NONINVASIVE MONITORING OF MUSCLE DAMAGE DURING RELOADING FOLLOWING LIMB DISUSE

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Introduction

Cast immobilization causes skeletal muscle disuse atrophy and an increased susceptibility to muscle damage. The objective of this study was to utilize T2-weighted imaging to monitor skeletal muscle damage and the subsequent repair mechanisms during reloading/reambulation following cast immobilization. In order to confirm that the inherent T2 relaxation properties provide a sensitive marker of loss of membrane integrity, in vivo MR measures were compared with standard in vitro markers of muscle damage and regeneration.

Methods

Animals: C57BL6 mice were placed in cast immobilization with the bilateral hindlimb plantartflexors in the shortened position for 2 weeks. T2 MR Measurements: Multiple T2 weighted spin-echo MR images were acquired prior to cast immobilization, during cast immobilization, immediately post-immobilization (prior to reloading), and at specific time points during reloading (days 1, 2, 3, 4, 6, 7, 9, 13 and 26). The lower mouse hindlimb muscles were MR imaged in a 4.7T horizontal bore Bruker AVANCE™ (Germany) spectrometer. The lower limb was inserted up to the knee into a 5-turn, 1 cm inner diameter single tuned 1H solenoid coil (200 MHz). Multiple slice, single spin-echo images were acquired with the following parameters: pulse repetition time (TR) = 2,000 ms, echo time (TE) = 14, 40 ms, field of view (FOV) = 10-20 mm, slice thickness = 0.5-1.0 mm, acquisition matrix size of 256x128, and 2 signal averages. Histology: IP injection of Evans Blue dye (0.1 g/ml/mg) 24 hours prior to sacrifice. Muscles were carefully dissected from the bilateral hindlimbs of the animals. Ten µm frozen sections were either stained with hematoxylin and eosin (H&E) or mounted for EBD visualization in an aqueous mounting media (Vector). Sections were visualized and digitized under both fluorescence and brightfield at 5 and 20X magnification on a DM LB microscope (Leica Microsystems).

Results and Discussion

Cast-immobilization in a shortened position induced a significant decrease (39%; P<0.01) in tetanic force in the soleus muscle. Examination of the MR images acquired during reambulation showed an increase in T2 contrast in the soleus muscle consistent with muscle damage (Fig. 1). Figure 2 displays the T2 time course during reloading for the soleus, gastrocnemius, and tibialis anterior muscles. Mean T2 values for the gastrocnemius, tibialis anterior, and soleus muscles at baseline (n=10) were 22.1±0.1 ms, 20.66±0.1 ms and 22.0±0.1 ms, respectively. As expected, in all muscles T2 was significantly elevated immediately following cast removal due to fluid shifts. However, at 1 day reloading only the soleus muscle demonstrated a dramatic further increase in T2 (p<0.0001; n=10). This marked increase in T2 with reloading did not occur in the gastrocnemius or tibialis anterior. The soleus T2 remained elevated compared to the other two muscles (tibialis anterior and gastrocnemius) from 1 to 6 days of reloading (Figure 1). Peak soleus T2 values were noted at 2 days reloading with an average T2 of 32.6±1.2 ms. Muscle damage was confirmed using in vivo Evans blue dye uptake. Note the massive macrophage infiltration and the presence of Evans Blue positive fibers in the soleus at 2 days of reambulation. A correlative study between EBD uptake and mean T2 values in the soleus muscle during the early stages of reloading (2-3days) confirmed that the inherent T2 relaxation properties provide a sensitive marker of loss of membrane integrity. We found a linear relationship between the number of EBD positive fibers and the mean T2 in the soleus and gastrocnemius muscle(y=4.38x-136; r = 0.87; n=18).

Conclusions

Two weeks cast-immobilization resulted in a significant loss of muscle force production. Reloading during cage-restricted reambulation induced specific muscle damage to the soleus, an important postural muscle. Increases in soleus T2 values were observed as early as one-day reambulation. A correlative study between in vivo T2 MR measures and the in vitro uptake of the vital dye EBD showed a strong correlation between noninvasive MR imaging and histological markers of muscle damage. These data confirm that T2 weighted MR imaging can be used to monitor skeletal muscle damage and muscle regeneration after cast-immobilization.

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