EDG-5506 is a Selective Inhibitor of Fast Skeletal Muscle Myosin

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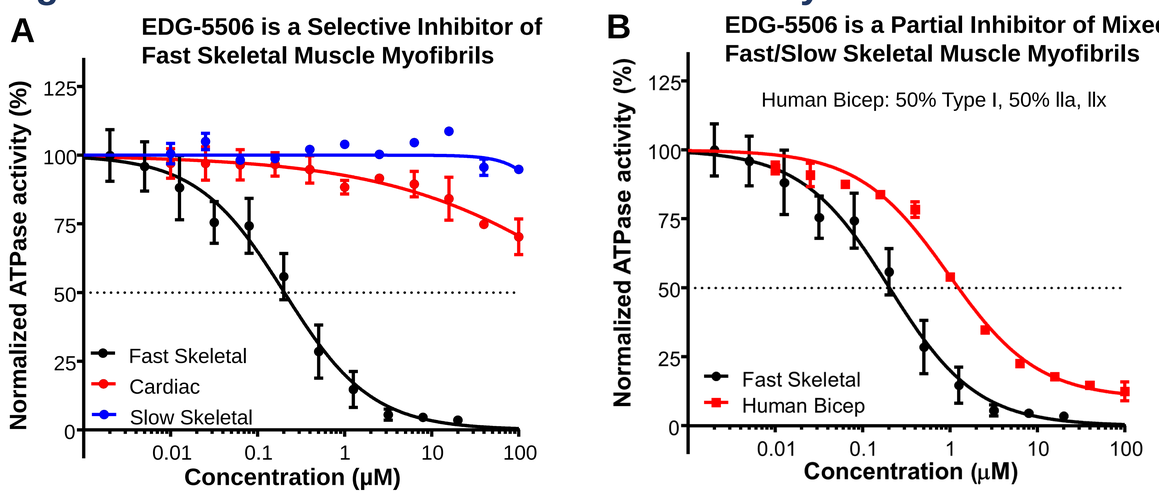
Introduction

Duchenne muscular dystrophy (DMD) is a disease characterized by the absence of dystrophin, a structural protein that links the contractile machinery of muscle to the extracellular matrix. In DMD, muscle contraction produces mechanical force, resulting in fiber stress and breakdown, especially in fast fibers. To understand the role of contraction stress in dystrophic muscle degeneration, we describe here the selectivity and specificity profile of a novel inhibitor of fast skeletal muscle, EDG-5506 which can be used to modulate muscle properties in a controlled manner.

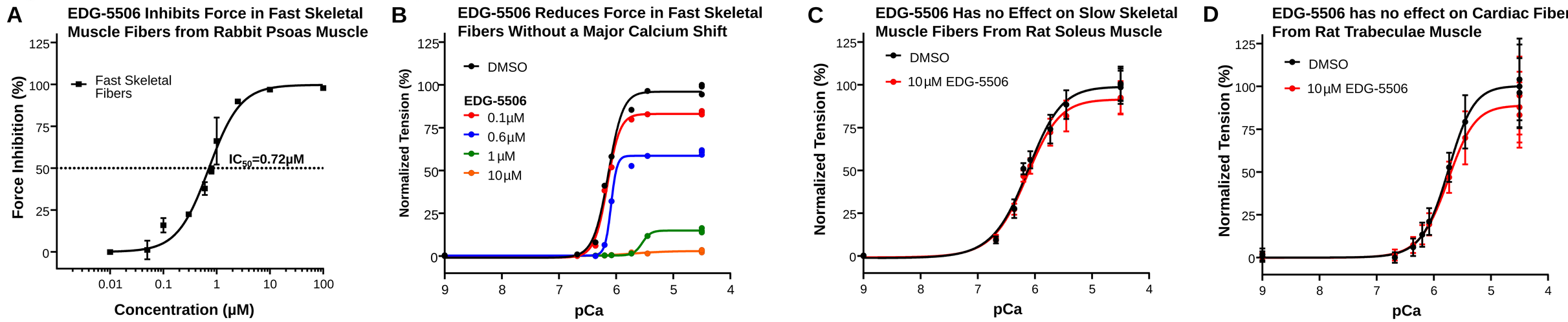
Figure 1: EDG-5506 Effects on ATPase Assay



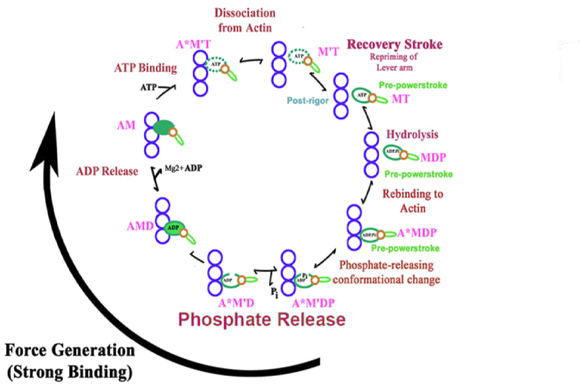
ATPase Methods: Using a NADH-coupled assay, the ATPase activity of purified rabbit fast skeletal psoas myofibrils, porcine ventricular myofibrils, and fast skeletal psoas chymotrypsin-digested myosin sub-fragment 1 (S1) was measured. EDG-5506 ATPase activity levels were normalized to DMSO, and inhibitory potency was calculated for each tissue type.

Figure 1: EDG-5506 potency and selectivity was evaluated in (A) fast and slow skeletal, and cardiac muscle myofibrils from animals, and (B) humans with a mixed fast:slow fiber type composition. Further, myosin S1 fragments were used to compare EDG-5506 effects on fast and smooth, as well as cardiac S1 preparations (C). EDG-5506 selectively inhibited fast skeletal myofibrils and myosin S1.

Figure 2: Force-calcium relationship



Chemo-mechanical Cycle



Methods: Phosphate release during the chemo-mechanical cycle was measured using fluorescent phosphate binding protein with skeletal muscle in a dual mixing stopped flow setup. Prior to mixing with 1 mM ATP, either 0 or 10 μM EDG-5506 was incubated with 6 μM fast rabbit skeletal muscle S1 myosin at 25ºC for 30 mins.

Figure 3: Phosphate release

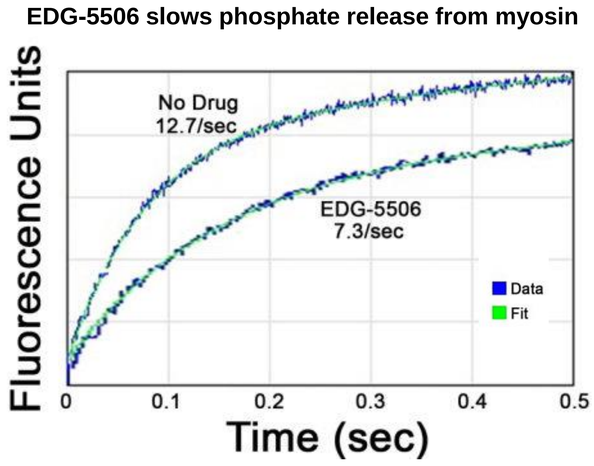


Figure 3: EDG-5506 effects on the crossbridge cycle were evaluated using stopped flow kinetic measurements in the presence of actin. A) Phosphate release in the chemo-mechanical cycle was slowed 43% at 10μM.

Fiber Methods: Striated muscles from fast (rabbit psoas), slow (rat soleus) and cardiac (rat trabeculae) muscles were used to prepare skinned single fibers and cardiac bundles. These fibers were mechanically tested to observe compound dose and force-calcium relationships under different contractile conditions.

Figure 2: EDG-5506 dose response was evaluated in fast rabbit psoas muscle fibers (A). The accompanying representative pCa curves at different concentrations of EDG-5506 are shown in (B), normalized to DMSO. The top concentration of EDG-5506 from (B) was used to compare with slow skeletal (C) and cardiac muscle pCa curves (D). N=2-6 fibers, +/- SEM).

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Conclusions

EDG-5506 is a selective fast myosin inhibitor that decreases ATPase and force in myofibrils and fibers and reduces phosphate release in the chemo-mechanical cycle, providing a mechanism to protect muscle against contractile stress in the absence of dystrophin.

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