

P3.48**Losartan improves muscle strength and ameliorates fibrosis in the dy2J/dy2J mouse model of merosin deficient congenital muscular dystrophy**

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Merosin deficient congenital muscular dystrophy (MDC1A) is the most common form of the congenital muscular dystrophies. In contrast to healthy muscle, dystrophic muscle is characterized by failure of regeneration and abundant proliferation of connective tissue with progressive loss of muscle function. Previous studies have shown that TGFbeta pathway is involved in enhancing fibrosis in muscular dystrophy. Losartan, an angiotensin 2 type 1 receptor antagonist, a medication most commonly used for treatment of hypertension, has anti-fibrotic properties by TGFbeta pathway inhibition. We evaluated the effect of 12 weeks oral Losartan treatment on fore and hind limb muscle strength, TGFbeta serum level, quantitative Sirius red staining of muscle fibrosis, and fibrosis (fibronectin & vimentin) and regeneration (myogenin & myo-D) markers in the dy2J/dy2J mouse model of merosin deficient congenital muscular dystrophy. Losartan treatment was associated with significant improvement in both fore and hind limb muscle strength. Significant quantitative reduction in muscle fibrosis was demonstrated on Sirius red staining associated with reduced expression of fibronectin & vimentin fibrosis markers and decreased serum TGFbeta level. While myo-D remained unchanged, the level of myogenin regeneration marker significantly decreased in the treated mice. We conclude that Losartan treatment was associated with prominent and statistically significant histological and clinical improvement in the dy2J/dy2J mouse model of congenital muscular dystrophy. This commercially available medication with a low side effect profile should be considered as a potential candidate for clinical trials in children with merosin deficient congenital muscular dystrophy.

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P3.49**The soy isoflavone genistein promotes muscle regeneration and function acting on cell cycle and apoptosis in mdx mice**

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Soy isoflavones have been reported to have antioxidant bioactivities and to inhibit several proinflammatory pathways known to be involved in Duchenne muscular dystrophy (DMD) pathogenesis. Moreover genistein has been shown to enhance cell proliferation promoting G1/S phase transition through the induction of cyclin D1 in different in vitro experimental models. The exhaustion of muscle regeneration in DMD muscle is one of the main targets of current therapeutic approaches. We tested whether genistein could have a beneficial effect on muscle function, morphology and biochemical pattern in mdx mice.

Five-week old mdx mice received for 5 weeks genistein (2 mg/kg i.p. daily) or vehicle. Genistein treatment (1) increased forelimb strength ($p < 0.05$) and strength normalized to weight ($p < 0.05$); (2) reduced serum creatine-kinase levels ($p < 0.01$); (3) increased cyclin D1 expression ($p < 0.05$); (4) showed an antiapoptotic effect,

modulating the expression of BAX and Bcl-2 ($p < 0.05$); (5) reduced muscle necrosis ($p < 0.01$) and enhanced regeneration ($p < 0.05$) with an augmented number of myogenin-positive satellite cells and myonuclei.

Our results suggest that this isoflavone might have a beneficial effect on muscle function and morphology in mdx mice promoting G1/S phase transition in muscle cell and inhibiting apoptosis. Further studies are needed to investigate the biochemical substrates of such encouraging preliminary results taking into account that this supplement could be easily introduced in the daily diet of patients with DMD.

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P3.50**Therapeutic drug screen using dystrophin deficient zebrafish**

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Zebrafish animal models of human diseases are powerful tools for screening a large number of chemical compounds for therapeutic efficiency. The zebrafish sapje mutant and sapje like (*sapc/100*) are excellent models of human Duchenne Muscular Dystrophy (DMD) and each shows disturbed muscle structure and a severe reduction of birefringence (the ability to refract polarized light). The birefringence is a result of dorsal skeletal muscle deterioration and weakness that ultimately results in lethality of the most sapje mutants at 10 dpf. However, a few dystrophin deficient fish can survive over 30 days.

To identify potential therapeutic chemicals for treating DMD, we undertook a chemical screen of a small, commercial molecular library using the sapje fish. Embryos from mating heterozygous sapje fish were cultured in fish water containing pools of the chemical library. To examine the effects of chemicals for the recovery of the muscle phenotypes, birefringence assays were examined at 4 dpf. Fish treated with some pooled chemicals in the chemical library that were genotypically confirmed as sapje mutants showed no defects in birefringence compared to the control, suggesting that we may have successfully identified compounds that are effective for the recovery of phenotypes caused by a dystrophin gene mutation. Individual chemical tests indicated that some chemicals in the small molecular library are capable of extending the lifespan of the embryos of sapje fish and can reduce muscle weakness. The candidate chemicals were also effective in the second allele of dystrophin deficient zebrafish, sapje like (*sapc/100*).

Our results of this screen using the zebrafish model of DMD may lead to new candidate molecules and pathways towards better therapeutic strategies for DMD.

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P3.51**Results of a Phase 2b, dose-ranging study of ataluren (PTC124®) in nonsense mutation Duchenne/Becker muscular dystrophy (nmDBMD)**

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