

subtypes. We have also identified two other classes of noncompetitive aptamers that are differentially selective to conformations of GluA2, a key AMPA receptor subunit that mediates excitotoxicity: one class uniquely inhibits the open-channel whereas the other inhibits the closed-channel conformation. To turn these aptamers into potentially useful drugs, we have now successfully generated a class of chemically modified aptamers that are biostable or resistant with ribonucleases so that these aptamers can be tested *in vivo*. Our results demonstrate the possibility of developing aptamers that have nanomolar affinity and are highly selective to both an AMPA receptor subunit and a unique receptor conformation. These aptamers are excellent water-soluble, nanomolar affinity templates for design of better inhibitors as drug candidates for a potential new ALS therapy.

doi:10.1016/j.nmd.2010.07.180

DYSTROPHINOPATHIES: PHARMACOLOGICAL APPROACHES; POSTER PRESENTATIONS

P3.39

Ca²⁺-permeable channel TRPV2 as a promising therapeutic target for muscular dystrophy and cardiomyopathy

Y. Iwata, S. Wakabayashi

National Cardiovascular Center Research Institute, Molecular Physiology, Suita, Japan

Disruption of dystrophin–glycoprotein complex caused by genetic defects of dystrophin or sarcoglycans results in muscular dystrophy and/or cardiomyopathy in human and animal models. Abnormal Ca²⁺ handling has been thought to be a key molecular event in the pathology of these diseases. However, the detailed mechanism for Ca²⁺ abnormality remains elusive. We have previously shown that the transient receptor potential cation channel TRPV2 is a principal candidate for Ca²⁺-entry pathways in dystrophic muscles. In order to determine whether TRPV2 is a crucial molecule for Ca²⁺-induced muscle damage, we developed two procedures to inhibit the endogenous TRPV2 activity, i.e., transgenic incorporation of dominant-negative TRPV2 and application of drugs inhibiting TRPV2. We found that these approaches significantly reduced the increase in basal intracellular Ca²⁺ concentration ([Ca²⁺]_i) as well as the increase in [Ca²⁺]_i induced by high Ca²⁺ and TRPV2 agonist, 2-aminoethoxy diphenyl borate, which were observed in dystrophic muscles. These findings indicate that these treatments resulted in the robust inhibition of TRPV2. Furthermore, we observed the 40–70% amelioration of impaired muscle function such as an increased number of central nuclei and fiber size variability, fibrosis, apoptosis, elevated serum creatine kinase levels, and of reduced muscle performance in dystrophic animals. Similar beneficial effects were also seen in cardiomyopathic animals. These results suggest that TRPV2 is a principal Ca²⁺-entry route leading to a sustained [Ca²⁺]_i increase and muscle degeneration, and that it is a common promising therapeutic target for muscular dystrophy and cardiomyopathy.

doi:10.1016/j.nmd.2010.07.181

P3.40

Potential role of sirtuin-1 as druggable target in muscular dystrophy: effect of a chronic resveratrol treatment on *in vivo* and *ex vivo* pathological signs of dystrophic mdx mouse

A. Cozzoli¹, R.F. Capogrosso¹, V.T. Sblendorio¹, S. Gagliardi², V. Longo³, M. Montagnani², B. Nico³, A. De Luca¹

¹ University of Bari, Dept. Pharmacobiology, Unit of Pharmacology, Bari, Italy, ² University of Bari, Dept. of Pharmacology and Human Physiology, Bari, Italy, ³ University of Bari, Dept. of Human Anatomy and Histology, Bari, Italy

Sirtuin1 (Sirt1) is a NAD⁺ dependent deacetylase modulating metabolic functions, reaction to stressors and longevity. Sirt1 also activates peroxisome proliferator-activated receptor γ coactivator 1 α (PGC-1 α), a key modulator of muscle metabolism; PGC-1 α overexpression in dystrophic mdx mice leads to milder signs of pathology and an improved function both in normal condition and after intense physical exercise (Handschin et al., Gen. Develop., 2007; Cantò et al., Nature, 2009). Then, drugs able to activate Sirt-1/PGC-1 α pathway may have positive effects in muscular dystrophy. We performed a proof-of-concept study by evaluating in treadmill-exercised mdx mice the effects of a chronic treatment with resveratrol (100 mg/kg; 6 days/week i.p. for 4–6 weeks), a known Sirt1 activator, in comparison with those of a similar treatment with α -methyl-prednisolone (PDN 1 mg/kg i.p.). *In vivo*, resveratrol and PDN similarly counteracted the exercise-induced decrease of maximal and normalized fore limb strength, while a partial amelioration of resistance to exercise was observed. *Ex vivo*, the resveratrol treatment slightly ameliorated mechanical threshold, an electrophysiological index of calcium homeostasis, but did not exert any significant effect on isometric twitch and tetanic tension of EDL muscle. However, in contrast with PDN, a significant reduction of plasma creatine kinase and lactate dehydrogenase was observed in resveratrol-treated animals. Also, resveratrol caused a 70% reduction of fibres positive to dihydroethidium (DHE), a marker of superoxide anion production, in tibialis anterior muscle. An improvement of histology profile was observed in gastrocnemius muscle, along with a slight decrease of NF- κ B positive fibres. The results suggest that resveratrol may exert protective effect in dystrophic muscles, likely by reinforcing the metabolic pathways that contrast oxidative stress (supported by Telethon-Italy and Charley's Fund).

doi:10.1016/j.nmd.2010.07.182

P3.41

In vivo studies on the effects of EGCG, a major polyphenol in green tea, on a mouse model of Duchenne muscular dystrophy

Y. Nakae, O.M. Dorchie, P.J. Stoward, U.T. Ruegg

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

At WMS2009, we reported that epigallocatechin-3-gallate (EGCG) given to young mdx mice in their diet or by subcutaneous (s.c.) injection reduces their serum CK activities and the numbers of lipofuscin (LF) granules, a marker of oxidative stress, per unit volume of diaphragm muscle. Concomitantly, the specific phasic twitch and tetanic tensions of triceps surae muscles are increased. These results suggest that EGCG limits the degeneration of mdx muscles. To extend our previous findings, we have investigated the effects of EGCG on mdx mice using additional assessment criteria. The EGCG doses used were 180 mg/kg/day in the diet and 2.9 or 5.7 mg/kg/day for s.c. injection. The mice were treated for 5 weeks beginning when they were 3-weeks-old. The integrated locomotor activities of the mice were then measured, and selected muscles removed at autopsy. The mean % area of the connective tissue in sections of EDL muscles of mdx mice given dietary EGCG was 15 \pm 3% less than in mdx controls not given EGCG. EGCG injection at both doses did not alter the amount of connective tissue in either EDL or diaphragm muscles. Dietary EGCG administration also had no significant effect on the