Long term treatment with naproxcinod, a nitric oxide-donating anti-inflammatory compound, shows significant therapeutic effects in *mdx* mouse model of dystrophy

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Duchenne muscular dystrophy (DMD) is the most common muscular dystrophy caused by mutations in the dystrophin gene. DMD is characterized by progressive muscle wasting and weakness, with the onset of symptoms occurring in the early childhood and consequently leading to paralysis and death for respiratory or cardiac failure. There is no cure and glucocorticoids are used to control the progression of the disease. In DMD patients and the *mdx* mouse model of DMD, dystrophin deficiency causes local inflammation and a decrease and mislocalization of muscle-specific neuronal nitric oxide synthase (nNOSμ), leading to a variety of functional impairments such as muscle ischemia and compromised myogenesis. Previous studies have shown that nitric oxide (NO) donation associated with anti-inflammatory action showed beneficial effects in dystrophic mouse models.

In this study, we have investigated the effects of naproxcinod, an NO donating naproxen, on skeletal and cardiac muscle function in *mdx* mice. 4-week-old *mdx* mice were orally treated for 9 months with three different doses of naproxcinod (10, 21 and 41 mg/kg/day) compared with 0.9 mg/kg of prednisolone. Functional and behavioral parameters using a grip strength meter and open field digiscan were measured at 3, 6, and 9 months of treatment using SOPs developed by TREAT-NMD. Additionally, optical imaging of inflammation, echocardiography and blood pressure were evaluated at the 9 months prior to sacrifice. Skeletal muscle histology was assessed in diaphragm muscle.

Naproxcinod treatment at 10 and 21 mg/kg resulted in significant improvements in hindlimb grip strength as well as approximately a 25-30% decrease in inflammation in fore and hind limbs measured by *in vivo* optical imaging in *mdx* mice. Histological analysis of diaphragm confirmed the beneficial anti-inflammatory effects exerted by NO donating naproxen, reducing significantly inflammatory infiltrates at the dose of 21 mg/kg. Additionally, naproxcinod induced significant improvements in heart function as evidenced by ameliorated fraction shortening and ejection fraction measured using echocardiography along with improvements in systolic blood pressure. Moreover, the long term detrimental effects of prednisolone typically observed in *mdx* skeletal and heart function were not observed at the effective doses of naproxcinod.

In conclusion, naproxcinod showed significant and persistent therapeutic effects improving muscle function, reducing muscle inflammation and improving heart function. Thus, naproxcinod seems to have significant potential as a safe therapeutic option for the treatment of muscular dystrophies.