Ultrastructural changes in the parotid gland of rats after intraglandular injection of botulinum toxin A

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Abstract

Introduction
Intraglandular injection of botulinum toxin A into the salivary glands is the effective treatment option for sialorrhoea in children with cerebral palsy or other neurological diseases without any severe side-effects. It results in transient denervation of the gland, which helps in reducing salivary secretion. The aim of this study was to evaluate histological changes in the rats’ parotid glands after accidental or intended intraglandular injection of botulinum toxin A in patients with bruxism or sialorrhoea.

Materials and methods
After 20 days of the botulinum toxin A injection, tissue specimens of the right parotid gland were obtained from 15 albino rats, while the specimens of left parotid gland were used as control. Ordinary light microscopy and electron microscopy were used to detect the morphological changes in the injected parotid glands.

Results
Morphological and ultrastructural analyses of the cell organelles and secretory granules showed a clear atrophy of the acini in glands injected with the botulinum toxin A. Acinar cells revealed significant morphologic variations in rough endoplasmic reticulum and degenerated mitochondria. There was a considerable variation in the size, shape and electron density of secretory vacuoles. The nucleus had an irregular shape and finely dispersed chromatin.

Conclusion
Intraglandular injection of botulinum toxin A induces structural and functional changes of the salivary glands, indicated by glandular atrophy. Thus, injection of botulinum toxin A into the salivary glands at a relatively low dose can be used as a treatment of choice for sialorrhoea. This is an easily performed procedure with low morbidity, and can be recommended as a first-line intervention in the treatment of adult sialorrhoea.

Introduction
Botulinum toxin, popularly known by its trade name Botox®, is a protein and a neurotoxin produced by Clostridium botulinum bacterium. It is used in various cosmetic and medical procedures. Justinus Kerner has described botulinum toxin as a ‘sausage poison’ and ‘fatty poison’ because the bacterium that produces this toxin often causes food poisoning by growing in improperly handled or prepared meat products. There are seven serologically distinct toxin types designated as A through G. Two botulinum toxin formulations can be found in the market: Dysport® (Speywood Pharmaceuticals Ltd, Maidenhead, UK) and Botox® (Allergan Inc., Irvine, CA, USA). It is estimated that one unit of Botox® is equivalent to 3 or 4

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Figure 1: Photomicrograph of albino rat’s parotid gland (control group) shows numerous round acini.
Botulinum toxin A injections have been used to treat saliva in adults with Parkinson’s disease, head and neck cancer, stroke, and neurodegenerative disease. Several studies have reported that botulinum toxin A injection into the salivary glands is an effective treatment option for saliva in children with cerebral palsy or other neurological diseases without any severe side-effects.\textsuperscript{6-9} Salivary gland secretion is controlled by the autonomic nervous system, mediated by adrenergic and cholinergic nerve endings, but primarily under parasympathetic cholinergic control. Major salivary glands (the paired parotid, submandibular and sublingual) are responsible for 95% of 1.5 litres of saliva secreted daily.\textsuperscript{10} Intraglandular application of botulinum toxin A has been shown to significantly decrease saliva production and is considered to be a safe treatment.\textsuperscript{11,12} It inhibits salivary production by binding to SNAP-25, a cytoplasmic protein involved in the fusion of synaptic vesicles with the presynaptic membrane. This ultimately disrupts the secretory pathway of acetylcholine and results in chemodenervation.\textsuperscript{13} Administering the botulinum toxin A injection has its own complications, which range from minor (bleeding, pain at site, flu-like symptoms, parotitis, and dry mouth) to severe (dysphagia, aspiration, facial nerve branch palsy, temporomandibular joint dislocation and vascular injuries).\textsuperscript{14} The purpose of this study was to evaluate the histological changes in the parotid glands of rats after accidental or intended intraglandular injection of botulinum toxin A in patients with bruxism or saliva in adults.\textsuperscript{5}

Materials and Methods
The protocol of this study has been approved by the relevant ethical committee related to our institution in which it was performed. Animal care was in accordance with the institution guidelines.
Research study

Materials
One millilitre of normal saline was used to dilute 50 units of botulinum toxin A (Botox®, Allergan Inc., Irvine, CA, USA). The right parotid gland was injected with 2 units of botulinum toxin A. A 25-gauge 1.5-cm needle was used to inject the required gland.

Animals and injections
Fifteen adult male albino rats weighing 150–200 g were used in the study. These animals were housed in the animal care centre in the Faculty of Veterinary, Mansoura University under controlled light and environmental conditions (12:12 h dark/light cycle; 23±1°C; 55% relative humidity). The animals were monitored for behaviour, food and water intake, and body weight was measured daily. In each animal, the right parotid gland was treated once with the toxin, while the left parotid gland of the same rat was used as a control. On day 20 of the experiment, the rats were euthanised. The parotid glands were dissected from the surrounding connective tissue and removed. From each gland, tissue samples were preserved for studying their histology under electron microscopy.

Methods
Tissue processing with haematoxylin and eosin
Tissue samples were fixed in 10% formalin and embedded in paraffin. Sections of 4-µm thickness were cut at the central region of each specimen to obtain maximum standardisation of the cutting surface. Sections were stained with haematoxylin and eosin to evaluate the ordinary histopathologic changes in both the groups.

Electron microscopy
For electron microscopy, glands were cut into small fragments that were immediately fixed in 4% glutaraldehyde fixative and processed for the transmission electron microscope.

Figure 4: Parotid gland of botox treated rats showed clear-cut signs of atrophy and degeneration. Acini appeared to be reduced in size, densely packed and with few numbers.

Figure 5: Most of the acinar cells lost their secretory granules. Their cytoplasm revealed extensive and coarse vacuoles.
duct systems were found (Figure 3). In botulinum toxin A-treated glands, acini appeared to be smaller in size, fewer in number and packed more densely as compared with those of the control group. Their shape was slightly elongated and the basal basophilic area containing the nucleus was more pronounced at the expense of secretory material (Figure 4). There were only a few secretory active acini, whereas most of the acini lost their secretory granules. The cytoplasm of the acinar cells revealed extensive and coarse vacuoles. Generally, the gland showed clear-cut signs of atrophy and degeneration (Figure 5). The interlobular spaces were wider than normal (Figure 6).

**Electron microscopy**

Acinar secretory cells of control glands had euchromatic nuclei. Their cytoplasm contained many secretory granules of homogeneous size. Rough endoplasmic reticulum (rER) was condensed and arranged parallelly (Figure 7).

Secretory cells in acini from glands that were treated with botulinum toxin A contained less secretory granules. The rER was dilated with bigger size, and showed several profiles, intermingled with degenerated mitochondria. The cytoplasm and some secretory granules showed some vacuoles (Figure 8). The secretory granules appeared altered, there were variations in size, shape and electron density. Along with these, large ovoid or polymorphic secretory vacuoles were also observed. The secretory granules appeared less dense than those seen in controls. Most of the nuclei had slightly irregular shape. There was a decrease in number of secretory material (Figure 9).

**Discussion**

Intraglandular injection of botulinum toxin A has been shown to significantly decrease saliva production and is considered a safe treatment for the treatment of sialorrhea.
An interesting observation of the present study was that parotid glands treated with Botulinum toxin A showed clear-cut signs of atrophy and degeneration. Their acini appeared to be smaller in size, densely packed with only few in numbers. Most of the acinar cells lost their secretory granules. Their cytoplasm revealed extensive and coarse vacuoles, while the interlobular spaces were wider than normal. These findings were in accordance with the reduction in the weight of the submandibular gland (reduced by 9.2% after treatment with botulinum toxin A), as reported by Teymoortash et al.15 Moreover, their morphometric evaluations indicated that the acinar cells were significantly smaller in glands treated with botulinum toxin A than in the controls. These effects may be due to glandular denervation induced by inhibition of the soluble N-ethylmaleimide-sensitive fusion protein attachment protein receptors involved in acetylcholine release at the neuroglandular junction and also inhibition of those involved in exocytosis of the granula of the acinar cells15. In this way, a temporary chemical denervation of the target organ is established16,17. This explanation is supported by Schneyer and Hall18 and Ekstrom and Reinhold19, who found that parasympathetic denervation by sectioning of the chorda tympani in rats resulted a decrease in the weight of the submandibular gland, by between 15 and 30%, and caused the acinar cells to shrink. This is because parasympathetic nerves exert trophic effect on salivary glands16,17. These changes cannot be attributed solely to a loss of acetylcholine. The mechanisms underlying the effects of denervation are still not clearly understood15. Therefore, it is thought that shrinkage of salivary glands might contribute to improvement in drooling20.

In the present study, the ultrastructural changes observed after intra-parotid injection of botulinum toxin A were highly morphologic option11,12. Botulinum toxin A inhibits salivary production by binding to 25-kD SNAP-25, a cytoplasmic protein involved in the fusion of synaptic vesicles with the presynaptic membrane. This ultimately disrupts the secretory pathway of acetylcholine and results in chemodenervation13.

Figure 8: Morphological changes of acinar cells of parotid gland after botulinum toxin A application. Rough endoplasmic reticulum (rER) is irregular and dilated. Degenerated mitochondria (M) are seen. Variation in electron density of secretory granules (G) with some is vacuolated. The cytoplasm contains some vacuoles (V).

Figure 9: The secretory granules (G) appear highly variable in size, shape and electron density. The nucleus had an irregular shape (N). Irregular dilated rough endoplasmic reticulum (rER). Decreased number of secretory materials is obvious.
variations in rER and degenerated mitochondria in acinar cells. There were significant variations in their size, shape and electron density. The nucleus had an irregular shape and finely dispersed chromatin. Several studies have reported that botulinum toxin A injection into the submandibular salivary glands exhibits clear compatibility with these findings\(^{15}\). Further studies are needed to understand the mechanism of action of botulinum toxin A.

**Conclusion**

The findings of this study suggest that injection of botulinum toxin A into the salivary glands at a relatively low dose can be treatment of choice for sialorrhoea. This is an easily performed procedure with low morbidity and can be recommended as a first-line intervention in the treatment of adult sialorrhoea.

**Abbreviations list**

rER, rough endoplasmic reticulum; SNAP-25, synaptosomal-associated-protein.

**References**


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