Diffusion tensor imaging of acute muscular injury in normal and dystrophic mice

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Introduction: Diagnosis of acute muscle strains is typically made based on physical exam and patient history, but muscle injuries can be detected with MR imaging methods. Thus far, muscle strains are revealed best by T2-weighted MRI images, which optimize contrast between injured muscles with edema (increased signal intensity) and normal uninjured muscles. Diffusion tensor imaging (DTI) is potentially an even more sensitive and earlier marker for muscle damage than T2-weighted MRI. The purpose of this study was to determine if variables calculated from DTI would serve as an earlier and more sensitive marker of damage after a muscle strain injury in dystrophic (mdx) and control mice.

Methods: Unilateral injury to the tibialis anterior (TA) muscle was induced by 15 maximal lengthening contractions using a previously-established model [1]. 5 adult healthy (C57BL/10ScSn) and 5 dystrophic (C57BLScSn-DM2mdx) male mice were imaged on Bruker 7T MRI system within 1 hour of injury. Additional mice (n = 3 each genotype) were injected intraperitoneally with Evans Blue Dye (EBD, Sigma/Aldrich) in buffered saline (1 mg EBD/0.1 ml PBS/10 g body mass) 24 h before injury to assess sarcolemmal integrity. In addition to standard structural T1- and T2-weighted imaging, spin echo (SE) diffusion tensor image data was acquired using 12 non-colinear directions: b-value = 350 s/mm−2, TE = 26 ms, TR = 4500 ms, in-plane resolution 150x150 µm, and slice thickness = 750 µm. Multi-slice multi-echo (MSME) T2 mapping image data using 16 TEs = 11.4 ms to 182.5 ms with ΔTE = 11.4 ms, TR = 10000 ms, in-plane resolution 150x150 µm, and slice thickness = 750 µm. Diffusion tensor reconstruction and tractography was performed using TrackVis (http://www.trackvis.org) to calculate mean diffusivity (MD), fractional anisotropy (FA), radial diffusivity (RD), and axial diffusivity (AD) images as shown in Figure 1. T2 mapping was performed using custom software written in MATLAB (The Mathworks; Natick, MA) using non-linear least squares to fit the measured data at each pixel to the canonical T2 signal equation. Tractography (shown in Figure 2) was used to guide bilateral segmentation of the TA muscles. Tracts were restricted to those traveling through several transverse slices of manually traced regions-of-interest within each of the left and right TAs. This was used to create an image mask for each muscle, which was then divided into proximal, middle, and distal sections of approximately equal length. Finally, the masks were used to calculate average measurements of MD, FA, RD, AD, and T2 within each section of the injured and uninjured muscle. These measurements were compared between normal and dystrophic mice for both the uninjured and the injured side using a Wilcoxon Rank Sum test.

Results and Discussion: While the mechanism of injury was identical between animals, injury was much more severe in dystrophic mice, with an average force loss of 85% compared to 42% (p<0.01) in normal mice. There were no differences in any of the measured parameters for the TA between normal and dystrophic mice in the uninjured side (Table 1). When comparing parameter differences on the injured side, dystrophic mice showed significantly increased MD and AD, and decreased FA (p<0.05) in the proximal and middle components compared to normal mice. Note that while not significantly significant, RD trended toward increased values. T2 was significantly increased in the injured proximal component of dystrophic mice. DTI and T2 findings are consistent with increased edema ([FA, ↑MD, ↑AD, trend toward ↑RD]). However, the lack of significant changes in RD may suggest increased diffusion along the sarcolemma as a result of cell swelling. Additionally, significant changes in DTI parameters were evident in the middle and proximal sections of the TA, whereas T2 changes were only seen proximally. In microscopic cross-sections of TA muscles (Figure 3), we see an increase in the number of EBD+ fibers (indicative of cell membrane damage) in the injured muscle of the dystrophic mice. No significant difference in EBD+ fiber count was detected between normal and dystrophic mice prior to injury.

Conclusion: These results suggest that DTI may be a more specific indicator than T2 in the assessment of acute muscle injury, even at early time points where the MR signal changes are dominated by local edema.

References: 1. Lovering et al, J Physiol Cell Physiol 2004