

Inhibition of the extracellular matrix protease adamts5 improves strength in fast-twitch hindlimb muscles from young, dystrophic *mdx* mice

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Duchenne muscular dystrophy (DMD) is caused by mutations in the dystrophin gene and is characterized by a high susceptibility to contraction-induced injury, ineffective repair and expansion of the extracellular matrix (ECM) leading to muscle loss and weakness. This ECM expansion is not just a consequence of the disease, but actively contributes to muscle degeneration. Versican is a transitional ECM chondroitin sulphate proteoglycan and is highly upregulated in dystrophic muscles from patients with DMD and in *mdx* mice, the mouse model of DMD. Cleavage of versican by A Disintegrin and Metalloproteinase with Thrombospondin motifs (ADAMTS) proteases regulates cell processes relevant to muscle repair and degeneration. The resultant DPEAAE fragment, versikine, is also bioactive and can stimulate apoptosis (McCulloch *et al.*, 2009). ADAMTS5 protein levels are upregulated in dystrophic muscles from *mdx* mice. Serum levels of ADAMTS5 are elevated in *mdx* mice and DMD patients and it has been suggested that ADAMTS5 is a highly informative biomarker of the pathogenesis of DMD (Coenen-Stass *et al.*, 2015). ADAMTS5 is an important arthritis drug target with antibody and pharmacological inhibitors in development.

Here we examined the role of versican processing by ADAMTS5 in the pathogenesis of DMD using young, growing *mdx* mice and assessed the consequences of ADAMTS5 inhibition on hindlimb muscle structure and function. All experiments were carried out in accordance with NHMRC guidelines with approval from the Animal Ethics Committee at Deakin University (G35-2013). Commencing at 4 weeks of age, male (N=7 treatment; N=9 IgG control) and female (N=9 treatment; N=11 IgG control) *mdx* mice received intraperitoneal injection with either an IgG control or an ADAMTS5 neutralising antibody (10 mg/kg; GlaxoSmithKline) once weekly for three weeks. To assess the effects of ADAMTS5 inhibition on muscle contractile properties, mice were anaesthetized *via* intraperitoneal injection (medetomidine 0.6 mg/kg; midazolam 5 mg/kg; fentanyl 0.05 mg/kg until unresponsive to tactile stimuli. Fast twitch *extensor digitorum longus* (EDL) and slow twitch *soleus* muscles were surgically excised, and muscle strength (determined from a force-frequency curve), fatigability (4 min of intermittent, submaximal stimulation at 60 Hz) and recovery from fatigue were assessed *in vitro* (1300A Whole Mouse Test System, Aurora Scientific). Anaesthetized mice were then euthanized by cervical dislocation and tissues were collected for molecular and histological analysis.

Body weight and *soleus* muscle mass was unaffected by either treatment. EDL muscle mass was reduced in female, but not male, *mdx* mice treated with the ADAMTS5 neutralising antibody ($P=0.02$, t-test). Inhibition of ADAMTS5, resulted in increased strength normalized to muscle size (sP_0) in EDL muscles from male ($P<0.0001$, main effect GLM ANOVA) and female ($P=0.049$, main effect ANOVA) *mdx* mice, as indicated by the upward shift of the force-frequency curve. This effect on strength that was not observed in *soleus* muscles. Treatment with the ADAMTS5 inhibitor antibody had minimal effects on fatigability and recovery from fatigue in both EDL and *soleus* muscle from male and female *mdx* mice. Histological and molecular analyses are ongoing to characterize the effects of ADAMTS5 inhibition on dystrophic muscle pathology. Our data are the first report of improved strength in dystrophic fast twitch muscles subjected to inhibition of ADAMTS5. We also show that targeting ECM remodelling is associated with distinct gender differences between male and female dystrophic *mdx* mice and this may have broader implications for understanding fibrosis and muscle degeneration.

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