking antibodies, which leads occasionally to clinical nonresponse, and hypersensitivity reactions, which cause local edema and erythema at the injection site [2]. Delayed hypersensitivity reactions to BoNT-A have not been reported in children.

We report the case of a 14-year-old girl with a history of Moyamoya disease. In 2014, she experienced a lacunar stroke, which resulted in spasticity affecting the left upper and lower limbs. In May 2019, she was prescribed intramuscular injections of Botox (onabotulinumtoxinA, Allergan Pharmaceuticals) in different muscle groups of the forearm, hand, and left leg (doses of 20 to 50 IU in each muscle group) every 3 months to improve spasticity and thus enhance motor function and quality of life. Antisepsis was with chlorhexidine 10 mg/mL, and no local anesthetics were used. The injections were guided by ultrasound protected with a latex

probe cover. In March 2021, she developed an erythematous and pruritic plaque at each of the injection sites 48 hours after treatment (a total of 4 plaques). On examination, the plaques measured 2×3 cm and were well defined. They lasted up to 2 weeks and were characterized by hyperpigmentation and superficial fine desquamation (Supplementary figure 1). The plaques resolved spontaneously. The same lesions appeared after the following dose of Botox. A histopathology study was not performed. After 2 delayed reactions to Botox, the patient 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. The patient's mother gave her written informed consent for the patient's data to be published in this case report.

Patch tests with Botox, abobotulinumtoxinA (Dysport, Ipsen Pharmaceuticals), and incobotulinumtoxinA (Xeomin, Merz Pharmaceuticals) toxins were applied at a 1:10 dilution. The results were all negative at the 48- and 96-hour readings. Prick and intradermal testing with the same dilution of the toxins were negative at the immediate and 48 hour readings. Negative results were recorded in the prick test and an open test with chlorhexidine. To determine the underlying mechanism, a lymphocyte transformation test (LTT) against Botox, Dysport, and Xeomin was performed 6 months after the appearance of skin lesions, as described elsewhere [3]. Mononuclear cells were isolated from heparinized venous blood samples (30 mL) using Lymphoprep gradient centrifugation (STEMCELL Technologies). The 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 a positive control; medium AIM V as a negative control (unstimulated condition); Botox; Dysport; and Xeomin. All stimuli were administered at 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 (37°C, 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

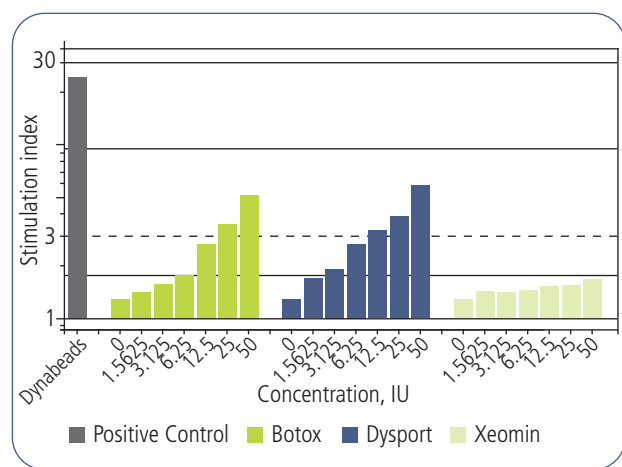


Figure. Lymphocyte transformation test with BoNT-A. IU, international units.

incorporation of radioactivity into DNA was measured using a liquid scintillation counter. The stimulation index (SI) was calculated as the ratio of the mean triplicate disintegrations per minute of the drug-stimulated cultures to the mean of triplicate disintegrations per minute of the negative controls. An SI value ≥ 2 (or ≥ 3 for β -lactams) is considered positive [4]. An LTT with Botox and Dysport yielded an SI ≥ 3 and a negative result with Xeomin (Figure). LTTs in 4 controls exposed to Botox and Dysport showed no response (data not shown). Three months later, the patient was treated with Xeomin administered at 35 to 70 IU in the same muscles as before, with no reaction.

Allergic reactions to BoNT-A are uncommon. Hypersensitivity to BoNT-A manifests mainly as IgE-mediated reactions caused by the toxin proteins themselves or by a common component such as gelatin used as solvent. The probability of allergy increases with the complexity of the proteins contained in the drug [5]. Xeomin has a purer formulation, in which hemagglutinins 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 patch testing have been described [2,7]. Reactions to Botox and Dysport have been reported in 2 cases where the patient tolerated Xeomin [8].

LTT is a highly useful in vitro test for the diagnosis of delayed hypersensitivity reactions [4], especially in the context of drug allergy [9]. It works particularly well in drug reaction with eosinophilia and systemic symptoms/drug-induced hypersensitivity syndrome and reactions to β -lactam antibiotics and with a testing period of 2 weeks to 2 months after the allergic event [4]. This technique complements patch testing, mainly in detection of cell proliferation with drugs, and is more likely to yield a positive result than patch testing [4,9].

We report a case of local delayed cutaneous reaction to Botox with negative in vivo results (intradermal and patch testing) to all 3 BoNT-A formulations tested, positive LTT results for Botox and Dysport and negative LTT results for Xeomin. Of note, LTT proved useful for confirming sensitization to Botox and Dysport (probable cross-reactivity with the former) and for confirming Xeomin as a safe alternative. Although LTT seems to be a promising tool, more studies are needed to validate the technique in this type of reaction. An appropriate confirmatory work-up after an adverse reaction is mandatory in order to identify the causes and offer alternative therapy.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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