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 of Fxo 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.

doi:10.1016/j.nmd.2011.06.977

**P4.14**

**Investigating the role of calcium-independent phospholipase A2b in store-operated calcium entry in mdx muscle**

A.K. Jacobson, O. Petermann, U.T. Ruegg

Department of Pharmacology, School of Pharmaceutical Sciences, University of Geneva, Geneva, Switzerland

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 A2b (iPLA2b) 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 bromoethanol lactone (BEL), a suicide inhibitor of iPLA2. It is also of interest to note that PLA2b Activity is elevated in DMD patients. The aim of this work has been to evaluate the involvement of iPLA2b in store-operated calcium entry in dystrophic muscles using mdx myotubes and FDB fibres. PLA2b activity has been assessed using either PED6, a fluorescent probe, or by measuring [3H]-labelled arachidonic acid release. The influence of PLA2b activity on calcium signalling has been studied using Fura-2 and [45]Ca²⁺. Both pharmacological tools and down-regulation have been employed to deduce the contribution of iPLA2b to store-operated calcium entry. Results showed that PLA2b activity could be substantially inhibited by both R- and S-BEL, selective for iPLA2b and iPLA2b 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 iPLA2b 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.

doi:10.1016/j.nmd.2011.06.979

**P4.15**

**Tamoxifen, an estrogen receptor modulator, is extremely potent on dystrophic (mdx5Cv) mice**


University of Geneva, Department of Pharmacology, Geneva, Switzerland

We evaluated tamoxifen (TAM), a selective estrogen receptor modulator (SERM), used to treat certain breast cancers, on the mdx5Cv 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

---

**P4.12**

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

Y.E. Jahnke, J.H. Van Der Meulen, H.K. Johnston, T. Partridge, E.P. Hoffman, K. Nagaraju

Children’s National Medical Center, Genetic Medicine, Washington DC, United States

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 Duchenne muscular dystrophy (DMD). As clinical trials are being conducted with EGCG on non-ambulatory Duchenne muscular dystrophy patients.

We evaluated tamoxifen (TAM), a selective estrogen receptor modulator (SERM), used to treat certain breast cancers, on the mdx5Cv 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
Evaluation of potential efficacy of GLPG0492, a novel selective androgen receptor modulator, in the exercised-mdx mouse model: Comparison with α-methylprednisolone and nandrolone

A. Cozzoli, R.F. Capogrosso, V. Sblendorio, C. Jaggerschmidt, F. Namour, A. De Luca

Abstracts / Neuromuscular Disorders 21 (2011) 639–751

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 \( Mn^{2+} \) enhancement compared to C57B110 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 moderate 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.

doi:10.1016/j.nmd.2011.06.982

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


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.

doi:10.1016/j.nmd.2011.06.983