Magnetic Resonance and Optical Imaging as Biomarkers for Muscle Disease and Injury

Chrzanowski, S.M. (UF, Physiology); Lee-McMullen, B.A. (UF, Biochemistry); Batra, A. (UF, Physical Therapy); Vohra, R.; Baligand, C. (UF, Physiology); Jiang, H. (UF, Biomedical Engineering); Vandenborne, K. (UF, Physical Therapy); Walter, G.A. (UF, Physiology)

Introduction

Duchenne muscular dystrophy (DMD) is caused by mutations in the dystrophin gene, ultimately resulting in physiological degeneration and clinical weakness of muscle. Current methods of assessing disease involvement in muscle include muscle biopsy and functional testing, but intrinsic variables such as invasiveness and patient compliance limit these two testing modalities, respectively. In this study, we demonstrate the ability of magnetic resonance imaging (MRI) and spectroscopy (MRS), as well as near infrared optical imaging (NIR-OI) to monitor diseased and damaged muscle in mice.

Experimental Design

Hindlimbs of *mdx* mice (6 weeks of age) were first compared to age matched unaffected control mice by MR and OI methods. MR and NIR-OI data were also collected from older *mdx* mice (12-32 weeks of age) that were subjected to a downhill treadmill running exercise bout to induce muscle damage. All mice were imaged on an Agilent 4.7T MR Scanner within the Advanced Magnetic Resonance Imaging and Spectroscopy (AMRIS) facility and an IVIS Spectrum In Vivo System to capture MR and NIR-OI data, respectively. Multiple slice, spin echo scans were acquired to assess MRI-T_2 relaxation and ^1H_2O spectroscopic relaxometry (TEs = 5 – 300 ms, 64 points exponentially spaced) was assessed using single voxel ^{1}H-STEAM spectroscopy within the soleus muscle, using non-negative least squares (NNLS-T_2) analysis of the spectroscopic data. Prior to NIR-OI, mice were intravenously injected with 100 µL of Indocyanine Green, an FDA approved fluorescent NIR contrast agent that binds serum albumin. 2D fluorescence images were captured using excitation and emission wavelengths of 745 and 820 nm, respectively on an IVIS Spectrum pre-clinical optical imaging system.

Results and Discussion

In *mdx* mice, MRI-T_2, ^1H_2O-T_2, and radiant efficiency were significantly elevated in both the hindlimb and forelimb muscles compared to unaffected control mice. Additionally, following downhill treadmill running, MRI-T_2, ^1H_2O-T_2, and radiant efficiency increased significantly in the post-treadmill cohort of *mdx* mice.

Conclusions

MR confirmed disease involvement and NIR-OI demonstrated the ability to detect muscle damage in a minimally invasive, sensitive, and specific manner in dystrophic muscle. Whereas MRI offers high-resolution imaging, it can be complimented by the use of contrast enhanced NIR-OI to track muscle damage.

Acknowledgements

A portion of this work was performed at the National High Magnetic Field Laboratory, which is supported by National Science Foundation Cooperative Agreement No. DMR-1157490, the State of Florida, and the U.S. Department of Energy. Additional funding was provided by the Department of Defense (MD 110050).

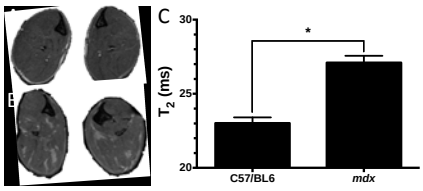

Fig. 1 – MRI's of control (A) and *mdx* (B) mice confirm differences in baseline T_2 relaxation times (C).

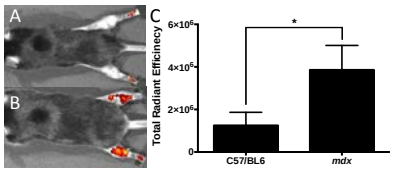

Fig. 2 – NIR-OI of control (A) and *mdx* (B) mice confirm differences in radiant efficiency (C).

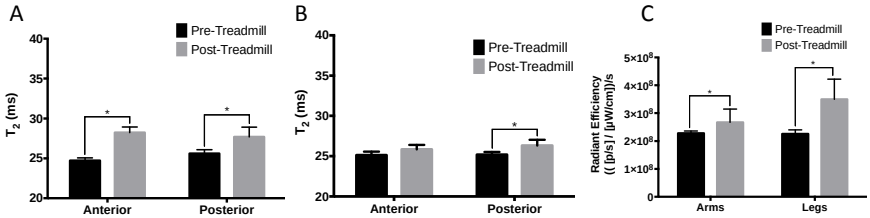

Fig. 3 – Treadmill exercise exacerbates muscle damage in *mdx* mice, shown by MRI in the forelimbs (A), hindlimbs (B), and NIR-OI of both forelimbs and hindlimbs((C)