Non-Invasive Markers Used to Quantify Acute Muscle Damage in \textit{mdx} Mice

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Introduction

Duchenne muscular dystrophy (DMD) is the most common form of muscular dystrophy and occurs from a mutation in the gene encoding for the sarcolemmal protein, dystrophin. The lack of dystrophin leads to a greater susceptibility to contraction-induced muscle damage, which has been observed in the \textit{mdx} mouse, a DMD homologue(1).

Experimental

The purpose of the experiment was to quantify acute muscle damage in \textit{mdx} mice after a single bout of eccentric exercise using magnetic resonance imaging (MRI). Wild type (C57BL6, n=4), and \textit{mdx} mice (n=4) underwent a bout of eccentric exercise by running them downhill on a motorized treadmill inclined at -14° at a speed of 8-10 m/min for 45 minutes. Multiple T2-weighted, MR images of the lower hindlimbs were acquired in a 4.7T, horizontal bore magnet (Bruker Avance) at 3 timepoints: before exercise, immediate post exercise and 24 hrs after exercise. Multiple slice, single spin-echo images were acquired with the following parameters: TR=2000ms, TE=14ms and 40ms, acquisition matrix=128 x 256 and two signal averages. Gastrocnemius (gastroc) and Tibialis anterior (TA) muscles, with elevated T$_2$ values (% damage), were quantified using in-house software. Evans Blue Dye (EBD) was injected at 24 hrs post-exercise and muscles were harvested at 48 hrs, to confirm the presence of damaged muscle fibers after eccentric exercise. The area of EBD positive fibers was expressed as a percent of total muscle cross-sectional area.

Results and Discussion

Prior to exercise, the percent of damaged muscle was 1.2±0.5 and 2.0±0.8 (TA and gastroc, respectively, mean±SD) in the \textit{mdx} mice compared to wild type mice (1.1±0.63 and 0.70±0.19, respectively). Immediately following exercise, muscle damage increased to 16.9±9.1% and 4.3±1.2% in the \textit{mdx} mice and remained elevated at 24 hours post-exercise in the TA muscle of \textit{mdx} mice (12.4±4.9%), whereas the gastroc muscle tended to decrease (2.9±0.7%). No change in muscle damage was observed in the wild type mice. The percent area of EBD positive fibers in \textit{mdx} mice was 32±9% (mean±SEM), whereas no EBD positive fibers were observed in the wild type mice.

(A) Pre Exercise Image, followed by, (B) Immediate Post Exercise Image, and (C) 24 hrs Post Exercise Image.

Conclusions

Eccentric exercise, from downhill running, results in acute muscle damage in \textit{mdx} mice but not in wild type mice. MRI can be used as a non-invasive, quantitative marker of muscle damage, which can be applied repeatedly to examine acute muscle damage and recovery.

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References