P4.12

AMPK and PPAR-delta agonists show beneficial effects in the mdx mouse model

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Mitochondria can sense signals linked to variations in energy demand to regulate nuclear gene expression. This retrograde signaling pathway is presumed to be involved in the regulation of myoblast proliferation/differentiation. Duchenne muscular dystrophy is a genetic disease inducing a severe muscle wasting characterized by rounds of degeneration and regeneration cycles. Enhancing mitochondria activity, in healthy mice, is known to increase muscle function and inhibit muscle wasting. The aim of the study was to determine whether an increase in mitochondrial activity using drugs that activate AMPK and PPAR-delta pathways contributes to improved muscle function in MDX mice. Twelve-weeks-old MDX mice were treated with two different metabolic remodeling agents (GW501516 and AICAR) separately or as a combination for 4 weeks. We found a gain in body and muscle weights in all treated mice. Histological analysis of the EDL muscle demonstrated a decrease in inflammation, number of fibers with central nuclei and an increase of total peripheral nuclei. Further, treated mice have significantly fewer activated satellite cells and regenerating fibers, along with an inhibition FoXO signaling (a marker of protein degradation), indicating a better regulation of myogenesis and inhibition of muscle wasting. Overall treatments showed significant improvements in overall behavioral activity, gain in forelimb and hindlimb grip strength of mdx mice. Our findings suggest that triggering mitochondrial activity provide beneficial effects of exercise to dystrophin deficient skeletal muscle. This approach may be useful to improve quality of life especially for non-ambulatory Duchenne muscular dystrophy patients.

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P4.13

Effects of green tea polyphenols and prednisolone in the mdx[5Cv] dystrophic mouse: Improvement of motor performance and calcium handling

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Oxidative stress and excessive calcium influx are thought to contribute to the pathogenesis of Duchenne muscular dystrophy. Indeed, we have previously shown that dietary interventions with powerful antioxidants such as green tea polyphenols (GTP) and EGCG (the major GTP component) improved muscle structure and function of the mdx[5Cv] mouse, a model for DMD. As clinical trials are being conducted with EGCG on DMD patients, we report additional effects of GTP and EGCG on the dystrophic mouse. Three-week old mice were given for ~6 weeks a chow enriched with GTP, EGCG or pentoxifylline (PTX), a TNFα release inhibitor used as a positive control. Prednisolone (PDN) was also tested alone or in combination with EGCG. All the interventions ameliorated spontaneous locomotor activity and performance in a wheel running assay, and decreased plasma creatine kinase levels (a marker of muscle membrane fragility). We measured the influx of calcium into muscle fibres isolated from FDB muscles and loaded with the calcium probe Fura-2. Treatment of the mice with GTP, EGCG, PTX, but not PDN reduced by up to two-thirds the excessive calcium influx in dystrophic muscle fibres in resting conditions. Acute exposure to these agents had no effect. Similar findings were obtained with diaphragm strips in influx experiments using a Ca[2+] radioisotope. The expression levels of candidate calcium channels and calcium binding proteins are being determined. Our findings suggest that GTP, EGCG, and PTX act through genome-dependent mechanisms to decrease the expression and/or the activity of calcium channels overactive in dystrophic cells. This likely contributes to the overall improvement of motor function on the awaken animal. Overall, PDN was less potent than EGCG. In some endpoints, PDN abolished the beneficial effects afforded by EGCG when given in combination. These findings may have a clinical impact regarding EGCG treatment in DMD patients treated with PDN.

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P4.14

Investigating the role of calcium-independent phospholipase $A_2\beta$ in store-operated calcium entry in mdx muscle

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Duchenne muscular dystrophy (DMD) is caused by lack of dystrophin. Characteristic signs of dystrophic muscles are membrane fragility and abnormally elevated levels of intracellular calcium, detrimental to the cells. Increased activity of store-operated calcium channels (SOC) has been proposed as a possible mechanism for the enhanced calcium concentration. Studies of SOC have indicated the enzyme calcium-independent phospholipase $A_2\beta$ ($iPLA_2\beta$) as an important mediator of calcium influx. We have previously shown that SOC entry in FDB fibres isolated from mdx mice is reduced in the presence of bromoenol lactone (BEL), a suicide inhibitor of $iPLA_2$. It is also of interest to note that PLA_2. Activity is elevated in DMD patients. The aim of this work has been to further investigate the involvement of $iPLA_2\beta$ in store-operated calcium entry in dystrophic muscles using mdx myotubes and FDB fibres. PLA_2 activity has been assessed using either PED6, a fluorescent probe, or by measuring $^{[3]}$H-labelled arachidonic acid release. The influence of PLA_2 activity on calcium signalling has been studied using Fura-2 and $^{[45]}Ca^{[2+]}$. Both pharmacological tools and down-regulation have been employed to deduce the contribution of $iPLA_2\beta$ to store-operated calcium entry. Results showed that PLA_2 activity could be substantially inhibited by both R- and S-BEL, selective for $iPLA_2\gamma$ and $iPLA_2\beta$ respectively, but no significant difference could be noted between the two enantiomers. Calcium influx induced by thapsigargin was observed to decrease markedly in dystrophic myotubes preincubated with BEL. Suppression of $iPLA_2\beta$ was successful as assayed by quantitative PCR and the PED6 assay and will serve to further highlight the importance of the enzyme in store-operated calcium entry.

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P4.15

Tamoxifen, an estrogen receptor modulator, is extremely potent on dystrophic (mdx^{5Cv}) mice

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We evaluated tamoxifen (TAM), a selective estrogen receptor modulator (SERM), used to treat certain breast cancers, on the mdx^{5Cv} dystrophic mouse, a model for Duchenne muscular dystrophy (DMD). We found that TAM (10 mg/kg/day, p.o.) given for 15 months markedly enhanced muscle force and resistance to fatigue, and decreased fibrosis of the diaphragm and plasma creatine kinase levels. The triceps of TAM-treated animals contracted and relaxed significantly slower than those of normal or dystrophic mice. This was supported by the increased proportion of oxidative fibres in certain muscles and a shift towards slow-fibre specific protein isoforms. We then evaluated the effects of TAM (0.1–10 mg/kg/day) and raloxifene (RAL, 10 mg/kg/day), another

SERM, given for 2 months to 6-month-old mdx^{5Cv} mice. Both TAM and RAL increased the spontaneous locomotor activity to near-normal values and caused a marked decrease in plasma creatine kinase activity. Overall, RAL was much less potent than TAM. TAM-treated dystrophic mice ameliorated their performance at a weekly grid test, reaching the scores of normal mice within about four weeks. In the triceps surae muscle, TAM fully normalized the phasic tension and corrected 65% of the tetanic tension deficit. TAM treatment conferred ~30% more resistance to fatigue as compared to untreated dystrophic mice. TAM also prevented muscle fibre damage in the diaphragm: The proportion of Evans blue permeable fibres decreased ~3-fold (from ~20% to ~7%) in the diaphragm of TAM-treated mice compared to untreated ones. In most instances, TAM effects were still evident at the lowest dose tested, suggesting the involvement of high affinity estrogen receptors in TAM effects. Importantly, we report here for the first time that estrogen receptors alpha and beta were both over-expressed several-folds in dystrophic muscle, possibly accounting for the exceptional responsiveness of dystrophic mice to TAM.

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P4.16

Evaluation of potential efficacy of GLPG0492, a novel selective androgen receptor modulator, in the exercised-mdx mouse model: Comparison with α-methyl prednisolone and nandrolone

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Anabolic drugs may counteract muscle wasting and dysfunction in Duchenne Muscular Dystrophy (DMD); however androgen steroids are controversial due to unwanted side effects. GLPG0492 is a new non-steroidal selective androgen receptor modulator (SARM), under development for cachexia. GLPG0492 (30 mg/kg, 6 day a week s.c., for 4 weeks) has been compared to the gold standard α-methylprednisolone (PDN; 1 mg/kg, i.p.) and the steroid nandrolone (NANDR, 5 mg/kg, s.c.), in the exercised-mdx mouse model of DMD (De Luca et al., Neurobiol. Dis., 2008). Similar to PDN and NANDR, GLPG0492 significantly increased mouse strength, with maximal forelimb grip strength values of 0.168 ± 0.006 kg ($n = 6$) vs. 0.142 ± 0.006 kg ($n = 7$; $p < 0.01$) for untreated mice. Importantly, in acute exhaustion tests, a surrogate of the clinical 6 min walking test used in patients, GLPG0492 preserved running performance over 4 weeks, whilst vehicle- or comparator-treated animals showed a significant increase in fatigue (30–50%). Ex vivo, all drugs showed a modest but statistically significant increase of tetanic tension in diaphragm with little, if any, effect on extensor digitorum longus (EDL). However, for EDL a significant drug-related improvement of chloride conductance and mechanical threshold, two electrophysiological disease biomarkers, was observed. Histologically, a decrease in non-muscular tissue area was observed in the diaphragm of GLPG0492-treated mice which was higher than that found with NANDR, while minor effects were seen with both compounds on gastrocnemius muscle. Preliminary results of a time and dose-dependent study (0.3–30 mg/kg s.c. up to 12 weeks) with GLPG0492, confirmed the effect on mdx mouse strength and resistance to fatigue and showed significant efficacy of lower drug doses on in vivo and ex vivo functional parameters. The results support further investigations of GLPG0492 as a potential treatment for muscular dystrophy (supported-Charley's Fund and Nash Avery Foundation, USA).

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P4.17

Manganese enhanced MRI (MEMRI) as an outcome measure for cardiac function in the mdx mouse

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Elevated intracellular calcium is thought to play a pivotal role in the development of muscular dystrophy. We have previously shown that at baseline, *Sgcd−/−*and mdx mice (models of LGMD2F and DMD, respectively) show elevated manganese 8(Mn^{2+}) enhancement compared to C57Bl10 controls, demonstrating for the first time in vivo that intracellular calcium is indeed elevated in these animals. We wished to investigate the functional relevance of this by examining Mn^{2+} enhancement in *Sgcd−/−*and mdx following drug treatments, which we have previously shown to modulate cardiac function. We have shown that metoprolol, a β1-selective adrenergic blocker, has divergent effects on cardiac function in mdx and *Sgcd−/−*. In addition, we have shown that the angiotensin-converting enzyme (ACE) inhibitor captopril has beneficial effects on function for mdx while enalapril, another ACE inhibitor is known to improve cardiac function in *Sgcd−/−*mice. We hypothesised that drugs that improve cardiac function would also normalise Mn^{2+} enhancement levels in heart. *Sgcd−/−*and mdx mice were treated from a presymptomatic age for two months with a clinically relevant dose of either captopril or metoprolol. Animals subsequently underwent MEMRI and their tissues were taken for histological analyses. Here we discuss the usefulness of MEMRI as an outcome measure for preclinical studies in mouse models of muscular dystrophy.

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P4.18

Prednisolone treatment does not influence antisense-mediated exon skipping in mdx mouse

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In Duchenne Muscular Dystrophy (DMD) dystrophin deficiency, leading to progressive muscular degeneration, is caused by frame-shifting mutations in the *DMD* gene. Antisense oligonucleotides (AONs) aim to restore the reading frame by skipping of the targeted exon(s), thereby allowing a slightly shorter, but largely functional protein to be formed, as is found in the much milder Becker Muscular Dystrophy (BMD). These are currently investigated in early clinical trials. Since most of the participating patients are treated symptomatically with corticosteroids (mainly prednisolone) and these stabilize the muscle fibers by, among other things, reducing inflammation, this might affect the uptake/efficiency of AONs. Therefore we investigated the effect of prednisolone on AON efficiency in cultured muscle cells and the *mdx* mouse model (on intramuscular and systemic AON injections). Both *in vitro* and *in vivo* AON uptake, skip efficiency and biomarker expression were comparable between saline and prednisolone-treated cells and mice. Furthermore low levels of dystrophin were detectable in all AON-treated mice. Western Blot analyses indicated slightly increased levels in prednisolone-treated mice, which might be explained by a better muscle condition. Overall these results show that the use of prednisolone forms no barrier to participate in clinical trials with AONs.

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