Pathological changes in levator palpebrae superioris muscle treated with botulinum toxin in a case of carotico-cavernous fistula

G G W Adams, P N Dilly

Abstract
We describe the case of a patient with carotico-cavernous fistula who had botulinum toxin A to induce a protective ptosis 4-5 days before death. The levator palpebrae superioris muscle from both sides and the superior rectus muscle from the injected side were obtained for examination. The preserved samples were stained with haematoxylin and eosin, Martius scarlet blue, Gleys, S100, dehydrogenase, ATPase, and toluidine blue as well as being examined by electron microscopy. Inflammation and oedema were found that were probably due to the carotico-cavernous fistula. Axonal and some myelin sheath damage were also seen.

Botulinum toxin A (BTXA) has been used clinically since 1979 in the USA and since 1982 in Britain. It was initially for the treatment of strabismus, but later uses have included the treatment of blepharospasm and hemifacial spasm. More recently it has been used to produce a ptosis as an alternative to tarsorrhaphy. The toxin is bound to nerve terminals that release acetylcholine, where it prevents the release of the transmitter and produces a flaccid paralysis of muscle.

Although work has been published on the effects of botulinum poisoning on animal muscle, we are unaware of any published data on the effects on the human extraocular muscles.

Results
Haematoxylin and eosin staining for the general morphology of the specimens demonstrates the LPS from the injected side to have inflammation and oedema (Fig 1). The muscle fibres, which were of uneven width, were widely separated from each other by pale irregular spaces indicative or oedema. These changes were not found in the control muscle, in which there was no 'bubbly' oedema; here the fibres were tightly packed and of regular width (Fig 2). The blood vessels on the injected side were very abnormal and appeared thrombosed, with hyaline-like fibrin clot and no identifiable red blood cells in many of the vessels. There was also perivascular cuffing, particularly with lymphocytes, but some plasma cells could also be seen (Fig 3). The congestion of the blood vessels and their thrombosis can best be explained as the result of the carotico-cavernous fistula, which at necropsy was found to be completely thrombosed. The vessels on the normal side did not show any inflammation, and there were generally RBCs rather than clot in them. They were perhaps slightly distended, an effect of back pressure from the fistula.

The superior rectus muscle from the injected side also showed similar changes to those found in its companion LPS (Fig 4).

Martius scarlet blue is a trichrome stain for connective tissue and muscle. In the sections from the injected side the muscle cell nuclei were centrally placed in contrast to those in sections of the control muscle, where they were found in their usual position in the periphery (Figs 5, 6). This change is usually associated with regeneration of necrotic muscle. The muscle striations were much more broken up in the treated than in the untreated muscle. There was also patchy
staining of muscle fibres, with disrupted organisation of sarcomeres. This is most likely to be a hypoxic change secondary to the fistula.

The Glees technique stains nerve fibre axons, but not their myelin sheaths. There appeared to be considerable damage to the nerves of the LPS on the injected side, as shown by the break up of the silver of the axons in comparison with the staining on the non-injected side (Figs 7, 8). There was much damage and very few if any staining intact axons. This finding of nerve fibre damage in BTXA treated muscle has not been reported from animal experiments.

S100 stains Schwann cells and so displays myelin rather than axons. Some sections of the nerves from the botulinum treated levator showed little content (Fig 10). The myelin sheaths appeared either granular or empty in comparison with the normal side (Fig 10). The changes in the Schwann cells were not marked and were more patchy in distribution than the nerve fibre change.

Dehydrogenase reductase is an indicator of mitochondrial distribution and activity. As type 1 muscle has larger numbers of mitochondria than other types of muscle, it can help to differentiate different muscle fibre types. This stain was thought to show a normal appearance in both the treated (Fig 11) and the control muscle (Fig 12).

The ATPase reaction with preincubation at different pHs disrupts the normal myofibrillar network of the muscle and allows estimation of the fibre types 2A, 2B, and 2C, while the type 1 fibres stain weakly with this method. There was some change in the sample preincubated at pH 4-3 but no difference in the others. This finding is consistent with a hypoxic effect (Figs 13, 14).

Toluidine blue was used as the stain to prepare tissue prior to electron microscopy. Some sections showed a snake-like pattern of fibril bundles.
on the injected side, with muscle fibres coming away from the sarcolemma (Figs 15, 16).

Electron microscopy shows PM change in the mitochondria. There was blurring of the Z lines in muscle on the BTXA side which may equate with the 'streaming' of Z lines in mouse work (Figs 17, 18).

Discussion
A carotico-cavernous fistula leads to reduced arterial pressure in the ophthalmic artery and increased pressure in the orbital veins. This reduces the A-V pressure gradient, causing tissue hypoxia from the lowered ocular pressure. Previous pathological examination of extraocular muscle from cases of carotico-cavernous fistula has shown infiltration with chronic inflammatory cells but no evidence of ischaemic necrosis. 4-5 days is sufficient time for BTXA to produce a protective ptosis. Obviously with a central tarsorrhaphy in situ this could not be proved, but the lids did appear to be laxer, suggesting that the toxin had worked.

The action of BTXA is said to be confined to the presynaptic terminals of the peripheral nervous system that release acetylcholine, with no destruction of the motor end terminals. Previous observations on animal material have not shown nerve fibre damage similar to what we found, and only minimal muscle damage. Spencer and McNeer have reported hypertrophy in the orbital fast muscles and reduced vascularity 14 days after botulinum injection. These fibres later faded before returning to normal. There was little change in other fibre types. In contrast our results showed more generalised muscle changes, with inflammatory vascular changes, which are more likely to be due to the tissue hypoxia from the fistula. Duchen has noted that slow fibres are affected earlier and recover more quickly after botulinum injection. We know that recovery does take place in man and experimental animals, and these differences may simply illustrate different times along the natural recovery pathway.

The apparent damage to the myelinated nerve fibre axons has not been reported by other
workers. It is possible that this contrast with our observations is due to transient changes that are followed by a full morphological recovery. Our case is complicated by the dual pathology of a carotico-cavernous fistula and botulinum injections. Little has been reported on the effects of carotico-cavernous fistula on the extraocular muscle, though they are known to cause anoxic damage. Thus some of our observations may be due to ischaemic pressure necrosis from thrombosis and not just BTXA poisoning.

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1 Duchen LW. Changes in the electron microscopic structure of slow and fast skeletal muscle fibres of the mouse after the local injection of botulinum toxin. J Neurol Sci 1971; 14: 61-64.