

The Pupillary Effects of Retrobulbar Injection of Botulinum Toxin A (Oculinum) in Albino Rats

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Botulinum toxin (BoTx) has been clinically used in the treatment of localized dystonic states such as blepharospasm, as well as in strabismus. Reported side effects have included primary excessive weakness of neighboring extraocular muscles. To evaluate possible involvement of the iris, we injected BoTx into the retrobulbar space of albino rats. Ipsilateral mydriasis with cholinomimetic supersensitivity developed in the treated animals. There was no apparent optic nerve dysfunction. The authors observed these effects using BoTx doses insufficient to cause clinical weakness or electrophysiological evidence of generalized neuromuscular dysfunction. The mydriasis disappeared spontaneously within 2–3 weeks. Higher BoTx doses resulted in severe neuromuscular paralysis and death. These findings were consistent with clinical botulism, which may include autonomic paralysis. The site of BoTx action could be the ciliary ganglion or cholinergic terminals in the iris. The authors concluded that side effects of BoTx were not necessarily limited to striated muscle weakness. Invest Ophthalmol Vis Sci 32:122–125, 1991

Since 1973, BoTx has been used to treat experimentally and routinely a wide range of clinical conditions in which deliberate paralysis of a particular muscle may have been beneficial. The latter conditions included strabismus^{1,2} hemifacial spasm,³ essential blepharospasm⁴ and spasmodic torticollis.^{5,6}

Several local side effects have been reported after the neurotoxin injection to a certain extraocular muscle in the rats: tearing, ptosis, dry eye with and without lagophthalmos, ectropion, entropion, and diplopia due to overcorrection.^{1,6,7} Elston found no involvement of the pupil after the injection of BoTx in the treatment of strabismus⁷; neither did Helveston and Pogrebniak,⁸ who injected BoTx retrobulbarly in the treatment of nystagmus. On the other hand, pupillary dilation and poor reaction to light and accommodation occur frequently in human botulism^{9,10} and tonic pupils were also reported in this condition.¹¹ Mydriasis after retrobulbar injections of 4 ng BoTx in squirrel monkeys was noted by Behrens et al,¹² and after retrobulbar administration of BoTx to rabbits, toxic effects to the ciliary ganglion were

recorded.¹³ Because of increased interest in BoTx, we have attempted to investigate the pupillary effects of retrobulbar injection of BoTx in albino rats, using clinically similar doses.

Materials and Methods

In preliminary experiments, the optimal dose of BoTx-causing mydriasis was studied by injecting gradually increased levels of BoTx (Sigma Chemical Company, St. Louis, MO) into the retrobulbar space of 30 albino adult rats (ICR strain), weighing 170–250 g. The rats were anesthetized by intraperitoneal injection of chloralhydrate (400 mg/kg), and retrobulbar injection was performed with a 27-gauge needle, using different dilutions of BoTx, in a constant volume of 0.1 ml of buffered saline (pH = 6.5). The neurotoxin dose was gradually increased from 40 pg to 10 ng/injection. The needle was inserted through the lateral area of the inferior fornix and directed toward the apex of the muscle cone, behind the globe. Proptosis was found 24 hr after the retrobulbar toxin injection, with the subsequent development of exposure keratitis in all animals. Corneal abscess and perforation were found in 20% of the animals. In the following experiments, lateral tarsorrhaphy was performed with 5-0 virgin silk sutures immediately after BoTx injection. Chloramphenicol drops were instilled twice daily. The tarsorrhaphy was opened daily during the first week after the retrobulbar injection, after which the antibiotic therapy was stopped and the sutures removed permanently. In those animals, keratitis had not developed and ocular

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All of the procedures used in this work conform to the ARVO Resolution on the Use of Animals in Research.

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palpation revealed the proptotic eyes to be soft and compressible.

After the preliminary experiments, BoTx was injected retrobulbarly into the right orbit of two groups of albino adult rats, each group consisting of 15 animals. The two groups received a different dose of the neurotoxin in 0.1 ml buffered saline: group A received 1.5 ng and group B received 2.5 ng. The eyes of each rat were examined after the injection, through a binocular microscope (Olympus, Tokyo, Japan). The pupillary diameter was measured in the injected, and in the control eye, by a divided scale fitted into one of the oculars.¹¹

During the first week after injection, the animals were examined daily, and subsequently once a week, for 3 months. Pharmacologic pupillary tests with arecoline 0.05% were performed to detect the presence of denervation supersensitivity.

As a control, a similar volume of 0.1 ml buffered saline (pH = 6.5) was injected into the retrobulbar space of the right eye of an additional 10 albino adult rats. Although no proptosis or exposure keratitis was subsequently noted, lateral tarsorrhaphy was done, and chloramphenicol drops were instilled twice daily for 7 days to imitate the experimental conditions described above. The pharmacologic tests were performed in these control animals.

The systemic effects of the neurotoxin were studied by using electromyography (EMG) on the gastrocnemius muscle in both legs of 10 treated animals. The

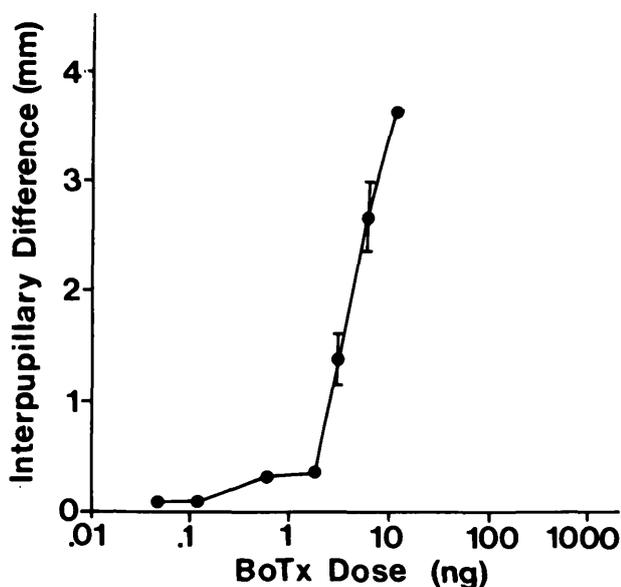


Fig. 1. Log-dose response curve of Botulinum toxin A (BoTx). The relationship between different doses of BoTx and the difference between the pupillary diameter of treated and untreated eyes, shows a steep rise at a dose of around 5 ng. The S.D. is very small in the extreme doses.

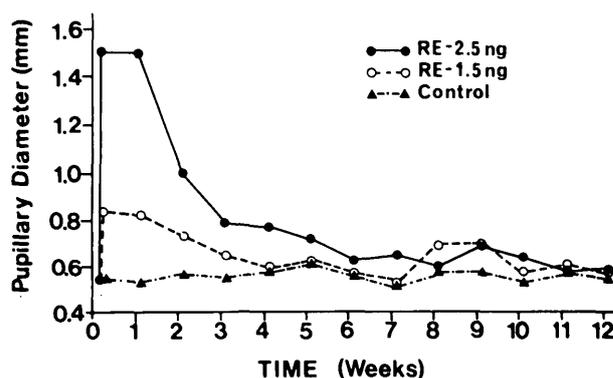


Fig. 2. Changes of pupillary diameter with time following retrobulbar BoTx injection at $t = 0$. Note the steep dose-dependent mydriatic effect of BoTx. Control values refer to the untreated eyes of the same animals.

EMG was performed by applying pairs of stimuli separated by an interval of approximately 8 msec to the muscles at a frequency of 1/sec.^{15,16}

Results

The lethal dose of BoTx, given by retrobulbar injection, was found to be 3.3 ng. No pupillary effect was found below an injected dose of 0.5 ng of the neurotoxin. Between 1.5 ng and 10 ng toxin per injection, there was a steep rise in the occurrence of mydriasis (Fig. 1), to almost complete pupillary dilation. After the injection of more than 3.3 ng of the neurotoxin, all the animals had severe systemic effects of botulism, such as generalized muscular weakness associated with respiratory difficulties. They died 24–96 hours after the retrobulbar injection. Subsequent experiments were performed using two different doses of BoTx: 1.5 ng per injection (group A) and 2.5 ng per injection (group B). These doses produced no apparent systemic effects.

The time-related, pupillary, mydriatic effect of BoTx in the two groups is shown in Fig. 2. The mydriatic effect was found to be dose dependent, 0.8 mm after 1.5 ng and 1.5 mm after 2.5 ng BoTx injection. The mydriasis lasted unchanged for 1 wk in both groups, then generally declined over 8 wk. In the control group, in which buffered saline was injected retrobulbarly, no pupillary effects were noted.

Before the denervation supersensitivity tests were started, the minimal concentration of arecoline, that causes pupillary constriction was studied in untreated rats. One drop of gradually decreasing concentrations of arecoline, from 10% to 0.01%, was instilled unilaterally on the cornea of normal albino rats and the pupillary diameter was measured every 15 min for 90 min. The latter data are presented in Fig. 3. The highest noneffective concentration of RE of arecoline was

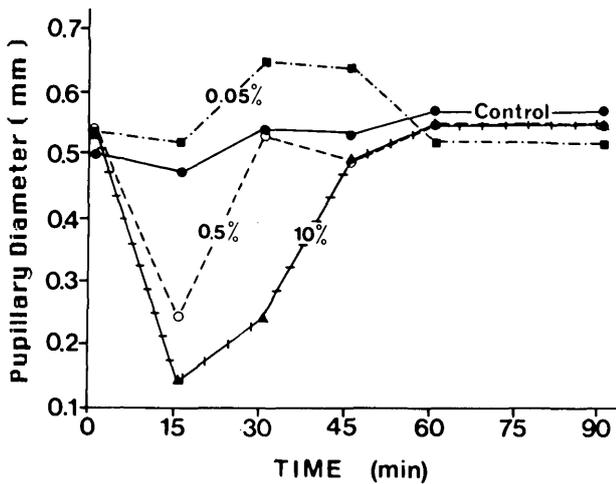


Fig. 3. Time related miotic effect of different concentrations of arecoline drops, instilled locally. Control animals were treated with normal saline solution.

found to be 0.1%, and a concentration of 0.05% was used in subsequent supersensitivity tests. One drop of arecoline 0.05% was instilled in both eyes of rats pretreated with unilateral BoTx injection. After instillation of arecoline, both pupils were examined under a biomicroscope and their diameters were measured every 15 min for 90 min.

Time-related dose sensitivity histograms are shown in Figure 4. By comparing the pupillary diameter immediately before and 15' after instillation of arecoline between the untreated and the treated eyes, it can be seen that maximal supersensitivity was present in both 1.5 ng and 2.5 ng BoTx-treated eyes on the fifth day after the neurotoxin injection. Subsequently in both groups, the pupillary sensitivity declined gradually from the end of the second week toward the 12th week after toxin injection. On the 13th week after injection, the pupillary diameter of the treated eye returned to its normal size, although minimal denervation supersensitivity was still noticed.

No EMG abnormalities were present in rats treated by the nonlethal doses of BoTx (1.5 ng and 2.5 ng). However, at doses of 3.3 ng and 5.0 ng, a typical larger amplitude of the response to the second stimulus was noted.

Discussion

The neurotoxic protein BoTx is produced as one of eight serologically distinct types by different strains of the bacterium, *Clostridium botulinum*. The most common form of the neurotoxin, and the one used in ophthalmology, is type A (Oculinum). The purified type A toxin moiety has a molecular weight of approximately 150,000 and is known to have an LD₅₀ (i.p.) of less than 1×10^{-5} μ g in mice.¹⁶ In vertebrate

species, this toxin is lethal by virtue of the flaccid paralysis it produces in the skeletal muscles, particularly of the respiratory system.

In 1949, Burgen et al¹⁷ obtained direct evidence for a presynaptic inhibitory effect of BoTx on evoked acetylcholine release in vitro. It is known that the botulinum neurotoxins act primarily on peripheral cholinergic synapses where they inhibit the release of the neurotransmitter.^{18,19}

Scott was the first to institute the use of BoTx for human therapy.^{1,2} The minimal effective dose of BoTx for the treatment of strabismus in adults is 0.75 U. One unit equals approximately 0.4 ng.²⁰ The amounts of the toxin delivered varied from 0.25 to 6.5 U in the work of Scott et al^{20,21} and up to 25 U according to Gammon et al.²² This potent microbiologic product, which appears to be safe, has been recommended as a treatment for a variety of dystonic conditions.²³

After BoTx injection, muscle weakness developed within 24-36 hours⁷ and lasted for 4-12 weeks.^{1,7} The muscle activity returned gradually to baseline within 2 to 3 months. No systemic side effects have been reported, but several local side effects were described. Pupillary involvement was never reported. Our goal was to determine whether BoTx could affect the ciliary ganglion after its retrobulbar injection in rats. Kupfer¹³ reported several years ago that retrobulbar BoTx injection to rabbits produced mydriasis. In this study, high doses were used, and it is unclear whether the effects could be elicited by lower doses and in other species. In addition, Kupfer has not examined whether cholinergic supersensitivity developed in his experimental animals.

Denervation supersensitivity of the pupillary sphincter has been demonstrated by Sachs et al²⁴ in

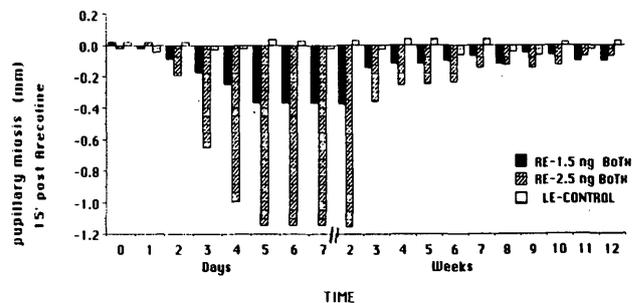


Fig. 4. The pupillary effects of arecoline (0.05%), 15' following bilateral instillation. A comparison between the pupillary diameter of BoTx treated and untreated eyes, following arecoline instillation, is shown along time. Data are given as differences from pupillary diameter before arecoline administration. Note the development of arecoline sensitivity during the first week and its gradual disappearance after two weeks. 2.5 ng of BoTx had markedly more pronounced effect than 1.5 ng, paralleling the appearance and disappearance of mydriasis.

cats after removal of the ciliary ganglion. The maximal supersensitivity was noticed on the fifth day after mechanical removal of the ganglion.²⁴ In the current experiments, maximal denervation supersensitivity of the pupil similarly appeared 5 days after retrobulbar BoTx injection. Four weeks after retrobulbar injection of BoTx, the diameter of the maximally constricted pupil, found 15 min after instillation of arecoline 0.05%, was significantly lower than the pupillary diameter of the control eye (Fig. 4). This is supportive evidence for denervation supersensitivity and, although we have shown indirect proof, we assume that the pupillary denervation supersensitivity resulted from the direct effect of BoTx on the ciliary ganglion. Twenty-four hours after retrobulbar BoTx injection, proptosis was found in all animals. It is probable that the toxin spread by diffusion to the extraocular muscles. As the latter muscles caused retraction of the eyeball in normal conditions, their paralysis was probably the cause of this proptosis.

Although BoTx caused immediate mydriasis, supersensitivity developed gradually over 1 wk (Figs. 2 and 4). Similar findings in humans show denervation supersensitivity several days after damage to the ciliary ganglion.²⁵

Our results show that the minimal effective dose that causes pupillary mydriasis in the rat was found to be 0.5 ng of BoTx. Scott²⁰ reported that the minimal effective dose for the treatment of strabismus in a human adult is 0.3 ng, which is 60% of the dose that affects the pupil in the albino rat. As multiple injections of BoTx are sometimes needed in dystonic conditions,^{4,6,23} and the cumulative doses of BoTx may reach 10 ng,²² mydriasis can be expected if the toxin is accidentally injected into the retrobulbar space or if it reaches the ciliary ganglion by diffusion.

Key words: botulinum toxin, mydriasis, cholinergic supersensitivity, iris, pupil

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