MRI monitoring for muscular dystrophy mice treated with gene therapy

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Target audience

Researchers who are interested in skeletal muscle MRI and muscular dystrophy research will benefit from this work.

Purpose

Muscular dystrophy is a group of inherited diseases that are characterized by progressive muscle weakness with no effective cure. Gene therapy is a promising treatment strategy because it has the potential to restore defective gene expression in dystrophic muscle and thereby improve muscle function and stabilize disease progression. This study was to investigate the efficacy of using quantitative MRI as a non-invasive tool for the monitoring of gene therapy for Duchenne muscular dystrophy (dystrophin mutations). We conducted longitudinal MRI at 14 Tesla (T) utilizing T2, apparent diffusion coefficient (ADC) and magnetization transfer ratio (MTR) in conjunction with high resolution 3 dimensional (D) imaging and histological analysis for the monitoring disease progression and treatment responses for a mouse model (*mdx*^{4cv}) of Duchenne muscular dystrophy.

Methods

We used *mdx*^{4cv} mice and age matched normal C57BL/6 mice as controls. All *mdx*^{4cv} mice were imaged at 10-11 weeks of age for the initial pre-treatment time point and then subsequently imaged at 8, 16 and 24-week time points post gene transfer. Multi-parametric ^{1}H MRI was carried out for the mice on a 14T MR scanner (Bruker Corp., Billerica, MA). The high resolution MRI protocol includes high resolution 2D imaging with 55 thin slices (200 micron thick) (multi-slice RARE: TR/TE=4 s/6 ms) for muscle volume evaluation, multi-slice multi-echo imaging (TR/TE = 4 s/ 6 ~ 100 ms, 16 echoes with 6.3 ms spacing) for T2 measurements, MT imaging (gradient echo; TR/TE = 935/5 ms, flip angle = 30 degrees), diffusion imaging with three b values of ~ 25, 586 and 1,111 s/mm^2 sequence (TR/TE = 3751/27.5 ms). Following completion of the MRI study, contractile properties were measured *in vitro* for force generation and protection from contraction-induced injury. In order to compare the MRI results with histopathology, the left hindlimbs were collected and fixed in formalin for both hematoxylin and eosin (H&E) and Masson's trichrome staining. Immunofluorescence was also conducted in mdx and control mice for the right hindlimbs collected after the final MRI measurements.

Results and Discussion

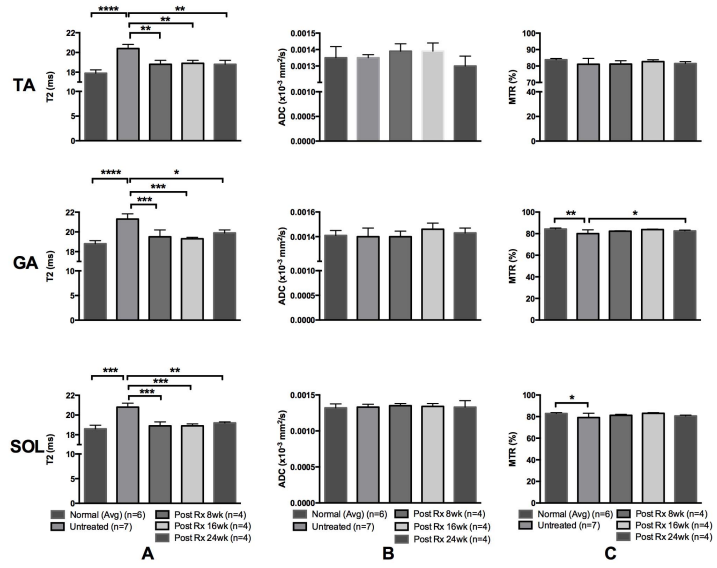

Figure 1. T2, ADC and MTR variations for skeletal muscles of all mice before treatment and at 8, 16 and 24 weeks post-treatment.

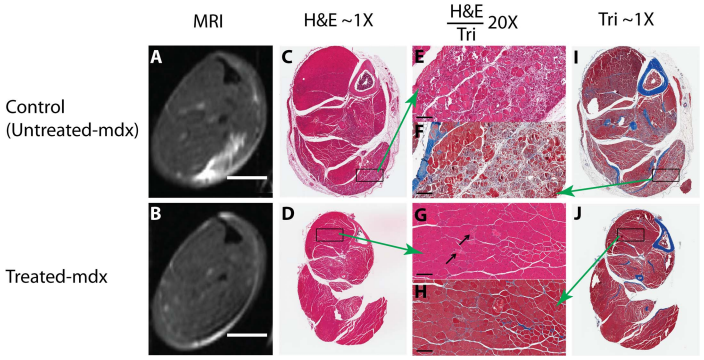

Figure 2. Tissue sample analysis: representative T2 images and histological images of H&E and Masson's trichrome staining.

T2, ADC and MTR values were on the 3 different muscle groups (TA: tibialis anterior, GA: gastrocnemius, SOL (soleus) muscles) at all time points (see Fig. 1). The analysis showed similar trends: the T2 measurements were significant for all three muscle types in regard to Pre-Rx (treatment) measurements vs Post-Rx measurements, along with the GA and SOL displaying significance between the untreated and treated groups. The MTR measurements for the same muscle groups also showed significance in several muscle groups. The MRI measurements taken were correlated with histopathology (utilizing H&E and Masson's trichrome staining) to visually represent the cellular processes of muscle degeneration and regeneration (Fig. 2).

Conclusions

Longitudinal parametric MRI utilizing T2, ADC and MTR measurements has feasibility for identifying and monitoring the disease progression and treatment responses in the *mdx*^{4cv} mouse model of muscular dystrophy and for future human clinical trials.