Botulinum Toxin Affects Muscle Function One Year After a Single Injection

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INTRODUCTION
Botulinum toxin type-A (BT-A) is widely used for the management of spasticity as well as other neuromuscular diseases. BT-A causes muscular paralysis by inhibiting the release of acetylcholine at the neuromuscular junction (NMJ) and the clinical effects of this toxin are reported to last to 6 months (1). However, a previous study indicated a tendency for neuromuscular junction abnormalities at 1 year after toxin injection (2). Thus, the purpose of this study was to try to understand the basis for the difference between clinical experience and the basic science literature by quantifying functional and structural properties of tibialis anterior (TA) muscle from 1 week to 1 year after a single injection of BT-A.

MATERIAL AND METHODS
Male Sprague-Dawley rats (390±3.05g; n=77) were divided into ten groups, that were submitted to either BT-A or saline solution injections and analyzed after 1 week (BT-A: n=8; Sal: n=4), 1 (BT-A: n=18; Sal: n=4), 3 (BT-A: n=8; Sal: n=4), 6 (BT-A: n=8; Sal: n=4) or 12 months (BT-A: n=9; Sal: n=10). Experimental methods were approved by the Institutional Animal Care and Use Committee of the Veterans Administration San Diego. After anesthesia induction (2% isoflurane, 2.0 L/min), isometric dorsiflexion torque was measured prior to injection by stimulating the common peroneal nerve (15V stimulus, 100Hz, 650ms train duration) and measuring torque with a custom-designed dynamometer. All rats then received a single 100μL injection of either BT-A (6.0 units/kg) or normal saline into the TA muscle. After the designated time point, torque from the injected hindlimb was remeasured. Immunohistochemical techniques were used to calculate average muscle fiber cross-sectional areas (CSA). One-way and two-way ANOVA tests with repeated measures were used to compare dependent variables across experimental groups over time. All values are reported as mean ± SEM.

RESULTS
Dorsiflexion torque in BT-A injected animals was significantly decreased at all time points compared to saline groups even up to one year (Fig. 1). Negative torque was observed one week after BT-A injection probably due to residual activity of the peroneal muscles (p<0.001). Torque gradually recovered over time increasing from 20% of saline values at one month to 44% at 3 months and 49% at 6 months (p<0.001). One year after injection, the BT-A group was still significantly lower (p<0.05), at only 73% of saline value.

The time course of fiber CSA was quite different compared to functional data. The greatest reduction in average fiber CSA was observed 1 month after injection, when CSA was only 28% that of the saline group (p<0.001; Fig 2). CSA gradually recovered to 68%, 71% and 92% at 3, 6 and 12 months, respectively, with no difference compared to the saline groups (p<0.02; Fig 2). Qualitative differences in muscle fiber CSA as well as changes in morphology were observed 1 and 3 months after BT-A injection compared to the other experimental groups (Fig 3).

DISCUSSION
The most significant finding of this study is that muscle function is compromised even one year after a single BT-A injection, at a time when muscle histology appears relatively normal. Previous studies reported normal gene expression of myogenic regulatory factors and nicotinic acetylcholine receptor subunits, as well as normal NMJ morphometry by 6 months following intramuscular injection of BT-A (2,3). However, our results showed that TA muscle function was still impaired one year after the injection, much longer than previous clinical studies suggest (1). This functional deficit was not explained by a reduction in fiber CSA. The process involved in the long-lasting effect of BT-A on muscle function is not known but may involve alterations in excitation-contraction coupling or ultrastructural level abnormalities not obvious using light microscopy.

LITERATURE REFERENCES

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