Motor Nerve Sprouting in Human Orbicularis Muscle after Botulinum A Injection

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The paralytic properties of botulinum A toxin have led to its use in humans in the treatment of strabismus and facial dystonias such as essential blepharospasm. Examination of orbicularis muscle from 10 patients with essential blepharospasm who received 2-18 injections of botulinum toxin 6 weeks to 3 years prior to surgery revealed characteristic nodal, terminal and ultraterminal “sprouting” of the motor axons. Orbicularis muscle from five individuals never exposed to botulinum failed to demonstrate these changes. The significance of persistent motor nerve sprouting in response to botulinum exposure remains to be elucidated. Invest Ophthalmol Vis Sci 31:964-967, 1990

Botulinum A toxin blocks neuromuscular transmission, producing profound but transient muscle paralysis. Since Scott and co-workers’ pioneering work on the selective weakening of extraocular muscles, botulinum toxin has found widespread use as an effective, though temporary, treatment for the symptoms of facial dystonias such as essential blepharospasm and spasmotic torticollis. Patients enjoy relief from their muscle spasms, although they require reinjection as the paralytic effects of the toxin wear off over 8–12 weeks.

Botulinum toxin injected into animals is known to produce motor axon outgrowth or “sprouting.” To our knowledge, sprouting has not been demonstrated in humans, either with food-borne botulism or after treatment for blepharospasm or other conditions. We evaluated intramuscular motor axons in the orbicularis muscle of 10 patients who had received local botulinum toxin injections for blepharospasm, to determine if axonal sprouting develops.

Materials and Methods

Patients

Orbicularis muscle was obtained from patients undergoing surgical myectomy for essential blepharospasm or Meige’s syndrome. Ten patients, ages 35–81 yr, had previously received 2–18 injections of 40–110 units of commercially available botulinum A toxin (Oculinum; obtained from Alan B. Scott, MD, San Francisco, CA; available from Allergan, Irvine, CA) for the treatment of blepharospasm. The last injection ranged from 6 weeks to 3 yr prior to surgery. Orbicularis muscle specimens were obtained also from two patients with blepharospasm who had never received botulinum toxin, and from three normal controls with no exposure to botulinum.

Procedure

Muscle specimens were snap-frozen in liquid nitrogen and sectioned to 40 nm. Intramuscular nerves then were evaluated with the silver-cholinesterase-immunocytochemistry (SCI) stain, which differentiates axons (silver), the motor end-plate (cholinesterase), and myelin (immunocytochemistry). The specimens were examined with light microscopy by an experienced investigator (KA) unaware of each patient’s prior history of botulinum injection.

Results

Orbicularis muscle from patients previously injected with botulinum toxin showed profuse axonal sprouting. Axonal sprouts arose from nodes of Ranvier of the preterminal motor axon (Fig. 1), from the terminal axon immediately proximal to the motor end-plate, and from the terminal axonal arborization over the motor end-plate (Fig. 2). Sprouts were seen to extend erratically through the muscle interstitium, with a preference for growth roughly paralleling the muscle fibers. Each muscle specimen examined showed several of each of the three types of sprouts. Identification of sprouts was facilitated by the visual-
Fig. 1. Preterminal sprouting in a patient 1 yr after botulinum administration. The preterminal motor axons are myelinated (large arrows). Numerous unmyelinated sprouts arise from the nodes of Ranvier and terminate in the interstitium between the muscle fibers (small arrows). Bar = 20 μm.

Fig. 2. Cholinesterase staining of a human orbicularis muscle fiber 1 yr after botulinum administration. Bar = 20 μm.

Fig. 3. Preterminal sprouting in a patient 1 yr after botulinum administration. The preterminal motor axons are myelinated (large arrows). Numerous unmyelinated sprouts arise from the nodes of Ranvier and terminate in the interstitium between the muscle fibers (small arrows). Bar = 20 μm.

Fig. 4. Cholinesterase staining of a human orbicularis muscle fiber 1 yr after botulinum administration. Bar = 20 μm.

Fig. 5. Preterminal sprouting in a patient 1 yr after botulinum administration. The preterminal motor axons are myelinated (large arrows). Numerous unmyelinated sprouts arise from the nodes of Ranvier and terminate in the interstitium between the muscle fibers (small arrows). Bar = 20 μm.

Fig. 6. Cholinesterase staining of a human orbicularis muscle fiber 1 yr after botulinum administration. Bar = 20 μm.

The fate of these sprouts was variable. In fields in which the sprout could be followed its end, some ended blindly at bulbous terminations in the interstitium (Fig. 1, small arrows). In other examples, these unmyelinated sprouts terminated over areas of cholinesterase staining on muscle fibers (Fig. 2, small arrows).

In the orbicularis oculi muscle from normals and from blepharospasm patients who had not received botulinum toxin, branching of the preterminal axon was rare, and these branches were well-myelinated (Fig. 3), and terminated at typical motor end-plates. Sprouts from the terminal axon were not observed in noninjected muscles.

Discussion

Botulinum toxin injection into orbicularis muscle produces abundant axonal sprouting in humans. This sprouting is expected based on the response of motor axons to botulinum toxin in animals,1,4-6 but has not previously been verified in humans.

As in experimental animals, axonal sprouts develop from three sites along the motor axon: 1) "nodal" sprouting from the nodes of Ranvier of myelinated motor axons; 2) "terminal" sprouts adjacent to the end plate and 3) "ultraterminal" sprouts from the axonal arborization over the end plate. Surprisingly, axonal sprouts persist for up to 3 yr, although the paralytic effect of the botulinum toxin is temporary.

Borodic and Ferrante9 have demonstrated increased cholinesterase staining over orbicularis muscle fibers after botulinum administration in humans. This expression of cholinesterase activity in response to botulinum has been attributed to a denervational effect.4,6,9 This study demonstrates persistence of unmyelinated axonal sprouts well beyond any expected period of neural remodeling in response to chemodenervation. Duchenh and Strich8 believed that the
Fig. 2. Ultraterminal sprouting in a patient 3 yr after administration of botulinum toxin. Sprouts (large arrows) extend from the terminal axon and axonal arborization. Some of these terminate over areas of dark (cholinesterase) staining activity on the muscle fibers (small arrows). Bar = 20 μm.

Fig. 3. Normal neuromuscular junction from a patient with essential blepharospasm but no history of botulinum exposure. Note that the translucent myelin (large arrows) envelops the motor axon up to the region of the motor end plate. The terminal axonal arborization is confined to the region of the darker cholinesterase patch of the motor end plate (small arrows). Bar = 20 μm.
cholinesterase staining with overlying nerve sprouts did not imply a functional connection between muscle and nerve, and interpreted their observations as a failure of the muscle and nerve to make functional end-plates. The nerve sprouting and muscle expression of cholinesterase observed here reopens questions about the mechanism and regulation of nerve sprouting and motor end-plate formation.

In conclusion, botulinum toxin induces exuberant distal motor axonal sprouting in human orbicularis oculi muscle. Axonal sprouts develop from the pre-terminal axon, terminal axon, and terminal axonal arborization. The fate of these sprouts is variable: some end blindly, whereas others terminate over areas of cholinesterase staining on muscle fibers, representing possible motor end-plates. The changes induced by botulinum toxin persist for at least 3 yr. Future research will examine the evolution of these changes and their significance in modulating the effects of subsequent botulinum toxin injections in these patients.

**Key words:** botulinum toxin, essential blepharospasm, nerve sprouting, muscle denervation, motor end-plate

**References**