Skeletal myosin inhibitor EDG-5506 protects dystrophic muscle from contraction-induced injury
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Introduction
DMD is a lethal, inherited muscle myopathy caused by the absence of dystrophin and destabilization of the dystrophin-glycoprotein complex (DGC) in the cell membrane. Dystrophin provides a structural link between the contractile elements of the sarcomere and the basement membrane of muscle. When dystrophin is absent, mechanical stress of muscle leads to the opening of membrane stress channels, calcium influx, muscle fiber injury and degeneration.

The relationship between contraction force, intracellular calcium, force drop and degeneration in lumbral muscles from mdx mice was examined ex vivo using a dual force/calcium system. To dissect the role of mechanical stress in this process, we pre-incubated muscles with different concentrations of EDG-5506, a novel, selective, fast myosin inhibitor currently in clinical trials for Becker muscular dystrophy. Two concentrations of EDG-5506 were tested (0.3 mM and 1 mM) and intracellular calcium concentration was monitored using the highly-sensitive fluorescent dyes, fura-2.

Methods
Contractile properties: Twitch pulse (0.2 ms) and tetanic contractions (125 Hz for 1.0 sec) were measured in lumbral muscles submerged in Tyrode solution (sarcomere length 2.5 μM) isolated from anesthetized control (C57BL/6) and mdx (C57BL/10 ScSn-Dmdmdx/J) mice (8–12 weeks of age).

Intracellular Ca²⁺: Muscles were incubated with calcium-sensitive fluorescent dyes for 30 min at 25°C. Pre-loading background fluorescence was subtracted from response fluorescence. All procedures were approved by the University of Michigan IACUC.

Results
Low concentrations of EDG-5506 protect dystrophic muscle fibers from injury related force loss, calcium dysfunction, membrane rupture, and clots.

(A) Specific force after 1-hr incubation with vehicle or EDG-5506 in mdx lumbral muscles (B) Change in force after repeated tetanic contraction of mdx lumbral muscles. (C) Inter-contractation fura-2 fluorescence ratio (left) and inter-contractation force creep (right) during repeated tetanic contraction. (D) Representative force (black) and fura-2 fluorescence ratio (blue, intracellular Ca²⁺) during 12 isometric contractions. Note the axis focus toward the main force traces show increases in resting force only in vehicle treated muscle. Error bars shown +/- SEM. (E) Top, representative images of mdx lumbral muscles before and after 12 contractions. Example clots circled in red. Bottom, quantification of muscle clots from retracted fibers (N=8-12). Significance calculated by one-way ANOVA with Dunnet’s multiple comparison (*<0.05; **<0.01; ***<0.001; ****<0.0001).

Calcium transients during contraction are unchanged

Resting calcium is related to force drop ex vivo

(A) Left, intracellular calcium transient (ICT) peak during contractions and right, ICT width (full width at half maximum) measured by mag-fura-2 fluorescence response after 1 hr incubation with DMSO or EDG-5506 in mouse lumbral muscle. (B) Force drop due to contractions as a function of inter-contraction calcium fluorescence, show a significant correlation in control group but not with treatment.

Conclusions
The small molecule EDG-5506 causes modest levels of specific force reduction by modulating skeletal myosin which is sufficient to protect mdx lumbral muscles from:
- Membrane leakage and clots
- Calcium influx
- Contraction injury related force deficits

These data show that membrane injury of dystrophic muscle occurs with active contraction via myosin, and this muscle damage can be prevented with EDG-5506.

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References