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Intraglandular Injection of Botulinum Toxin A Reduces Tear Production in Rabbits

Anna M. Demetriades, M.D., Ph.D.^{*}, Ilya M. Leyngold, M.D.^{*}, Sam D'Anna, M.S.^{*}, Allen O. Eghrari, M.D.^{*}, Dave G. Emmert, B.S.^{*}, Michael P. Grant, M.D., Ph.D.[†], and Shannath L. Merbs, M.D., Ph.D.[†]

^{*}Department of Ophthalmology, The Wilmer Eye Institute, The Johns Hopkins Hospital, Baltimore, Maryland, U.S.A

[†]Division of Oculoplastic and Reconstructive Surgery, The Wilmer Eye Institute, The Johns Hopkins Hospital, Baltimore, Maryland, U.S.A

Abstract

Purpose—To develop an animal model and investigate the dose-dependent effect of an intraglandular injection of botulinum toxin A (BTX-A) on tear production.

Methods—In a volume of 0.1-ml, 0.625-, 1.25-, or 2.5-U BTX-A was injected transconjunctivally in the superolateral lobe of the lacrimal gland of adult New Zealand white female rabbits. In the contralateral lacrimal gland, 0.1 ml of 0.9% sodium chloride was injected. Prior to injection and at 1-week postinjection, photographs were taken to evaluate pre- and postoperative eyelid position. Fluorescein and Rose Bengal stain were used to evaluate the corneal surface, and Schirmer test was used to assess tear production.

Results—Glands injected with the intermediate (1.25 U) and the highest (2.5 U) doses of BTX-A displayed a statistically significant decrease in tear production ($p = 0.002$ and 0.007 , respectively) compared with the contralateral saline-injected glands at 1 week. No corneal pathologic factors from excessive dryness were observed following the injection. While postinjection ptosis was observed ($p = 0.025$), no difference was seen between BTX-A and saline-injected eyes.

Conclusions—In rabbits, intraglandular injection of BTX-A resulted in decreased tear production at 1 week. No additional reduction in tear production was seen with a BTX-A dose greater than 1.25 U, suggesting glandular receptor saturation at this dose. Despite suppression of tear production, no corneal pathologic factors were observed. Further studies are needed to refine this animal model with the ultimate goal of determining optimum delivery route and concentration to reduction in tear production while minimizing side effects in patients.

Botulinum toxin A (BTX-A) is a neurotoxin produced by *Clostridium botulinum* bacteria. It reversibly blocks the release of acetylcholine from the cholinergic nerve terminals of the neuromuscular synapse and autonomic nerve fibers. Reversibility occurs because of collateral sprouting of axons and regeneration of the end plate.¹ In conditions such as strabismus, dystonias, torticollis, and blepharospasm, BTX-A has been used to weaken

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Address correspondence and reprint requests to: Shannath L. Merbs, M.D., Ph.D., The Wilmer Eye Institute, Johns Hopkins University School of Medicine, 600 North Wolfe Street, Maumenee 505, Baltimore, MD 21287. smerbs@jhmi.edu. Drs. Demetriades and Leyngold contributed equally to this article.

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overactive muscles.² In addition, it has been used to control hypersecretion from glands supplied by cholinergic neurons.³ Injection in the lacrimal gland blocks the presynaptic release of acetylcholine, which is required for tear secretion.

Botulinum toxin A has been successfully used to treat excess tear production in the setting of gustatory hyperlacrimation, also known as the crocodile tear syndrome, which can occur after injury to the proximal seventh nerve.⁴ Fibers originally supplying mandibular and sublingual glands reinnervate the lacrimal gland by way of the greater superficial petrosal nerve and stimulate tear production with chewing. Since the first successful treatment of crocodile tear syndrome using BTX-A in 2 patients in 1998,⁵ eleven additional articles described the successful use of BTX-A in a total of 51 patients with crocodile tear syndrome.^{6–16} All these studies reported complete or near-complete resolution of aberrant tearing within 1 to 2 weeks of treatment, with only infrequent and reversible complications including ptosis, lagophthalmos, diplopia, conjunctivitis, and dry eye. Botulinum toxin A has also been used to a lesser degree to minimize symptomatic tearing in patients with lacrimal obstruction^{16,17} and functional tearing.¹⁸

The amount of BTX-A injected in these studies ranged from 1-U to 60-U Botox (Allergan, Inc., Irvine, CA)^{8,9,11,13–18} and 10-U to 20-U Dysport (Ipsen Ltd., Slough, United Kingdom)^{5,7,10,12} in differing or nonspecified concentrations. No apparent benefit of higher doses was seen, although no actual dose or concentration comparisons have been published. Reported injection routes include transconjunctival injections in the palpebral lobe of the lacrimal gland^{6,7,11–14,16–18} and transcutaneous injection in the orbital lobe.^{5,8–10,15} Montoya et al.¹² suggested that a transconjunctival injection was preferred due to the ability to directly visualize the lacrimal gland during injection. More recently, a meta-analysis found the transconjunctival approach to be associated with fewer complications.⁷ Patients who experience relief of tearing report that it lasts anywhere from 3 to 12 months after the injection, and the injection can safely be repeated multiple times.⁶

The authors' limited clinical experience with the use of BTX-A in tearing patients has suggested that tear reduction and complications such as ptosis may be concentration- and/or dosage-dependent. To allow them to systematically study the relationship among the administered dose, reduced tear production, and associated side effects, the authors developed an animal model. Although BTX-A has not been used in an animal model before, intraglandular injection of BTX-B has been shown to suppress normal tear production and create an animal model of dry eye in mice.¹⁹ The authors chose to use rabbits because of their larger eyes and lacrimal glands for their investigation of the dose-dependent effect of a transconjunctival intraglandular injection of BTX-A on normal tear production.

MATERIALS AND METHODS

Animals

Studies were performed using New Zealand white sexually mature female rabbits, weighing about 3 kg. Rabbits were sedated with 1.25-ml intramuscular injection of 60% ketamine (100 mg/ml) and 40% xylazine (20 mg/ml). All animals were handled in accordance with the Association for Research in Vision and Ophthalmology statement for the Use of Animals in Ophthalmic and Vision Research and according to an Institutional Animal Care and Use Committee approved protocol.

Intraglandular Injection of BTX

Transconjunctival injections were performed with a 30-gauge needle, using a Desmarres retractor for exposure, in the superolateral lobe of the lacrimal gland to reproduce the anatomical location of the palpebral lobe in humans (Fig. 1A). A 0.625-, 1.25-, or 2.5-U

BTX-A in 0.1 ml of 0.9% sodium chloride solution was injected on the right side, and 0.1 ml of 0.9% sodium chloride solution was injected on the left as control. Four animals were in each dose treatment group.

Measurements

Measurements were performed under sedation just prior to injection and 1 week after injection.

Schirmer test with topical anesthesia was used to measure baseline tear production as follows. Topical proparacaine ophthalmic solution 0.5% (Akorn Inc., Buffalo Grove, IL) was applied to each eye. A 5 × 25-mm strip of filter paper was inserted in the lower conjunctival cul-de-sac with the end of the filter paper hanging over the edge of the lower eyelid (Fig. 1B). The degree of wetting at 5 minutes was measured.

Corneal staining was used to assess for ocular surface pathologic factors as follows. After topical application of 1% sodium fluorescein (Sigma-Aldrich, St. Louis, MO), the eyes were examined under cobalt blue light and photographed using a digital camera with a macro lens. Rose Bengal strips (Akorn Inc.) were then applied to the inferior fornix, and the level of Rose Bengal staining was documented by an additional photograph.

Upper eyelid measurements were used to evaluate for possible ptosis caused by the BTX-A injection as follows. External photographs were taken of each eye to document the upper eyelid position. From each photograph, the vertical intrapalpebral height and the horizontal corneal diameter were measured, and the ratio between the 2 measurements was calculated. The eyelid positions were assessed at baseline prior to anesthesia, after anesthesia but prior to injection, and at 1 week following injection.

Lacrimal Gland Histology

Rabbits were euthanized, and the orbital contents were harvested for histologic study of the lacrimal gland. The tissue was fixed in 2.0% paraformaldehyde; 2.0% glutaraldehyde in 0.1-mmol/l cacodylate solution and embedded in paraffin. Eight micron sections were stained with hematoxylin and eosin to determine the extent of lymphocytic or inflammatory cell infiltration of the stroma and acini of the lacrimal gland and surrounding tissues following injection.

Statistical Analyses

Linear mixed-effects model was used to compare the difference in tear production between OD and OS (i.e., the wetting length from the BTX-A-injected eye minus the wetting length from the saline-injected eye). The model took into consideration the correlation between the preinjection and 1-week differences in tear production from the same rabbit. A *p* value of less than 0.05 was considered statistically significant.

RESULTS

One week after intraglandular injection of BTX-A, a statistically significant decrease in tear production was observed at the intermediate (1.25 U) and the highest (2.5 U) doses tested (average difference in wetting length: −7.00 mm, *p* = 0.002 and −6.00 mm, *p* = 0.007, respectively) compared with the contralateral eye injected with saline (Table). There was no difference seen in tear production at the lowest dose of BTX-A (0.625 U).

Despite suppression of baseline tear production at the higher doses, there was no evidence of dry eye or corneal pathologic factors observed after application of either fluorescein or Rose

Bengal (Fig. 2). Mild ptosis was observed in some rabbits (Fig. 3), and there was a significantly smaller ratio of intrapalpebral distance to horizontal corneal diameter in eyes postinjection compared with the preinjection measurements ($p = 0.025$). No significant difference between right and left ratios (BTX-A and control eyes) was observed ($p = 0.020$). On gross pathologic factors following dissection, hemorrhage in the lacrimal gland was noted in some rabbits; however, this did not correlate with the presence or absence of ptosis. On histologic examination, no inflammation or changes in gland architecture were observed after the injection of either BTX-A or saline (Fig. 4).

DISCUSSION

The authors sought to develop an animal model to systematically investigate how modifying the dose, concentration, and route of injection of BTX-A for tearing affects the success and complication rates of the treatment. A successful animal model would reproduce the decrease in tear production seen in patients and the side effects. In rabbits, the tissues involved in lubrication of the ocular surface include the lacrimal gland, Harder's gland which lies adjacent to the lacrimal gland, and the nictitans gland which lies buried in the nictitating membrane adjacent to Harder's gland.²⁰ To recapitulate a transconjunctival injection in the palpebral lobe of a patient's lacrimal gland, the authors injected transconjunctivally in the lacrimal gland of the rabbit. In the experiment described here, the authors held the injection volume constant (0.1 ml) and varied the dose of BTX-A, finding a statistically significant decrease in tear production compared with the saline-injected eye with the 1.25- and 2.5-U doses.

In humans, the aqueous layer of the tear film is produced by the main and accessory lacrimal glands located anterolaterally within the orbital part of the frontal bone and conjunctival stroma, respectively. Traditionally, the main lacrimal gland was thought to be responsible only for reflex tear production and the accessory glands (lacrimal glands of Krause and Wolfring) for basal tearing. More recent data, however, suggest that main and accessory glands significantly contribute to the 2 types of tear production mechanisms.²¹ Botulinum toxin A injected in the main lacrimal gland of patients with aberrant facial nerve regeneration has been shown to reduce pathologic excess production and relieve tearing symptoms.^{5–16} Additionally, in patients without excess tear production, BTX-A injected in the main lacrimal gland should suppress reflex lacrimation and basal tear production.

An ophthalmologist frequently encounters patients who report epiphora even with a patent nasolacrimal outflow system, and some of these patients still have functional tearing despite conventional interventions. Whittaker et al.¹⁸ investigated the usefulness of BTX-A in these patients and was able to achieve symptomatic relief in 8 of 11 patients with transconjunctival injection of 2.5-U to 5-U BTX-A in the palpebral lobe of the lacrimal gland, causing transient ptosis and diplopia in 2 patients. It is unclear whether modifying the dose, concentration, or route of injection would have improved the success rate or reduced the complication rate of the treatment.

The authors assessed for 2 possible adverse outcomes, ocular surface pathologic factors attributable to excessive drying and ptosis of the upper eyelid. In the BTX-A-injected eyes, the authors found no evidence of excessive dry eye in the rabbits, as demonstrated by a lack of fluorescein or Rose Bengal staining. This is in contrast to the mouse model of dry eye, in which 1.25-U BTX-B injected in the lacrimal gland causes corneal fluorescein staining as early as 3 days after injection.¹⁹ One explanation for this difference may be that BTX-B is known to have a quicker onset of action and greater diffusion than BTX-A.²² Another explanation is the size and the anatomical differences between the 2 animals. In the rabbit, the lacrimal gland consists of 2 distinct glands, the main lacrimal gland and the infraorbital

gland; in rodents, these 2 glands are 2 portions of single gland.²⁰ To produce corneal drying in future studies, it may be necessary to inject both glands in the rabbit.

Upper eyelid ptosis was seen more frequently after injection, most likely due to either a volume effect from the hemorrhage the authors saw on gross pathologic factors around some of the lacrimal glands or actually trauma to the levator complex during the injection. Because there was no difference in postinjection ptosis between the BTX-A and saline-injected eyes, it seems less likely that the ptosis resulted from paralysis of the levator muscle by the BTX-A.

Histologic examination of the lacrimal glands revealed no pathologic factors, particularly no evidence of inflammation from injection of either BTX-A or the saline control. This is similar to what has been observed for BTX-B injections in the mouse lacrimal gland¹⁹ and for BTX-A injections in the orbicularis of patients with blepharospasm.²³

While intraglandular injection of BTX-A in patients may provide a viable therapeutic alternative for patients with functional epiphora, further studies are required to determine the optimum dose, concentration, and route of delivery. The authors used BTX-A to reduce normal, baseline tear production in a rabbit with the idea of developing an animal model, in which the authors could systematically vary the dose, concentration, and route of delivery to reduce tear production while minimizing side effects.

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FIG. 1.

A, Intraglandular injection is accomplished by exposing the superolateral lobe of the lacrimal gland with a Desmarres retractor and injecting with a 30-gauge needle. **B**, Baseline tear production is measured by placing a Schirmer strip in the inferior fornix and leaving in place for 5 minutes. The amount of wetting is measured in millimeter.

FIG. 2.

Anterior segment photographs of a rabbit's eyes 1 week after intraglandular injection of either BTX-A (**A**, study eye) or saline (**B**, control eye). Photographs of the same eyes showing an absence of fluorescein staining (**C**, study eye; **D**, control eye) and an absence of Rose Bengal staining (**E**, study eye; **F**, control eye) 1 week after injection.

FIG. 3.

A and B, Normal upper eyelid position in 2 rabbits before injection. Photographs of the same animals, respectively, 1 week after injection (**C**) with and (**D**) without ptosis.

FIG. 4. Histology of the lacrimal gland (**A**) and Harder's' gland (**B**) 1 week after BTX-A injection (2.5 U). No inflammation or hemorrhage is seen (hematoxylin-eosin, $\times 10$).

Table

Reduction of tear production after BTX-A injection

Dose (U)	Average preinjection wetting right-left (control) (<i>p</i> , 95% CI)	Average 7 days postinjection wetting right (BTX-A)-left (control) (<i>p</i> , 95% CI)
0.625	-0.88 (0.7, -5.2 to 3.5)	-0.25 (0.9, -4.6 to 4.1)
1.25	0.25 (0.9, -4.1 to 4.6)	-7.00 (0.002, -11.4 to -2.6)
2.5	1.00 (0.7, -3.4 to 5.4)	-6.00 (0.007, -10.4 to -1.6)

BTX-A, botulinum toxin A; CI, confidence interval; U, units.