Fast Skeletal Myosin Modulator EDG-5506 Confers Additional Protection to mdx Mice Receiving Glucocorticoid Treatment

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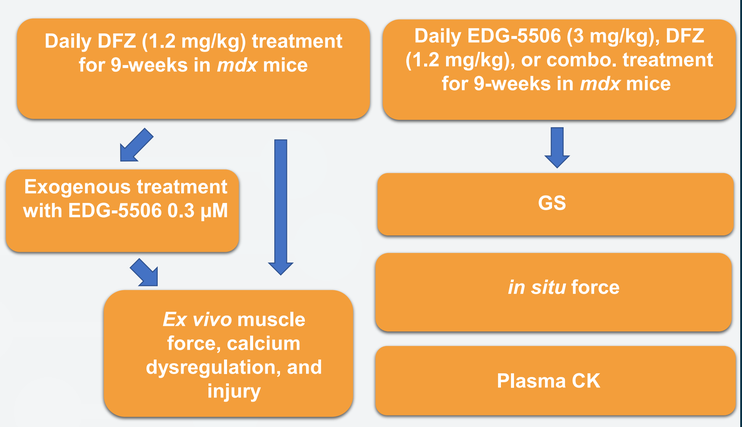
1. Background

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[1]. 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*$^{mdx/J}$ (*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 lumbrical 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 lumbrical muscles submerged in Tyrode solution (sarcomere 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 25ºC. 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

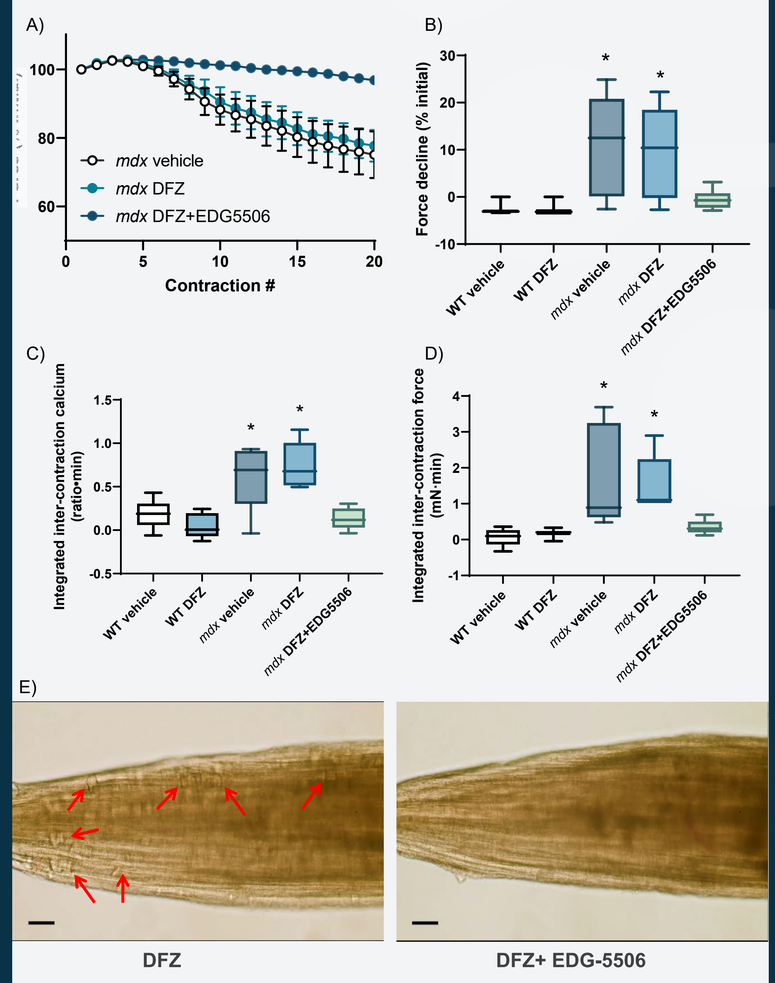


Figure 1. A) Force loss over twenty contractions, error is SEM. B) Box plots showing total force loss after twenty contractions. C) Box-plots of inter-contraction calcium during the *ex vivo* protocol. D) Box plots of inter-contraction force during the *ex vivo* protocol. E) Morphologically damaged muscle fibers indicated by the red arrows. Concentration of EDG-5506 is 0.3 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[3]. 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

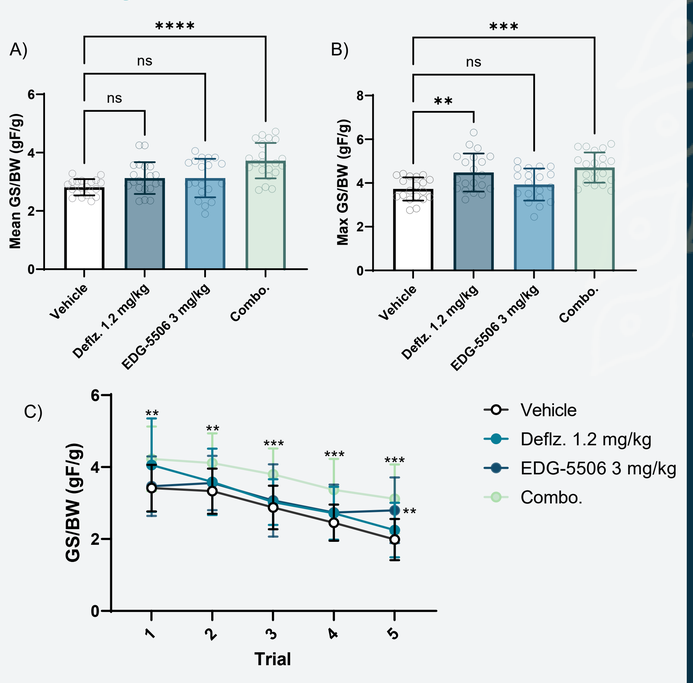


Figure 2. A) Mean GS normalized to BW ($p < 0.05$, ANOVA. B) Max GS normalized to BW ($p < 0.05$, ANOVA) C) Mean GS normalized to BW across trial ($p<0.05$, two-way ANOVA). For all figures the error is the SD.

Combo.-treated mice have greater GS (mean and maximal) than vehicle-treated mice. Across GS trials dystrophic muscle display fatigue (force drop across trials).

3. Results (Continued)

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.

Combo. treatment improves muscle force *in situ* and lowers plasma CK, a marker of muscle injury

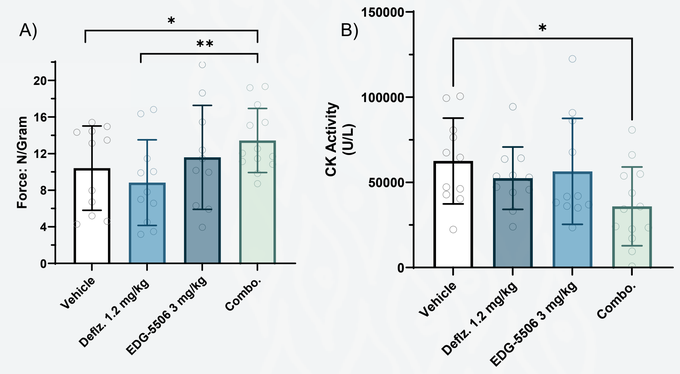


Figure 3. A) *in situ* force (150 Hz) normalized to muscle weight ($p < 0.05$, t-test). B) Plasma CK levels taken after *in situ* lengthening injury. $p < 0.05$, one-way ANOVA. For all figures the error is the SD

Force measurements (*in situ*) showed combo.-treated mice have greater force than the DFZ only or vehicle group. Dystrophic muscle injury is associated with elevation of circulating CK[2]. The combo. treatment lowered plasma CK activity compared to vehicle treated mice, and DFZ or EDG-5506 alone groups. These data support myosin modulation with EDG-5506 improves muscle function in the background of GC treatment.

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 has positive effects both with and without GC background therapy. This finding has relevance to its use in DMD (The LYNX trial, NCT05540860)**

References

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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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