Multi-parametric MRI at 14T for muscular dystrophy mice treated with gene therapy

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Introduction

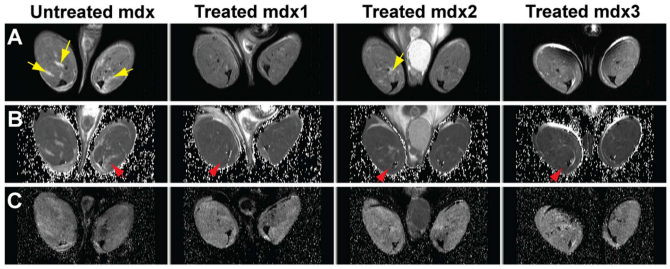
Duchenne muscular dystrophy (DMD) is one of the most common forms of muscular dystrophy in humans. There is no cure for this deadly disease. Although magnetic resonance (MR) has emerged as a noninvasive tool capable of generating valuable information on tissue characteristics, clinical muscle MRI has been dependent on T_1 and T_2 weighted imaging to monitor inflammatory myopathies in patients with muscular dystrophy (1,2). These methods are useful because of their sensitivity to a wide range of mechanisms, but this generality means that they may not be able to identify specific cellular processes in the affected areas. Here we performed multi-parametric MRI to monitor treatment responses for *mdx* mice after adeno-associated virus (AAV) vector mediated gene therapy. We used quantitative T_2, diffusion and magnetization transfer (MT) MRI to evaluate treatment effects for the AAV vector medicated gene therapy.

Methods

Eleven *mdx* mice and three normal C57Bl/6 mice were used for this study. Among 11 *mdx* mice, 7 were systemically treated with 10^{13} vg of AAV virus containing a codon-optimized micro-dystrophin at 12 weeks of their ages. Multi-parametric ^{1}H MRI was carried out for *mdx* mice on a Bruker 14T Avance MR spectrometer (Bruker Corp., Billerica, MA). The high resolution MRI protocol includes scout imaging (gradient echo; TR (recycle delay)/TE (echo time) = 30/1.3 ms), planning for image planes (multislice RARE (rapid acquisition with refocused echoes): TR/TE = 668/4.5 ms), high resolution 2 dimensional imaging with thin slices (200 micron thick) (multi-slice RARE: TE/TE = 4000/6 ms) for muscle volume evaluation, multi-TE RARE imaging (TR/TE = 4000/ 6 ~ 75 ms, 12 echoes) for T_2 measurements, magnetization transfer imaging, diffusion imaging with three b values of 0, 500 and 1000 s/mm^2 sequence (TR/TE = 4000/25 ms) and diffusion tensor imaging (DTI) with 4 shot echo planar imaging and b value of 1000 s/mm^2 (TR/TE = 4000/18 ms).

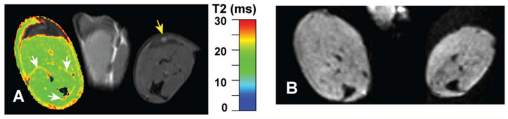
Results and Discussion

T_2 and apparent diffusion coefficient (ADC) values were measured for both treated and untreated *mdx* mice and compared to those for normal mouse. Figure 1 shows T_2 weighted images (TR/TE = 4 s/ 12.6 ms), T_2 maps (TR/TE= 4 s/ 6.3 ~ 75.4 ms, 12 echoes), ADC maps acquired at 14T for untreated (A) and treated muscles (B, C) of mice with muscular dystrophy (*mdx*). T_2 and ADC values were measured on tibialis anterior (TA) muscles (Fig. 1D). Three *mdx* mice (columns 2 - 4 in Fig. 1) were systemically treated with adeno-associated virus (AAV) mediated microdystrophin genes while their litter mate control *mdx* mouse was untreated (1st column in Fig. 1). T_2 values of TA muscles were determined to show approximately 37% T_2 decrease and up-to 16% ADC reduction on TA muscles for treated *mdx* mice compared to those for untreated control mouse. Also, T_2 weighted (T_2w) images and the T_2 and ADC values for the treated mice are close to those acquired for normal mouse muscles (see Figure 2 for high resolution MR images for a normal mouse). The results demonstrate that the systemic micro-dystrophin treatments have high potential of an effective treatment approach for muscular dystrophy and that T_2w, T_2 and diffusion measurements would be important components in an MR protocol to monitor treatment responses. Figure 2 shows some results of T_2 and DTI acquisitions conducted at 14T for *in vivo* hind-limb muscles of a normal C57BL/6 mouse. The corresponding T_2 map and fractional anisotropy (FA) map were also generated by multi-slice multi-echo and DTI sequences, respectively.



D	Untreated TA	Treated TA1	Treated TA2	Treated TA3
T2 (ms)	25.3 ± 5.9	18.5±1.1	18.5 ± 1.4	20.0 ± 2.0
ADC (x 10^{-3}mm^2/s)	1.53 ± 0.19	1.45 ± 0.21	1.31 ± 0.27	1.33 ± 0.22

Figure 1. T2 weighted (T2w) images showing fatty infiltrated regions (yellow arrows) in muscles (A), and T2 and ADC values measured on TA muscles (red arrows) in their corresponding T2 maps (B) and ADC maps (C). T2 and ADC values measured on TA muscles are summarized in (D).



	TA	GA
T2 (ms)	17.6 ± 1.0	18.3 ± 1.0
ADC (x 10^{-3}mm^2/s)	1.24 ± 0.06	1.22 ± 0.06
FA	0.16 ± 0.05	0.16 ± 0.05

Figure 2. A colorized T2 map of a right leg was overlaid on top of a high resolution image for hindlimb muscles of both mouse legs. Connective tissues (white arrows) and a popliteal lymph node (yellow arrow) are clearly seen from the T2 map and the high resolution image acquired at 14T, respectively (A). A diffusion weighted image (B) was selected from a DTI acquisition. T2, ADC and FA values measured for both tibialis anterior (TA) and gastrocnemius (GA) muscles are summarized in the table.

Conclusions

Our results show T_2, apparent diffusion coefficient (ADC) and MT ratio values would be potential MR markers to evaluate the treatment efficacy of gene therapy for muscular dystrophy. These noninvasive MR markers may be useful to timely monitor treatment responses for muscular dystrophy, which facilitates development of effective approaches to treat the deadly disease.

References

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