DECREASED LOADING DELAYS THE DEVELOPMENT OF THE SUPRASPINATUS TENDON TO BONE INSERTION

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INTRODUCTION: During post-natal development, a fibrocartilaginous insertion zone forms at the tendon-bone interface [e.g., 1, 2]. We have shown that this transition zone effectively reduces stress concentrations at the insertion and reduces the risk for failure [3]. During tendon to bone healing, on the other hand, a fibrocartilaginous transition zone does not develop at the interface [4]. Unlike tendon enthesis development, inferior scar tissue fills the repair. Understanding the differences between the developing insertion and the healing insertion will allow us to develop solutions to augment tendon-bone healing. The fibrocartilaginous site develops post-natally, implicating a role for loading cues. In addition, compressive loads have been shown to change adult tendon to a more fibrocartilaginous composition in vivo [e.g., 5]. Therefore, the overall objective of this study was to study the role of the stress environment on the development of a fibrocartilaginous transition. To evaluate the role of loading on the development of the enthesis, we developed a novel in vivo animal model where shoulders of neonatal mice are paralyzed using botulinum toxin A. We hypothesized that decreased loading across the supraspinatus tendon would delay the formation of a fibrocartilaginous insertion site.

METHODS: Animal model: 26 CD-1 neonatal mice were used. All animal studies were approved by the Institutional Animal Care and Use Committee. A supraspinatus intramuscular injection of botulinum toxin A (BOTOX, Allergan Inc) was used to paralyze the left shoulders of each animal (N=16) within 24 hours of birth. The supraspinatus muscles of right shoulders were injected with an equal volume of saline to serve as contralateral controls. Based on pilot studies to determine optimal dose for paralysis, a botulinum toxin dose of 0.05U per gram body weight was used, delivered intramuscularly in a 10μl volume using a 30 gauge needle. The injections were repeated every 3 days to maintain local paralysis. A separate group of neonatal mice (N=10) were injected every three days with saline in the right shoulders and served as fully mobile controls. Mice were sacrificed 14, 21, and 28 days after birth. Activity level observations: Following botulinum toxin injection, the mice were graded for paralysis by two independent observers once a day until sacrifice. The grading was based on degree of observed paralysis of the experimental forelimb compared to the contralateral forelimb on a scale of 0-3 (0=full abduction, 1=partial abduction, 2=no abduction, 3=full limb paralysis). Mice were weighed at 14, 21, and 28 days after birth. Weights were statistically compared between botulinum A toxin injected mice and saline injected mice using an analysis of variance followed by a Fisher’s least squares differences test. Histology: Specimens were fixed overnight in 4% paraformaldehyde and decalcified in 14% EDTA. Specimens were paraffin embedded, sectioned at 5μm, and dried for 1h at 60°C. Sections were then stained with 0.1% Toluidine blue. Sections were evaluated by two observers, blinded to group, for insertion site morphology and maturation.

RESULTS: Activity level observations: 90% of shoulders that received injections of botulinum toxin A were paralyzed within 24 hours after birth. Paralysis was maintained through the sacrifice date. The average grade for paralyzed shoulders was 3+/−0.50. The average weight of botulinum injected mice was significantly less than saline injected mice at 21 and 28 days (Fig. 1) (p<0.05). Weight increased over time in both groups. Botulinum injected mice were unstable during walking compared to saline injected mice. Histology: There were clear differences when comparing the maturation and morphology of the supraspinatus insertion in shoulders injected with botulinum toxin A to the contralateral saline injected control shoulders. There were no apparent differences between the shoulders of mice which received saline injections and the saline control shoulders of the botulinum injected mice. The maturity of the insertion site was delayed in the botulinum injected shoulders compared to their contralateral controls (Figs. 2 and 3). Hypertrophic chondrocytes remained at the insertion of the paralyzed shoulders through at least 21 days. In comparison, contralateral saline injected shoulders had mature insertion sites at 21 days, without any hypertrophic chondrocytes present.

DISCUSSION: We presented a novel animal model of tendon enthesis development. We showed that:
1. We can eliminate the majority of loading across the supraspinatus tendon to bone insertion from birth through joint maturity.
2. The development of a fibrocartilaginous insertion is sensitive to its mechanical environment. A reduction in load delays the development of the insertion by delaying the mineralization and maturation of hypertrophic chondrocytes.
3. Botulinum toxin A caused a decrease in body weight during development. However, our model provides local paralysis of one joint, allowing us to use the contralateral joint for comparison. Future studies will examine the differences in gene expression (e.g., type X collagen) between paralyzed shoulders and their contralateral controls.

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