muscle fibres and decreased levels of TIMP-1 in the serum. The chronic dosing study also showed a reduction in inflammation, restoration of a more normal fibre type pattern and reduction in fibre size variability. These studies show the potential of the PPMO formulation to significantly improve the results of exon-skipping in clinical trials.

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P11.2
Naproxcinod, a nitric oxide-donating anti-inflammatory compound, is effective in two mouse models of muscle dystrophy
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Nitrile oxide (NO) plays a critical role in skeletal muscle function, including control of blood flow and muscle repair. In muscular dystrophies, synthesis of NO in the skeletal muscle is known to be defective therefore contributing to damage progression. In the present study, we evaluated the effects of naproxcinod, an NO-donating anti-inflammatory compound, in two models of muscular dystrophy, the alpha-sarcoglycan (alpha-SG) null mice, a model for limb-girdle muscle dystrophy, and the mdx mouse model for Duchenne muscle dystrophy (DMD).

Naproxcinod (10 and 30 mg/kg/day) was orally administered for 7 months to mdx mice and for 4 months to alpha-SG null mice starting at 4 weeks of age. Muscle function was assessed by treadmill test at 4 months (both mdx and alpha-SG null mice) and 7 months of treatment (mdx mice). Serum creatine kinase (CK) was measured as index of skeletal muscle damage. Inflammatory infiltrates, as well as muscle regeneration were studied in diaphragm and tibialis anterior muscles.

In mdx mice 30 mg/kg/day of naproxcinod significantly improved muscle function in terms of resistance to exercise with a recovery score of 12%; significantly reduced skeletal muscle inflammation and serum CK activity; and increased muscle regeneration. Likewise in alpha-SG null mice, 4 months of naproxcinod treatment led to a significant improvement of resistance to fatigue (recovery score of 59%), and of reduction of muscle inflammation and damage. Furthermore, both diaphragm and tibialis anterior muscles appeared fully regenerated, thus supporting the marked beneficial effects observed for muscle function.

The results demonstrate that naproxcinod, through NO donation together with anti-inflammatory activity, produces significant and persistent therapeutic effects improving muscle function, reducing muscle inflammation and maintaining regeneration capacity of the muscle in two models of muscular dystrophies.

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P11.3
Long term treatment with naproxcinod significantly improves skeletal and cardiac function in mdx mouse model of dystrophy
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There is evidence that nitric oxide (NO) plays a critical role in skeletal muscle. In Duchenne muscular dystrophy (DMD) patients and the mdx mouse model of DMD, dystrophin deficiency causes a decrease and mislocalization of muscle-specific neuronal nitric oxide synthase (nNOSa), leading to a variety of functional impairments such as muscle ischemia and compromised myogenesis. Previous studies have shown that 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, in vitro EDL force contraction, optical imaging of inflammation, echocardiography and blood pressure were evaluated at the 9 months prior to sacrifice. 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. 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 seems to have significant potential as a safe therapeutic option for the treatment of muscular dystrophies.

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P11.4
Altered mechano-transduction in dystrophic muscle is accompanied by changes in function and expression of CIC-1 chloride channel
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The understanding of the molecular mechanisms underlying exercise susceptibility of mdx mice may help to identify new drug targets for muscular dystrophy. An enhancement of in vivo weakness and fatigability occurs in mdx mice undergoing 6–12 weeks of treadmill running; in parallel ex vivo studies show that exercise increases the resistance to eccentric contraction in wildtype (wt) extensor digitorum longus (EDL) muscles, while the adaptation is not observed in mdx ones, which remain weaker than controls. For clarifying the mechanism involved in the different exercise adaptations of the two genotypes, we focused on chloride channel conductance (gCl), crucial for excitation–contraction coupling and myofiber-type profile. A decrease in gCl is a typical hallmark of mdx diaphragm (DIA) myofibers, while it occurs in EDL ones only as a consequence of exercise. RT-PCR experiments show a 30–35% reduction of CIC-1 mRNA in both DIA and EDL of mdx mice, irrespective to the exercise regimen. In parallel, genes involved in slow-oxidative phenotype such as type 1 myosin heavy chain, peroxisome proliferator-activated receptor γ coactivator 1α and sirtuin-1 are more expressed in both EDL and gastrocnemius muscles of non-exercised mdx mice vs. wt ones. The exercise-selective alteration of gCl in mdx EDL muscle suggests post-transcriptional mechanisms of channel modulation. We investigated the role of mechanosensitive mediators of inflammation and oxidative stress, such as Angiotensin II (Ang-II). The application of Ang-II to wt EDL fibers reduces gCl in a concentration-dependent manner (IC50 = 60 nM). The effect is mediated by activation of the AT1-receptor and inhibited by chelerythrine, a protein kinase C-inhibitor, N-acetylcysteine and apocynin, an inhibitor of NADPH-oxidase. Then, transcriptional and post-transcriptional changes may occur in mechano-sensitive targets in mdx muscles in relation to metabolism, inflammation and oxidative stress (supported by DPP/ NL).

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