epidermal junction in skin. Here we report for the first time that epidermal melanocytes express dystrophin. Dystrophin full-length muscle isoform (mDp427) is clearly detectable in skin sections at RNA analysis. By immuno-histochemistry, dystrophin is selectively expressed at the basal layer of melanocytes where it co-localizes with basement membrane (BM) components. Dystrophin is absent in the epidermis of DMD patients, while dystroglycans and BM components were normally expressed. Interestingly, cultured DMD melanocytes display decreased adhesive capacity with respect to controls. Moreover, ultrastructural analysis of skin from DMD patients reveals melanocytes miss-localization, morphological nuclear heterogeneity and degenerative changes. Our study suggest that epidermal dystrophin acts in stabilizing melanocytes adhesion to the BM and that this function is impaired in DMD patients. Considering that melanocytes cultures can be easily obtained by conventional skin biopsy, they may represent a feasible and reliable cellular model for studying and monitoring dystrophinopathies.

doi:10.1016/j.nmd.2011.06.788

P1.29
SOC, SAC, iPLA2 and NOX involvement in abnormal calcium handling and muscle function in isolated fast and slow dystrophic muscles
H.M. Ismail a, V. Dufour b, O.M. Dorchies a, U.T. Ruegg a
a University of Geneva, Pharmacology, Geneva, Switzerland; b University of Geneva, Geneva, Switzerland

A number of studies have reported chronic elevation in intracellular Ca2+ concentration in skeletal muscle fibers or in cultured myotubes from mdx mice, a model for Duchenne muscle dystrophy. Two of the pathways that might be involved in the Ca2+ overload are store operated channels (SOC) and stretch activated channels (SAC). Earlier results suggest that both channels are controlled, at least partly, by the Ca2+-independent form of phospholipase A2 (iPLA2). Several groups also reported that the increment in intracellular Ca2+ concentration modulates reactive oxygen species (ROS) production and the increased ROS reciprocally regulates these channels. NADPH oxidase (NOX) has been implicated to be a major source of ROS. In the present study, we investigated the effect of various inhibitors of SOC, SAC and NOX on the activity of iPLA2, Ca2+ handling and ROS production in EDL-MDX-2 myotubes. We also examined the effects of these inhibitors on force production, fatigue characteristics and recovery capabilities in isolated fast and slow twitch muscles under isometric and eccentric conditions. Preliminary data showed reduced fatigue and increased recovery capabilities in isolated fast and slow twitch muscles under isometric and eccentric conditions. Preliminary data showed reduced fatigue and increased recovery capabilities in isolated fast and slow twitch muscles under isometric and eccentric conditions. A general alteration in dystrophic muscles. Therefore, the pharmacologic inhibition of sarcoplasmic reticulum Ca2+ leak is an additional therapeutic strategy for which the GRMD dog could help to demonstrate its clinical relevance.

doi:10.1016/j.nmd.2011.06.790

OUTCOME MEASURES: POSTER PRESENTATIONS

P1.31
Outcome measures in animal models of mild spinal muscular atrophy and duchenne muscular dystrophy: Introducing a novel standardized physical activity paradigm and a proprietary in vivo behavior screening platform (SmartCube) in adult rodents
PsychoGenics, Neurodegeneration, Tarrytown, United States

Fatigability, is a common symptom observed in Spinal Muscular Atrophy (SMA) and Duchenne Muscular Dystrophy (DMD) patients and shared by almost all other neuromuscular disorders (NMD). We raised the question whether fatigability could be integrated as an outcome measure in animal models of NMD such as SMA and DMD. For this aim, we have recently developed a novel, Standardized Physical Activity paradigm for mice and have obtained encouraging preliminary data from mouse model displaying mild form of SMA (the “C” line) and DMD (the mdx mouse). The physical challenge component of this paradigm overcomes the disadvantages of common fatigue approaches when applied in pre-clinical drug screening studies and provides a reliable objective evaluation of exercise capacity, relatively easy to administer and sensitive to fatigability-dependent changes while being resistant to adverse drug effects. This physical challenge was able to expose motor phenotypes in the mdx mice which would show no response deficiency in open field chambers. SmartCube, our proprietary in vivo behavior screening technology platform, was applied to identify behavior features in the mdx mouse model across ages and drug treatments. Animal responses were recorded via Digital videos and processed through computer segmentation algorithms allowing visualization of behavioral responses difference between drug treatments and genotype (mdx vs. control mice). SmartCube assessment provides interesting and
A primary outcome measurement for evaluating efficacy in clinical trials of Duchenne muscular dystrophy (DMD) is strength. Because of the challenges of measuring strength, the Cooperative International Neuro-muscular Research Group (CINRG) adapted a fixed system called the CINRG Quantitative Measurement System (CQMS). A non-fixed method is a Hand-Held Myometry (HHM) device, by Microfet. This study compared the intra and inter rater reliability of these two methods in order to assess which of the two quantitative muscle testing systems is more reliable for future DMD clinical trials. This study design evaluated two devices in 4 muscle groups (knee extension (KE), knee flexion (KF), elbow flexion (EF), elbow extension (EE)). Each of 2 testing days included 2 sessions of randomized order of each of the 2 devices and a different clinical evaluator (CE) on each day. Muscle group testing was in a standardized sequential order. A total of 30 children with DMD were evaluated 8 times, on each device, by a combination of 9 experienced clinical evaluators at 5 CINRG sites. A mixed effects model confirmed that fatigue was not a factor in strength assessments (p > .16 for fatigue in all muscle groups models). Inter-rater reliability was high in both devices (>.88). Intra-rater reliability showed more variation with the following ranges across all CEs: KE CQMS (.81–.99), HHM (.85–.97); KF CQMS (.72–.94), HHM (.67–.93); EE CQMS (.83–1.0), HHM (.92–.99); EF CQMS (.92–.99), HHM (.82–.99). Confidence bands around the loess regression lines showed CQMS to have less variability in the younger age group and less difference between measures in the older age group, 3/4 muscles. The study shows comparable inter-rater reliability and associated intra-rater reliability. Knee flexion had the least inter and intra-rater reliability while elbow flexion had the best reliability. These results may impact the experimental design and sample size calculations in future clinical trials.

doi:10.1016/j.nmd.2011.06.792