• Does Muscle Fibrosis Contribute to Contracture Formation Following Neonatal Brachial Plexus Injury?

INTRODUCTION:
Neonatal brachial plexus injury (NBPI) occurs in 1-3 per 1,000 live births, leaving permanent neurological sequelae in up to 40% of affected children. In these children, disabling contractures commonly occur at the shoulder and elbow. The pathogenesis of these contractures is incompletely understood, although previous work in our laboratory has demonstrated that functional shortening of denervated muscle plays a primary role in contracture pathogenesis. Muscle fibrosis is variably present in these neonatally denervated muscles, but its role in contracture pathogenesis is unknown. Muscle fibrosis has been implicated in contracture formation in Duchenne’s muscular dystrophy and cerebral palsy, raising the possibility that it also contributes to contractures following neonatal denervation. Using an established mouse model of elbow flexion contracture following NBPI, the present study assesses the relationship between elbow flexor muscle fibrosis and elbow flexion contractures, with the null hypothesis that muscle fibrosis does not play a primary role in the pathogenesis of contractures following NBPI.

METHODS:
Animal model of NBPI: All protocols were approved by the Institutional Animal Care and Use Committee. Unilateral upper (C5-6) or global (C5-T1) brachial plexus injuries were created by surgical extraforaminal nerve root excision in 5-day-old mice under general anesthesia. Appropriate neurological deficits were confirmed by motor examination immediately post-operatively. Animals were then sacrificed at time points specified by each experiment. Except where specified, elbow passive range of motion was measured immediately post-sacrifice by a validated digital photography technique. Muscle fibrosis was assessed using Masson’s trichrome staining of axial muscle sections, with digital analysis of color-deconvoluted images to determine the collagen: muscle area ratio. For each muscle, the fibrosis index (FI) was calculated by normalizing the collagen: muscle ratio of the denervated muscle to that of the contralateral control muscle.

Time course of fibrosis versus contracture development: 94 mice underwent C5-6 excision and were sacrificed either immediately post-operatively or at 4 subsequent weekly time points. Passive range of motion was measured in 4-8 mice per time point, and biceps and brachialis fibrosis were measured each in 5-7 mice per time point.

Relative contributions of biceps and brachialis to contractures: Four weeks following C5-T1 excision, 8 mice were sacrificed and positioned supine in a custom load chamber with fixed tension loads applied simultaneously to both abducted forelimbs. Radiographs were taken for measurement of passive elbow extension under this applied load prior to and following sequential excision of the biceps and brachialis muscles. In 4 mice, the biceps was removed first; in 4 mice the brachialis was removed first. Global brachial plexus injuries were used to standardize the degree of denervation of the two muscles. Mice displaying elbow flexion motor recovery at four weeks were not included in the analysis.

Correlation between fibrosis, distribution of neurological injury, and contracture severity: In the above 8 mice following C5-T1 excision and in an additional 17 mice following C5-6 excision, passive elbow range of motion was measured and biceps and brachialis fibrosis was histologically assessed 4 weeks post-operatively.

Effect of antifibrotic therapy on fibrosis and contractures: 37 mice underwent C5-6 excision and were then randomized to halofuginone (3ug/g) or PBS intrapectineal injections 3x/week for four weeks. The halofuginone dose was selected based on failure of a lower dose (1ug/mg) to influence fibrosis following NBPI. Four weeks post-operatively, elbow range of motion and biceps and brachialis fibrosis were measured.

Statistical Analysis: Continuous variables were compared using Student t-tests (paired when using the contralateral limb as control), and categorical variables were compared using the Fisher exact test. Correlations were tested using Spearman’s correlation. Samples sizes for each experiment were set to provide at least 80% power for each variable tested.

RESULTS:
Following C5-6 excision, elbow flexion contractures (defined as >10 degree difference between sides) were identified as early as 2 weeks post-operatively, whereas the biceps and brachialis did not display significant increases in collagen: muscle ratio until four weeks post-operatively (p=0.039, p=0.033, respectively).

In elbow flexion contractures present 4 weeks following C5-T1 excision, removal of the brachialis provided a mean 44% improvement in elbow extension under constant load compared to a 20% improvement after removal of the biceps (p=0.006), regardless of which muscle was excised first. In contrast, the brachialis muscles displayed significantly lower collagen: muscle ratios than the biceps (p=0.020). Therefore, when similarly denervated, the brachialis was less fibrotic than the biceps, yet contributed more to the elbow flexion contracture.

Following both upper and global plexus injuries, neither biceps nor brachialis fibrosis index correlated with the degree of elbow flexion contracture present four weeks post-operatively. While the biceps fibrosis index was not significantly different between upper and global plexus injuries, the brachialis fibrosis was significantly lower following global injury than following upper plexus injury (p=0.038).

Halofuginone treatment at 3ug/mg following C5-6 excision produced characteristic phenotypic skin changes, yet did not prevent contractures. There were no significant differences between halofuginone- and placebo-injected mice in the severity of contractures or in the proportion of mice with contractures >10 degrees. Fibrosis index was similarly not decreased by halofuginone in either the biceps or brachialis.

DISCUSSION:
Previous investigators have identified histopathologic findings consistent with muscle fibrosis in human muscle biopsies following neonatal brachial plexus injury, leading to the assumption that muscle fibrosis is the cause of NBPI-induced contractures. However, the results of the current experiments suggest that muscle fibrosis alone cannot explain the pathogenesis of contractures following neonatal denervation. First, contracture development precedes the development of fibrosis, in contrast to the impairment of longitudinal muscle growth that we have previously identified within one week following neonatal denervation. Second, the elbow flexor muscle most responsible for the elbow flexion contracture is the less fibrotic of the two, even when the degree of denervation is standardized. Third, histologically quantified fibrosis does not correlate with contracture severity, again in contrast to the consistent correlation between functional muscle shortening and contracture severity previously identified. Fourth, the antifibrotic agent, halofuginone, which has been shown to reduce fibrosis in the mdx mouse model of muscular dystrophy, did not reduce fibrosis or prevent contractures following NBPI. While this failure of halofuginone to prevent contractures following NBPI cannot yet be mechanistically explained, it suggests a potential difference between the fibrotic states of dystrophic versus neonatally denervated muscles.

SIGNIFICANCE:
The current study cautions against the extrapolation of findings from other conditions into the quest to understand the pathogenesis of and improve treatment for contractures following neonatal brachial plexus injury. Instead, the current findings support the need for further research into specific cellular and molecular perturbations of muscle growth and development in this unique pediatric neuromuscular condition.