

HISTOLOGICAL INVESTIGATION AFTER REPEAT INJECTIONS OF BOTULINUM TOXIN A FOR RAT BLADDER

Hypothesis / aims of study

Many cases of clinical success of botulinum neurotoxin-A (BoNTA) as a method of management of refractory overactive bladder have been reported. The effect lasts for 9 to 12 months. When the patients develop botulinum toxin resistance, repeated injections are needed to maintain the effect. Some papers say repeated injections are less effective than earlier one.¹ In this study, we investigated the histological features in rat bladder after repeated BoNTA injections.

Study design, materials and methods

Adult female S.D rats (200-250g) were used in this study. BoNTA or saline were injected into the anterior walls with a 10 ul Hamilton micro syringe in every 1 week total 5 times. The amount of injected BoNTA per one time was 2 units with diluted 0.2ml saline. The tissue was harvested after 3 and 5 times injections, sectioned, methylene blue, trichrome, and TUNEL by immunohistochemistry and examined by electron microscopy.

The photographs of methylene-blue staining were taken with a Nikon E 800 microscope. The image was transferred via Photoshop software (Adobe System Inc.). The intensity of smooth muscle was evaluated by measuring the square pixels with IPLab software (Scanalytics Inc.).

Results

The bladder wall tissue after 5 times injections did not demonstrate apoptosis by TUNEL staining. However, muscle fiber became thinner and fibrosis was demonstrated after 5 times injections by methylene blue and trichrome staining. The area of muscle fibers has gradually decreased over the time course. Fascicles of bladder smooth muscle have become atrophic and muscle intercellular distance has become wider after injections by electron microscopy. (Figure 1)

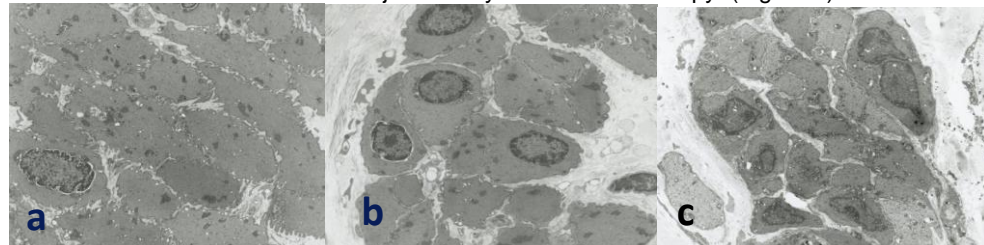


Figure 1: Electron microscopic findings show fascicles of bladder smooth muscle have become atrophic and muscle intercellular distances have become wider after repeat injections ($\times 5,000$). a ; control b ; after 3 times injections c ; after 5 times injections

Interpretation of results

Clinically, repeat injections for refractory OAB at least 3 to 4 times are as effective as the first one.² However, in this study repeat injections have induced histological change in rat bladder. There are some possibilities rather larger amount BoNTA units compared with human, shorter injection intervals, or different species might induce these results.

Concluding message

When repeat injections are performed, treatment dose and treatment interval should be carefully taken considerations. More detail examines are needed in the future study.

References

1. *Mov Disord* (1994) 9; 213-217.
2. *Eur Urol* (2005) 47; 653-659.

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Is this a clinical trial?	No
What were the subjects in the study?	ANIMAL
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	Yes
Name of ethics committee	Animal subjects: This study followed the guideline for care and use of laboratory animals.