1. Background

Duchenne muscular dystrophy (DMD) is characterized by progressive muscle weakness due to repeated cycles of injury, incomplete regeneration, and fibrosis. Glucocorticoids (GC), such as deflazacort (DFZ) and prednisone, slow disease progression in DMD and are considered standard of care. Preclinical data in mdx mice suggest that GC improve membrane repair and muscle regeneration via upregulation of annexin repair proteins. The fast skeletal muscle myosin modulator, EDG-5506, protects skeletal muscle in models of muscular dystrophy by reducing contraction-induced injury to prevent degeneration. We sought to understand whether the previously reported protective effects of EDG-5506 would be retained in the background of GC administration.

2. Study design

8-week-old C57BL/10ScSn-Dmd-mdx (mdx) received daily oral treatment with DFZ (1.2 mg/kg) for 9-weeks and ex vivo muscle force, calcium dysregulation, and morphological damage were assessed in the lumbar muscles. In a separate cohort, mice were treated with daily oral DFZ (1.2 mg/kg), EDG-5506 (3 mg/kg), or a combo. For 9-weeks. At the end of the study, forelimb grip strength (GS), in situ TA/EDL muscle force, and plasma creatine kinase (CK) were measured.

3. Methods

Drug treatment

Mice were given a daily oral dose of a drug or vehicle suspension (1% methyl cellulose and 0.1% Tween80) for 9 weeks.

Ex vivo protocol

Contractile properties: Twitch pulse (0.2 ms) and tetanic contractions (125 Hz, 1.0 sec) were measured in lumbar muscles submerged in Tyrode solution (sarcromere length 2.5 µm) isolated from anesthetized control (C57BL/10J) and mdx mice. Intracellular Ca²⁺: Muscles were incubated with calcium-sensitive fluorescent dye (fura-2) for 30 min at 25°C. All procedures were approved by the University of Michigan IACUC.

GS

The forelimb GS test consists of 5 trials spaced 30 seconds apart (Columbus Instruments, OH, USA).

In situ protocol

Isometric force (300 ms, from 25-175 Hz) was measured in the TA muscle (Aurora Scientific, ON, Canada; Chalgren. Enterprises Inc, CA, USA) of anesthetized mice on a 37°C surgical platform. All procedures were approved by the University of Colorado Boulder IACUC.

Plasma Creatine Kinase

After the in situ protocol, blood samples were collected for circulating CK (Pointe Scientific, Thermo Fisher).

3. Results

Treatment with DFZ + exogenous EDG-5506 prevents ex vivo force loss and dysregulated calcium associated with muscle injury

Figure 1. A) Force loss over twenty contractions, error is SEM. B) Box plots showing total force loss after twenty contractions. C) Box-plots of inter-contraction force during the ex vivo protocol. D) Box plots of inter-contraction force in the ex vivo protocol. E) Morphologically damaged muscle fibers indicated by the red arrows. Concentration of EDG-5506 is 0.5 µM. mdx vehicle and DFZ were significantly different from the other groups, which were not different from each other (p < 0.05, ANOVA).

Treatment with DFZ + exogenous EDG-5506 prevented force loss with injury ex vivo. Injured dystrophic muscle displays dysregulated calcium and aberrant force production (increased inter-contraction calcium and force production) which leads to further muscle damage. The combo. treatment prevented this more than vehicle or DFZ alone. This shows that EDG-5506 protects muscles via a different mechanism than DFZ. Treatment with the combo. restored force loss and markers of injury nearly to healthy (WT) levels.

Combo. treatment improves muscle force measured by GS (continued)

At each trial, combo.-treated mice had greater GS than vehicle-treated mice. At the end of the protocol, mice treated with EDG-5506 alone had higher GS than vehicle-treated mice. Fatigue associated with dystrophic muscle was improved with the combo. treatment and EDG-5506 alone.

4. Conclusions

- Sarcomere stabilization with EDG-5506 protects muscle via a different mechanism than DFZ (ex vivo).
- Combo. treatment showed greater improvements in muscle force (GS, in situ TA/EDL) and injury response (CK, and ex vivo) than DFZ alone.
- Combo. treatment restored dystrophic muscle to healthy levels (ex vivo) when GC alone did not.
- These data support myosin modulation with EDG-5506 improves muscle function in the background of GC treatment.

References


At Edgewise, patients are at the core of everything we do.

Competing Interests: AKP, YQ, BNS, SS, ME, and AR are employees of Edgewise Therapeutics and hold financial interests (stock and/or stock options)

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