THE MECHANISM OF MUSCLE RECOVERY FOLLOWING INTRAMUSCULAR INJECTION OF BOTULINUM TOXIN A: A STUDY IN JUVENILE RATS

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Introduction:
Botulinum toxin A (BoNT-A) is a potent biological toxin widely used for the management of skeletal muscle spasticity or dynamic joint contracture. Intramuscular injection of BoNT-A causes muscle denervation, paresis and atrophy. This clinical effect of botulinum toxin A lasts three to six months, and injected muscle eventually regains muscle mass and recovers muscle function. The goal of the present study was to characterize the molecular and cellular mechanisms leading to neuromuscular junction (NMJ) regeneration and skeletal muscle functional recovery after BoNT-A injection.

Methods:
Fifty-six one-month Sprague-Dawley rats were used. Botulinum toxin A was injected into the left gastrocnemius muscle at a dosage of 6 units/kg body weight. An equivalent volume of saline was injected into the right gastrocnemius muscle to serve as control. The gastrocnemius muscle samples were harvested from both hind limbs at 3 days, 7 days, 15 days, 30 days, 60 days, 90 days, 180 days, and 360 days after administration of toxin. In addition, the gastrocnemius muscles from one-month old rats with no injections were harvested to serve as uninjected control group. Muscle samples were processed and mRNA was extracted. Real-time PCR (polymerase chain reaction) and gene microarray technology were used to identify key molecules involved in NMJ stabilization and muscle functional recovery.

Results:
More than 28,000 rat genes were analyzed and approximately 9,000 genes are expressed in the rat gastrocnemius muscle. Seven days following BoNT-A injection, 105 genes were up-regulated, and 59 genes were down-regulated. Key molecules involved in neuromuscular junction (NMJ) stabilization and muscle functional recovery were identified and their time course of gene expression following BoNT-A injection were characterized (Figure 1-5).

Discussion:
This animal study demonstrates that following intramuscular injection of BoNT-A, there is a sequence of cellular events that eventually leads to NMJ stabilization, remodeling, and myogenesis and muscle functional recovery. This recovery process is divided into two stages (aneural and neural) and that the IGF-1 signaling pathway play a central role in the process (Figure 6).

Figure 1: Time course of nAChR-alpha gene expression in the gastrocnemius muscle following BoNT-A injection in juvenile rats (n=3) at 6 units/kg of body weight (*: p< 0.05).

Figure 2: Time course of MRF4 gene expression following BoNT-A injection in juvenile rats (n=3) at 6 units/kg of body weight (*: p< 0.05).

Figure 3: Time course of myogenin gene expression following BoNT-A injection in juvenile rats (n=3) at 6 units/kg of body weight (*: p< 0.05).

Figure 4: Time course of IGF-1 gene expression in the gastrocnemius muscle following BoNT-A injection in juvenile rats (n=3) at 6 units/kg of body weight (*: p< 0.05).

Figure 5: Time course of MuSK gene expression in the gastrocnemius muscle following BoNT-A injection in juvenile rats (n=3) at 6 units/kg of body weight (*: p< 0.05).

Figure 6: Proposed mechanisms of neuromuscular junction remodeling, regeneration and skeletal muscle functional recovery from BoNT-A induced muscle paresis and atrophy.

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