Sarcomeric Adaptations to Botulinum Toxin A Injection and Neurectomy during Muscle Lengthening in Rats

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INTRODUCTION:
Botulinum toxin A is used as an anti-spasticity agent for the treatment of many upper motor neuron syndromes. Specifically, it has been used for the treatment of contractures in children with cerebral palsy (Corry, Cosgrove et al. 1998; Kay, Rethlefsen et al. 2004; Kinnett 2004) Botulinum toxin A effects a pharmacological denervation of skeletal muscle when injected intramuscularly. It does so by inhibiting the release of acetylcholine at the neuromuscular junction. This temporarily paralyzes the muscle and allows external forces, including braces, static and dynamic casting, and physical therapy to stretch the muscle and joint to restore functional range of motion. The clinical effect of botulinum toxin A lasts three to six months (Kay, Rethlefsen et al. 2004).

Many previous studies have demonstrated the ability to lengthen skeletal muscle by increasing the number of sarcomeres in series using skeletal lengthening devices or serial casting (Goldspink, Tabary et al. 1974; Williams and Goldspink 1978; Williams, Simpson et al. 1999; Gajdosik 2001; Caiozzo, Utan et al. 2002; De Deyne 2002; Lindsay, Makarov et al. 2002; Coutinho, Gomes et al. 2004). However, a question remains as to whether or not this sub-cellular effect is accelerated or impaired by muscle denervation (Goldspink, Tabary et al. 1974; Gajdosik 2001). The clinical difficulty of limb lengthening in polio patients suggests that muscle denervation inhibits the process of muscle lengthening. If this inhibitory effect is also seen following the administration of botulinum toxin A, then its ubiquitous use in the spastic population undergoing stretching for muscle contractures needs to be reevaluated.

The purpose of this study was to investigate the sarcomeric responses to muscle lengthening with and without neurological impairments by studying the adaptation of serial sarcomeres in the skeletal muscles of rats subjected to lengthening after receiving either botulinum toxin A injection or neurectomy. The sarcomeric adoptions observed in these muscles would give us insight into muscle behavior at the fiber level with the goal of determining whether or not botulinum toxin A injection is a worthwhile treatment for patients suffering from fixed contractures/spasticity who may be undergoing slow stretching.

METHODS:
Twelve adult male Sprague-Dawley rats (401.42 ± 28.68 g) were used in this study and were divided into four groups (lengthening only, n=4; botox injection + lengthening, n=3; neurectomy + lengthening, n=3; control, n=2). Each of the rats underwent placement of an external fixator on the right tibia followed by a tibial osteotomy (Fig. 1). An incision was made over the lateral aspect of the tibia in each animal. A 1.1mm diameter Kirschner wire with threaded tip (Synthes Inc., West Chester, PA) was inserted by hand in a bicortical fashion into the distal metaphysis of the tibia. No additional procedures were performed on the rats in the control or lengthening only groups. In the Botulinum toxin + lengthening group, the soleus and tibialis cranialis muscles were infiltrated with Botulinum toxin. In the neurectomy + lengthening group, the ischiadicus nerve was identified and transected sharply.

After no latency period, all experimental limbs except those in the control group were lengthened at a rate of 0.5 mm/day for 20 days. On the 21st day after surgery, the rats were sacrificed and the experimental legs with the fixatures on were removed and preserved in 10% neutral buffer formalin for 48 hours. Following the fixation, tibialis cranialis and soleus were dissected from all surrounding tissue. The in situ lengths of both muscles were measured both including and excluding the tendons. The number of sarcomeres in series was counted for fiber bundles dissected from the muscles. The protocol was approved by the Animal Care and Use Committee.

ANOVA followed by post hoc tests were used to determine if statistical significance between groups was present. The level of significance was set at p < 0.05.

RESULTS:
The number of serial sarcomeres in the soleus muscle fibers of the lengthening only group showed significantly more addition of sarcomeres in series (9525 ± 812) compared that of the Botox + lengthening group (8138 ± 357, P=0.001), the neurectomy + lengthening group (8586 ± 798, P = 0.035), and the control group (7988 ± 953, P = 0.003) (Figure 2). There was no statistically significant difference in the serial sarcomere numbers among the control group, the botulinum toxin + lengthening group, and the neurectomy + lengthening group. Additionally, there was no statistically significant difference between the sarcomere numbers in the botulinum toxin + lengthening group and the neurectomy + lengthening group. The numbers of sarcomeres added in the muscle fibers of the tibialis cranialis muscles in each group showed a similar trend to those in the soleus muscle; however these changes did not achieve statistical significance, given the samples available.

DISCUSSION:
Although botulinum toxin is commonly used as an adjunct to the treatment of patients with contractures/spasticity, the present study is the first to evaluate the sarcomeric adaptation to muscle lengthening with botulinum toxin or neurectomy. The results of this study indicate the neurectomy and Botox may inhibit the process of sarcomere serial addition under lengthening, which is consistent with the observed clinical difficulty of limb lengthening in polio patients with muscle denervation.