DMD is a progressive myopathy with a reduced life expectancy. Gene replacement for the dystrophin gene is only about to evolve. Standard therapy with glucocorticosteroids is limited due to side effects. Immunomodulation by immunoglobulin G (IgG) could be an alternative. Twenty one day old mdx mice received two intraperitoneal injections of human IgG at a dose of 2 g/kg or an equal amount of human albumin or saline as controls every 4 weeks. Mice were housed in separate cages with a running wheel that was connected to a computer and continuously recorded all parameters. Bodyweight and forelimb grip-strength were measured weekly. After 8 weeks, animals were sacrificed and blood, diaphragm and lower limb muscles were removed for quantitative PCR, histological analysis and ex vivo muscle contraction tests. Treatment of mdx mice with IgG compared to controls improved voluntary running: the maximum velocity was enhanced and the total running time as well as the distance were significantly increased. Eight weeks after the first injection, ex vivo muscle contraction assessment displayed significantly reduced fatigability in mice treated with IgG compared to controls. Upon IgG compared to controls, mRNA expression levels of relevant inflammatory markers such as transforming growth factor (TGF)-β, chemokine CCL-2 and secreted phosphoprotein 1 were reduced in the diaphragm and skeletal muscle. IgG significantly ameliorated myopathic changes including the number of fibers with internalized nuclei. Detection of macrophages and T-cells was performed by iFluorescence microscopy and immunohistochemistry. Bodyweight, grip strength and CK levels in the blood did not display significant changes between the treatment groups. Collectively, treatment with human IgG profoundly improves the running performance of mdx mice and reduces myopathic changes in the muscle and of IgG in serum and muscle was determined by ELISA.

In dystrophic muscles, fibrosis is a pathological feature observed in heart and diaphragm of patients with Duchenne muscular dystrophy and in mdx mice. We investigated the effects of suramin, a transforming growth factor beta 1 blocker, on cardiac muscle of aged mdx mice. Mdx mice (n = 17; 8 months old) received intraperitoneal injections of suramin (60 mg/kg body weight), twice a week for 3 months. Control mdx mice (n = 12; 8 months old) were injected with saline. Suramin decreased heart total fibrosis (6.0 ± 1% in control vs 2.8 ± 1.5% in suramin-mdx, p < 0.05) and inflammation (12.0 ± 9.5% in control vs 3.0 ± 2.8% in suramin-mdx, p < 0.05). Cardiac functional analysis (electrocardiogram) showed that suramin ameliorated the abnormal S and R waves amplitudes seen in control mdx mice, leading to a 3-fold increase in the S/R amplitude ratio, possibly related to its anti-fibrotic effect. We further evaluated potential effects of suramin on metalloproteinases and components of the dystrophin-glycoprotein complex in the diaphragm of the aged mdx mice. Suramin reduced by 30% metalloproteinase-9 activity (zymography) and immunoblotting showed that suramin caused a 50% increase of b-dystroglycan. In addition, suramin prevented the loss of diaphragm muscle force over time in in vitro diaphragm strip preparations, possibly due to decreased fibrosis (11.6 ± 3.2% in control vs 8.0 ± 5.4% in suramin-mdx, p < 0.05; Masson’s trichrome). Urine total protein content was similar in control and suramin-mdx mice (0.3 g/L). Most urine samples were negative for ketones. These results indicate that suramin exerts a positive effect during later stages of the disease by improving the histopathological features of cardiac muscles of aged mdx mice. In skeletal muscle, potential effects of suramin on the balance of metalloproteinases may help maintain key components of the dystrophin-glycoprotein complex.

Ectopic calcification (EC) is often found in mdx mouse skeletal muscle. We have been studying the mechanism of EC in mdx mice. We previously reported that EC is the deposition of hydroxyapatite, a calcium phosphate. In vitro, C2C12 cells cultured in a high-phosphate medium shifted from myogenesis to osteogenesis. In present study, we discovered the relationship between dietary phosphate and the deposition of EC in mdx mice. We fed diets with different amounts of phosphate (a high-phosphate diet; 2.0% P, a moderate-phosphate diet; 1.0% P, a low-phosphate diet; 0.7% P) to mdx mice and control mice (B10) from weaning to 90 days old. Mdx mice on a high-phosphate diet increased EC in skeletal muscle especially in lower limbs and diaphragm, and the calcified regions had been extensive by age. Serum phosphate and calcium levels of these mdx mice were significantly higher than that of B10 mice on the same diet and mdx mice on the other diets. This result indicated that mdx mice fed a high-phosphate diet were associated with hyperphosphatemia and developed kidney failure. In addition, isometric contraction of gastrocnemius and soleus muscles of these mdx mice was weakened. Mdx mice on a low-phosphate diet decreased EC in lower limbs, and the deposits were not developed in diaphragm. Also, serum phosphate and calcium levels of mdx mice on this diet were similar with those of B10 mice on the same diet. These findings suggest that dietary phosphate intake and unusual muscle regeneration/ degeneration cycles of mdx mouse play important roles in the production of EC and muscle function. It is important that developing healthy eating habits could be a beneficial complementary therapy for patients of Duchenne muscular dystrophy and improve their quality of life.
to cellular constituents such as protein, lipids and DNA. However, ROS have other consequences in tissue, including affecting protein function through the reversible oxidation of protein thiols. Multiple protein targets have been characterised in cell culture studies of oxidative stress, and these have the potential to disrupt cell function through effects on processes including muscle contraction, immune function and molecular signalling. We have measured the level of protein thiol oxidation in skeletal muscles from the mdx mouse model of DMD and found that protein thiol oxidation was higher in dystrophic myofibres. Proteomic analysis of mdx myofibres shows multiple proteins affected by thiol oxidation. The increase in protein thiol oxidation may account for the general lack of success of anti-oxidant clinical trials since many of the agents tested do not target protein thiol oxidation. To test this hypothesis, the thiol containing antioxidant N-acetylcysteine (NAC) was used to treat mdx mice in vivo. NAC decreased protein thiol oxidation and prevented exercised induced myofibre damage (measured by blood creatine kinase and myofibre necrosis) after a single 30 min bout of treadmill exercise. Notably, NAC did not affect irreversible damage as measured by damage to proteins (protein carbonyls) or membranes (malondialdehyde). This study highlights the potential significance of protein thiol oxidation in dystrophopathy and provides a basis for potential clinical translation of thiol containing antioxidants such as NAC for treatment of muscular dystrophies.

Inhibition of signalling via the activin receptor IIB (ActRIIB) is considered a promising therapeutic strategy for Duchenne muscular dystrophy (DMD). A soluble form of activin receptor IIB (ActRIIB fused to the Fc portion of IgG=ActRIIB-Fc) blocks ActRIIB signalling by capturing several ligands of the TGF-beta family of growth factors such as myostatin, which stimulates muscle growth. We treated adult nondystrophic mice (NOD-SCID) and the DMD mouse model mdx with this soluble receptor. Treatment of non-dystrophic mice with rodent specific sActRIIB-Fc strongly stimulated muscle growth and tetanic force, and importantly, there was no increased fatigability. However, treatment of dystrophin deficient mdx mice with sActRIIB-Fc had much lower positive effects on muscle mass and function compared to non-dystrophic mice. Combined treatment with sActRIIB-Fc and AAV-U7-mediated exon skipping to restore dystrophin expression did not increase the effect of sActRIIB-Fc on mdx muscle. We furthermore performed a placebo-controlled and blinded preclinical study on the canine model of DMD and treated 2.5 months old GRMD dogs for 4 months with canine specific sActRIIB-Fc. Results of this preclinical study will be presented.

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The role of activin receptor IIB signalling on skeletal muscle and the possible therapeutic implication for Duchenne muscular dystrophy

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Progressive muscle wasting in Duchenne muscular dystrophy is a consequence of cycles of degeneration-regeneration in parallel with chronic inflammation and oxidative stress, leading to satellite cell senescence. Small molecules enhancing regeneration efficiency may prove beneficial. Pentoxifylline (PTX), an aspecific phosphodiesterase (PDE) enzyme inhibitor, ameliorates disease-related indices and increases muscle area in active regeneration in dystrophic mdx mouse (Burdi et al., J. Appl. Physiol., 2009). No clear data are available concerning the role of cyclic nucleotides and PDE enzymes in satellite cell activation and myogenesis. We therefore evaluated the effects of PTX on C2C12 myoblasts. Preliminary results show that PTX (3–30 μM) exerts a modest but significant concentration-dependent stimulation of Cyclic GMP (cGMP) appearance in 20% serum growth medium (GM). Forty eight hours incubation enhanced cell number by 10% (3/48) and 50% (4/48) at 10 and 30 μM, respectively. This was confirmed in a BrdU proliferation assay; 2 h drug incubation induced a significant 30% increase in cell proliferation at 100 μM (3/15). Both cAMP and cGMP appear to stimulate Cyclic GMP. Moreover, acute PTX reduces H2O2 induced cell death, although the protection is lower than that of the classical anti-oxidant N-acetylcysteine (1–20 mM). Due to the lack of data about PDE isoforms expressed in myoblasts, we performed preliminary PCR experiments (n = 3) focusing on PDE5a1 and PDE4d1. The results suggest greater expression of PDE5a1 vs PDE4d1 in the proliferative phase. PDE5a1 expression was maintained at GM level during differentiation, while PDE4d1 expression peaked at 24 h, declining.

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Glatiramer acetate (Copaxone) decreases macrophage infiltration and increases muscle strength in mdx mouse model of Duchenne muscular dystrophy


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Glatiramer acetate (GA) is an extensively used approved drug for multiple sclerosis. Although the exact mechanism of action of GA is unknown, it is involved in immune modulation in various different pathways and in outside the nervous system. In previous studies we showed that GA improves hind limb muscle strength and motor performance in the dy2J/dy2J mouse model for MDC1A. In addition it increases the expression of regeneration transcription factors MyoD and myogenin, and attenuates the fibrosis markers vimentin and fibronectin. In the current study we evaluated the effect of GA in the mdx mouse model for Duchenne muscular dystrophy (DMD), the most common muscular dystrophy in childhood. In early stages of the disease following treatment from 4 to 8 weeks of age, GA significantly decreased macrophage infiltration in the diaphragm in sedentary mdx. T cell proliferation remained unchanged. Seven weeks treatment, starting at 8 weeks of age, resulted in significantly increased fore and hind limb muscle strength (p < 0.01, p = 0.0001 respectively) in exercised mdx mice. Furthermore, increased skeletal muscle regeneration was suggested by increased central nucleation in the gastrocnemius muscle. We conclude that increased muscle strength and amelioration of inflammation in the mdx mouse support previously results of beneficial effect of GA treatment in the dy2J/dy2J mouse. Thus, GA could be considered as a potential therapeutic agent for congenital and Duchenne muscular dystrophy.

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