

**PW 25:  
Pharmacological  
therapies –  
Evaluation and animal  
models**

PW25-305	<p><b><u>THERAPEUTIC EFFICACY AND MECHANISM OF ACTION OF NITRIC OXIDE-RELEASING NON STEROIDAL ANTI-INFLAMMATORY DRUGS IN MUSCULAR DYSTROPHY</u></b></p> <p>SCIORATI C<sup>1</sup>, AZZONI E<sup>1</sup>, ONGINI E<sup>2</sup>, MONOPOLI A<sup>2</sup>, BRUNELLI S<sup>1</sup>, COSSU G<sup>1</sup>, CLEMENTI E<sup>3</sup></p> <p>(1) Stem Cell Research Institute-San Raffaele Scientific Institute, Milano, ITALY. (2) NicOx Research Institute, Bresso, ITALY. (3) E. Medea Scientific Institute and Department of Preclinical Sciences, University of Milano, Milano, ITALY.</p>
To contact the author:: emilio.clementi@unimi.it.	<p>Duchenne muscular dystrophy is a relatively common disease that affects skeletal muscle leading to progressive paralysis and death. There is currently no resolute therapy. We have developed a novel strategy based on the combination of nitric oxide (NO), which has beneficial effects in skeletal muscle, with non steroidal anti-inflammatory drugs (NSAIDs). To this end we used a new class of NO-releasing NSAIDs (NO-NSAIDs). We report the results of long term (one-year) oral treatment in the mouse model for limb girdle muscular dystrophy (<math>\alpha</math>-sarcoglycan null mice) with two such NO-NSAIDs, nitroibuprofen and nitroparacetamol. Both drugs significantly ameliorated the morphological, biochemical and functional phenotype in the absence of secondary effects, efficiently slowing down disease progression and were significantly more effective than the corticosteroid prednisolone analyzed in parallel. NO-NSAIDs acted by reducing inflammation, preventing muscle damage and preserving the number and function of satellite cells. To assess the mechanisms of NO-NSAID action we dissected the contribution of NO and NSAID activities, by analyzing the effects of the NSAID ibuprofen and of isosorbide dinitrate that release NO with a pharmacokinetic profile similar to that of NO-NSAIDs. The NO-NSAIDs were significantly more effective than either isosorbide dinitrate or ibuprofen. Of importance, the NO-NSAIDs were more effective therapeutic agents also with respect to the combination of isosorbide dinitrate and ibuprofen, suggesting that NO-NSAIDs have properties additional to NO release and NSAID activity.</p> <p>The new therapeutic strategy we propose is not selective for a subset of mutations, provides ground for immediate clinical experimentation with NO-NSAIDS, which are approved for use in humans.</p> <p>.</p>

PW25-306	<p><b>GREEN TEA POLYPHENOLS DISPLAY POTENT ANTI-FIBROTIC ACTIVITY ON PRIMARY CULTURES OF DYSTROPHIC MUSCLE CELLS</b>  DORCHIES O<sup>1</sup>, COMYN S<sup>1</sup>, RUEGG U<sup>1</sup>  (1) University of Geneva, Geneva, SWITZERLAND.</p>
<p>To contact the author::  olivier.dorchies@pharm.unige.ch.</p>	<p>Muscular dystrophies are characterized by fibrosis, a process leading to abnormal accumulation of materials of fibroblastic origin in the skeletal muscles as necrosis-regeneration cycles take place. Fibrosis results from alterations in a multifactorial balance involving cytokines and growth factors, expression of their receptors at the cells' surface, oxidative stress, production of extracellular matrix components, release of matrix-metalloproteinases (MMPs) and of their endogenous inhibitors TIMPs (tissue inhibitors of MMPs), all collectively regulating extracellular matrix turnover and fibroblast invasiveness. Fibrosis severely impairs skeletal and cardiac muscle functions both in patients suffering from Duchenne muscular dystrophy (DMD) and in the mdx mouse, the most commonly used model for DMD. Published data indicate that green tea polyphenols (GTP) exhibit anti-fibrotic properties in several cell types and tissues, and pentoxifylline (PTX), a well-known inhibitor of TNF<math>\alpha</math> release, has been proposed to reduce fibrosis in the dystrophic mdx mouse. Treatment of primary cultures of mouse muscle at the myotube stage with GTP or PTX (2.5 to 25 <math>\mu</math>M) inhibited the expression and/or the release of the fibrosis modulators TNF<math>\alpha</math>, TGF<math>\beta</math>1, and PDGF-BB. When needed, fibrosis was induced in the cultures with TNF<math>\alpha</math> or TGF<math>\beta</math>1 (30ng/mL). GTP dose-dependently decreased both basal and TNF<math>\alpha</math> or TGF<math>\beta</math>1-induced expression of <math>\alpha</math>-smooth muscle actin (<math>\alpha</math>SMA, a marker for activated fibroblasts), collagen, TGF receptor type 2, and CTGF (connective tissue growth factor, a key mediator of TGF<math>\beta</math>1 actions). As determined by zymography, the expression of pro- and active forms of MMP-2 and MMP-9 were not changed with GTP and PTX. However, the MMP-2 and MMP-9 gelatinase activity in the supernatants was significantly decreased and correlated with a marked elevation of TIMP-1 levels. Overall, PTX was less potent than GTP on these endpoints. Our results indicate that GTP profoundly alter the expression and/or the activity of proteins involved in the fibrogenic process.</p>

PW25-307	<p><b><u>OXIDATIVE INJURY OF MYOBLASTS: SEARCH FOR A PROTECTIVE EFFECT BY A GLYCOSAMINOGLYCAN MIMETIC (OTR4120)</u></b>  VAN ZOGGEL J<sup>1</sup>, ALABANESE P<sup>1</sup>, JACOBS MS<sup>1</sup>, COURTY J<sup>1</sup>, PAPY-GARCIA D<sup>1</sup>, MORIN C<sup>1</sup>, MARTELLY I<sup>1</sup>  (1) CRRET Laboratory, Université Paris-Est, Faculté de Sciences et Technologie, Paris 12, Créteil, FRANCE.</p>
To contact the author:: martelly@univ-paris12.fr.	<p>Overproduction of reactive oxygen species has been implicated in cellular defect that may lead to apoptosis. Skeletal muscle cells are frequently submitted to oxidative stress upon excessive exercise or in disease. Although cells withstand oxidative exposure by developing antioxidant defences, their resistance capacity could be sometimes overcome, resulting in cell entering apoptosis. It is therefore important to investigate mechanisms occurring in skeletal muscle cells in response to oxidative stress. Among defence mechanisms, it has been suggested that glycosaminoglycans (GAGs) could exert a protective effect. Therefore, we have explored how myoblasts react <i>in vitro</i> to oxidative stress and whether treatment with a synthetic GAG mimetic (namely OTR4120) would protect them against oxidative injury.</p> <p>An oxidative stress induced by oxygen peroxide (H<sub>2</sub>O<sub>2</sub>, 10pmol/cell, 30min) was applied on proliferating myoblasts (C2.7 cell line). Cell survival was decreased by about 70% within 24 hrs. Compared to untreated cells, oxidative stress induced apoptosis assessed either by the presence of apoptotic features of nuclei stained with diaminophenyl indol, or by the presence of subG1-DNA observed by Flow cytometry or by an increase in caspases-3, 9 and 8 activities at 48hrs. This was accompanied by a two times increase in the amount of total sulphated GAGs produced by stressed cells compared to untreated cells, without change in the overall GAG composition. However, as shown by immunocytology, these GAGs seemed to remain in the intracellular compartment instead of being addressed to the cellular membrane.</p> <p>Treatment with the GAG mimetic OTR4120 (1.0 µg/ml) added just after the oxidative stress period partially prevented such features associated with apoptosis, in part restored GAG localisation at membranes and allowed cells to differentiate after 72hrs. Possible mechanisms of action of OTR4120 are being further explored.</p> <p>These data support the working hypothesis that GAG mimetics could protect myoblasts from oxidative stress injury.</p>

PW25-308	<p><b><u>MELATONIN PREVENTS OXIDATIVE-STRESS MEDIATED MITOCHONDRIAL PERMEABILITY TRANSITION AND DEATH VIA MAINTENANCE / ENHANCEMENT OF REDUCED PYRIDINE NUCLEOTIDES AND GLUTATHIONE IN MOUSE SKELETAL MUSCLE CELLS</u></b></p> <p>HIBAOUY Y<sup>1</sup>, ROULET E<sup>1</sup>, RUEGG UT<sup>1</sup></p> <p>(1) Laboratory of Pharmacology, Geneva Lausanne School of Pharmaceutical Sciences, University of Geneva, Quai Ernest Ansermet 30, CH-1211 Geneva 4, SWITZERLAND.</p>
To contact the author:: youssef.hibaoui@pharm.unige.ch.	<p>Oxidative stress-induced mitochondrial dysfunction has been shown to play a crucial role in the pathogenesis of a wide range of diseases including muscle disorders. Protecting mitochondrial function, therefore, is vital for cells to survive. In this study, we demonstrate that melatonin, the main secretory product of the pineal gland, readily rescued mitochondria from oxidative stress-induced dysfunction and effectively prevented subsequent apoptotic/necrotic events and death in C57BL/6J myotubes. In particular, melatonin potently prevented myotube death induced by <i>tert</i>-butylhydroperoxide (t-BHP) in a concentration-dependent manner (<math>10^{-4}</math>-<math>10^{-6}</math> M). This protective effect was more potent than that of N-acetyl-L-cystein, a well known antioxidant that increases cellular pools of free-radical scavengers. Moreover, melatonin maintained plasma membrane integrity (t-BHP-induced membrane blebbing) after t-BHP exposure and prevented t-BHP-induced fissions of the long mitochondrial filaments and inhibited mitochondrial swelling that was clearly visible after t-BHP treatment. To determine if the mitochondrial protection provided by melatonin was due to the inhibition of the formation of reactive oxygen species (ROS), intracellular ROS levels were measured using fluorescence imaging. Application of t-BHP produced a rapid and significant increase in free-radical generation in myotubes. This effect was concentration dependently prevented by pretreatment of the myotubes with melatonin. Considering that t-BHP cytotoxicity was also prevented by cyclosporin A, a mitochondrial permeability transition pore (mPTP) inhibitor, we investigated the effect of melatonin on mPTP. Melatonin prevented t-BHP-induced mitochondrial depolarization and protected the pyridine nucleotides and glutathione (two regulators of mPTP opening under conditions of oxidative stress) against t-BHP-induced stress. Using isolated mitochondria, we found that melatonin (<math>10^{-8}</math>-<math>10^{-6}</math> M) desensitized the mPTP to <math>Ca^{2+}</math> and prevented t-BHP-induced mitochondrial swelling, pyridine nucleotides and glutathione oxidation, and enhanced mitochondrial function. In conclusion, our findings suggest that inhibition of the mPTP may essentially contribute to the protective effect of melatonin against oxidative stress in myotubes.</p>

PW25-309	<p><b>"BENEFICIAL" EFFECTS OF GINKGO BILOBA ON MOTOR ENDPLATES (NMJ), NEUROMUSCULAR TRANSMISSION AND MUSCLE CONTRACTILITY: THERAPEUTICAL APPROACH OF SARCOPENIA IN RAT.</b></p> <p>KOENIG H<sup>1</sup>, BAUCHÉ S<sup>2</sup>, MOLGO J<sup>3</sup>, ROUCHE A<sup>2</sup>, DE LA PORTE S<sup>3</sup>, PIGNOL B<sup>4</sup>, CHRISTEN Y<sup>5</sup>, HANTAÏ D<sup>2</sup>, KOENIG J<sup>2</sup>, KOENIG J<sup>6</sup></p> <p>(1) Institut Pierre et Marie Curie et CNRS UMR 7091, Hôpital Pitié, Paris, FRANCE. (2) Institut Pierre et Marie Curie et INSERM U582, Hôpital Salpêtrière, Paris, FRANCE. (3) CNRS UPR 9040, NBCM, Institut A Fessard, Gif sur Yvette, FRANCE. (4) IPSEN, Les Ulis, FRANCE. (5) Ipsen-Beaufour, Paris, FRANCE. (6) Université Bordeaux 2, Bordeaux, FRANCE.</p>
To contact the author:: koenig@chups.jussieu.fr.	<p>The aging process in mammals, including humans, is associated with a decline in neuromuscular function and performance. The main aspect of this decline, known as sarcopenia, is the reduction in skeletal muscle mass, related to a loss of muscle strength. As no pharmacological treatment was reported at present, we analyzed the effects of a Ginkgo biloba extract (EGb 761, IPSEN), which exhibits biochemical and pharmacological effects, mostly beneficial, in the nervous system, among other tissues. Recently, it was also shown that EGb 761 modifies the expression of many aged rat muscles genes (group of J.Mallet, CNRS UMR 7091). After a 2 months oral treatment, the 2 years old rats showed that EGb 761 had "rejuvenating" effects on aged muscles 1) the ratio muscle/rat weight increased, close to young rat values 2) muscle creatine kinase content decreased as compared to control muscles 3) the highly fragmented NMJ of aged control muscles recovered a more juvenile pattern 4) as a consequence, the axonal sprouting was reduced 4) the number of terminal Schwann cells doubled 5) the percentage of degenerating NMJ was decreased 6) in connection with these adaptative morphological responses to the treatment, EGb761 increased the evoked quantal transmitter release (Ach) and 6) as a result, the muscle contractile strength was enhanced. However, the muscle fibers diameter and the ratio of type 1 vs type 2 fibers was not modified in the treated slow and fast muscles. Similarities and differences between a slow-twitch muscle (Soleus) and a fast twitch muscle (EDL) will be emphasized.</p> <p><i>In conclusion, the muscles of rats treated with an herbal extract, Ginkgo biloba, show several "rejuvenated" patterns of neuromuscular junctions and of the major functions of muscle cell physiology, which could provide a pharmacological treatment of sarcopenia in humans.</i></p>

PW25-310	<p><b>RECOVERY OF SKELETAL MUSCLE MASS AND PHENOTYPE AFTER EXTENSIVE MUSCLE INJURY IN RATS: NO POSITIVE EFFECTS OF CREATINE SUPPLEMENTATION</b></p> <p>KOULMANN N<sup>1</sup>, CRASSOUS B<sup>1</sup>, RICHARD-BULTEAU H<sup>1</sup>, DELDICQUE L<sup>2</sup>, SERRURIER B<sup>1</sup>, PASDELOUP M<sup>1</sup>, FRANCAUX M<sup>2</sup>, BIGARD X<sup>1</sup></p> <p>(1) Département des facteurs humains, Centre de Recherches du Service de santé des Armées, La Tronche, FRANCE. (2) Département d'éducation physique et de réadaptation, Université catholique de Louvain, Louvain-la-Neuve, BELGIUM.</p>
To contact the author:: nkoulmann@crssa.net.	<p>Recent studies have shown that creatine supplementation (Cr) may enhance muscle functional capacity in patients with neuromuscular diseases, disuse atrophy or muscular dystrophies. Because it has been shown in culture cells that the fusion of myogenic satellite cells is largely enhanced while Cr is added to the culture medium during the differentiation phase, we hypothesized that Cr-supplementation may have beneficial effects during the early steps of regeneration following muscle injury, then may accelerate the recovery of both muscle mass and phenotype.</p> <p>Degeneration of left soleus muscle was induced by notexin injection in rats supplemented or not with Cr both in powder food and drink. At days 1, 3, 7, 14, 21, 28, 35 and 42 after injury, we studied in regenerated compared with contralateral intact muscles, muscle weight and protein levels of the Proliferator Cell Nuclear Antigen (PCNA) as a marker of initial cell proliferation, and expression of MRFs as markers of differentiation. We also studied the myosin heavy chain (MHC) profile and activities of citrate synthase (CS) and lactate dehydrogenase isozymes (LDH), as markers of the maturation of muscle phenotype.</p> <p>Cr-supplementation allowed to progressively recover the creatine content within regenerated muscles. However, we observed, without differences between Cr-treated and non-treated rats, that: 1) regenerated muscles did not recover weight values similar to intact muscles 42 days after injury; 2) PCNA and MRFs expression strongly and early increased in regenerated muscles; 3) the MHC profile of regenerated muscles was recovered 35 days after injury; 4) a full recovery of CS activity was observed from day 14, while the specific H-LDH activity remained lower than in intact muscles until 42 days.</p> <p>In contrast with results from <i>in vitro</i> experiments, Cr-supplementation had no beneficial effect <i>in vivo</i> on the time course of recovery of skeletal muscle mass and phenotype after notexin-induced injury.</p>

PW25-311	<p><b><u>TREATMENT WITH A SOLUBLE ACTIVIN RECEPTOR TYPE IIB ATTENUATES MUSCLE WEAKNESS IN YOUNG MDX MICE.</u></b>  LACHEY J<sup>1</sup>, PULLEN A<sup>1</sup>, WONG V<sup>1</sup>, PEARSALL RS<sup>1</sup>, SEEHRA J<sup>1</sup>  (1) Acceleron Pharma, Cambridge, USA.</p>
To contact the author:: jlachey@acceleronpharma.com.	<p>Dystrophin-deficient, or <i>mdx</i>, mice contain a mutation in the dystrophin gene and are therefore the genetic homolog of Duchenne muscular dystrophy. While the consequences of dystrophin deficiency in mice are less severe than what is seen in DMD patients, <i>mdx</i> mice exhibit a period of muscle degeneration that manifests as muscle weakness before subsequent regeneration restores strength to wild-type levels. Activin type IIB receptors (ActRIIB) are one of the receptors responsible for TGF<math>\beta</math> signaling and are capable of binding myostatin as well as other negative muscle mass regulators in the TGF<math>\beta</math> superfamily. Treatment with a soluble ActRIIB molecule blocks the ability of these ligands to signal and results in increased lean tissue mass. Here we describe the ability of a soluble ActRIIB fusion protein (RAP-031) to increase lean tissue mass and restore strength in young <i>mdx</i> mice. RAP-031 treatment significantly increases body weight in both C57BL/10 and <i>mdx</i> mice. NMR scans revealed increased lean tissue gain accompanied the higher body weights. RAP-031 treated C57BL/10 mice gained 35.2% and the RAP-031 treated <i>mdx</i> group gained 48.3% more lean tissue mass than their respective control cohorts. Further, the effect of RAP031 treatment on strength was assessed. Vehicle treated <i>mdx</i> mice grip strength scores were 15.7% lower than the vehicle C57BL/10 cohort thereby illustrating the muscle weakness associated with dystrophin deficiency. In contrast, the RAP-031 <i>mdx</i> mice improved their grip strength compared to the <i>mdx</i> vehicle group, and attained grip strength measurements which surpassed C57BL/10 vehicle mice and reached the level of the RAP-031 C57BL/10 grip strength scores (vehicle <i>mdx</i>: 0.140 <math>\pm</math> 0.01 KgF; RAP-031 <i>mdx</i>: 0.199 <math>\pm</math> 0.02 KgF; vehicle C57BL/10: 0.166 <math>\pm</math> 0.03; RAP-031 C57BL/10: 0.205 <math>\pm</math> 0.02 KgF). These data support the idea that inhibition of ActRIIB signaling could have important clinical applications in Duchenne muscular dystrophy.</p>

PW25-312	<p><b><u>CREATINE AND L-ARGININE TREATMENTS IMPROVE SARCOPLASMIC RETICULUM FUNCTION AND CONTRACTILE PERFORMANCE OF DIAPHRAGME FROM MDX MICE.</u></b></p> <p>LAFOUX A<sup>1</sup>, BERTRAND J<sup>2</sup>, GERVIER P<sup>1</sup>, HUCHET-CADIOU C<sup>1</sup></p> <p>(1) UMR CNRS 6204 "Biocatalyse, Biotechnologie et Biorégulation", Nantes, FRANCE. (2) UMR 6187 "Institut de Physiologie et Biologie Cellulaires", Poitiers, FRANCE.</p>
To contact the author:: aude.lafoux@univ-nantes.fr.	<p>In duchenne's muscular dystrophy, that is characterized by a progressive skeletal muscle fiber necrosis, the membrane-stabilizing protein dystrophin is missing, and this leads to altered total Ca<sup>2+</sup> content in muscle fibers. This study investigated the potential therapeutic benefits of pharmacological tools in reversing the Ca<sup>2+</sup> sequestration function of the sarcoplasmic reticulum and improving the functional capacity of skeletal muscle of <i>mdx</i> mice. Creatine (CrM: 2,15 mg/mL/day) and L-arginine (L-Arg: 3,75 mg/mL/day) were administered per os to male <i>mdx</i> mice (12 weeks) during 4 weeks. Then, the effectiveness of the treatment was investigated on the contractile protein properties, the sarcoplasmic reticulum Ca<sup>2+</sup> uptake and the expression of SERCA1 and calsequestrin in diaphragm muscle of <i>mdx</i> mice. The data demonstrate that after treatments, the maximal Ca<sup>2+</sup> activated tension developed by skinned fibers were increased approximately by 30% (Tmax <i>mdx</i>= 46.8 ± 4.5 mN.mm<sup>-2</sup>; CrM= 60.3 ± 5.7 mN.mm<sup>-2</sup>; L-Arg= 61.0 ± 2.8 mN.mm<sup>-2</sup>). The study of sarcoplasmic function, assessed in skinned fibers and vesicle preparations, shows that the Ca<sup>2+</sup> uptake was improved in <i>mdx</i> diaphragm muscle after both pharmacological treatments (Ca<sup>2+</sup> uptake <i>mdx</i>= 20.3 ± 1.1 nmole Ca<sup>2+</sup>.s<sup>-1</sup>.mg<sup>-1</sup>; CrM= 22.1 ± 0.6 nmole Ca<sup>2+</sup>.s<sup>-1</sup>.mg<sup>-1</sup>; L-Arg= 26.4 ± 1.1 nmole Ca<sup>2+</sup>.s<sup>-1</sup>.mg<sup>-1</sup>). Furthermore, the overall force of the musculature was tested in living animals using the grip strength test, the wire test and the rotarod. The results show that the locomotor activity was enhanced and that <i>mdx</i> mice were more resistant to fatigue. During the grip test, the force developed by the four limbs was ameliorated in <i>mdx</i> mice after both treatments (Strength <i>mdx</i>= 4.0 ± 0.1 g.g<sup>-1</sup>; CrM= 4.2 ± 0.2 g.g<sup>-1</sup>; L-Arg= 4.7 ± 0.2 g.g<sup>-1</sup>). In conclusion, the data show that creatine and L-arginine treatments significantly normalized many functional and biochemical parameters by acting on events that are related to Ca<sup>2+</sup> homeostasis.</p>

PW25-313	<p><b>TAMOXIFEN IMPROVES THE STRUCTURE AND THE FUNCTION OF SKELETAL MUSCLE IN MDX MICE</b>  DORCHIES O<sup>1</sup>, REUTENAUER J<sup>1</sup>, VUADENS O<sup>1</sup>, COMYN S<sup>1</sup>, RUEGG U<sup>1</sup>  (1) University of Geneva, Geneva, SWITZERLAND.</p>
To contact the author:: olivier.dorchies@pharm.unige.ch.	<p>Data from the literature indicate that tamoxifen (Tam), a selective estrogen receptor modulator (SERM) used in the treatment of certain types of breast cancers, displays antioxidant, anti-apoptotic and anti-fibrotic properties and stabilises biological membranes. We hypothesised that Tam might improve both the structure and the function of dystrophic muscles. When applied to primary cultures of dystrophic myotubes, Tam and 4-hydroxy-Tam (<math>10^{-8}</math> to <math>10^{-6}</math>M) were not toxic, reduced collagen content slightly, and exhibited a limited antioxidant activity against hydrogen peroxide. Mdx dystrophic mice, the most common model for Duchenne muscular dystrophy, were treated for about 15 months following weaning with 0.01% tamoxifen mixed into the food pellets (Tam intake approx. 10mg/kg/day). Tam-treated mdx mice showed a significant decrease in body weight, likely due to the important reduction in white adipose tissue compared to untreated mice. The mass of the triceps surae (composed of soleus, plantaris and gastrocnemius muscles) was lowered to normal values, while the EDL and the diaphragm showed significant hypertrophy compared with both normal and untreated dystrophic animals. Using the horizontal wire test, the Tam-treated mdx performed as well as the normal mice and 3 times better than the untreated mdx mice. Isometric muscle force of the triceps surae was recorded. Remarkably, specific phasic and tetanic twitch tensions were increased by 100% and 70%, respectively, compared with untreated mdx, resulting in values higher than those of normal animals. The rates of contraction and relaxation were much slower than in the untreated mdx, and the force-frequency curve was significantly shifted to the left, suggesting a marked change toward a slower phenotype. In the Tam-treated mdx, the resistance to repetitive tetanisations was improved by 44% and creatine kinase levels were lowered by 50% compared with the untreated mdx. On-going histological analysis suggests a normalisation of the mean fiber diameter.</p>

PW25-314	<p><b><u>BENEFICIAL EFFECT OF PENTADECAPEPTIDE BPC 157 ON DENERVATED MUSCLE IN RATS</u></b>  MIHOVIL I<sup>1</sup>, RADIC B<sup>1</sup>, BRCIC L<sup>2</sup>, BRCIC I<sup>2</sup>, DRMIC D<sup>1</sup>, VUKOJA I<sup>1</sup>, ILIC S<sup>1</sup>, BOBAN BLAGAIC A<sup>1</sup>, SEIWERTH S<sup>2</sup>, SIKIRIC P<sup>1</sup>  (1) Department of Pharmacology, University of Zagreb Medical School, Zagreb, CROATIA. (2) Institute of Pathology, University of Zagreb Medical School, Zagreb, CROATIA.</p>
To contact the author:: lbrcic@mef.hr.	<p>AIM: Since it was previously reported that pentadecapeptide BPC 157, applied without a carrier, improved the healing of transected quadriceps muscle, as well as muscle healing in rats with muscle crush injury, we believe it could also be an effective treatment for denervated muscles.</p> <p>METHODS: The left opturatorius nerve of Wistar Albino rats was transected through opturatorius canal. Animals received either 10 ug/kg of BPC 157 (GEPPPGKPADDAGLV, M.W. 1419, manufactured by Diagen, Ljubljana, Slovenia) or 5 ml/kg of 0.9% NaCl, intraperitoneally every day. Functional evaluation (muscle function index and walking pattern) and physical examination of hind limbs were performed every month. After 1 year animals were sacrificed, muscles gracilis were isolated and weighted, followed by patohistological and morphometrical analyses using ISSA software (Vamstec, Croatia)..</p> <p>RESULTS: One year after m. gracilis denervation measurements showed difference in muscle function index values, being statistically better in BPC 157 treated animals (p&lt;0.05), as well as higher muscle weight in the latter group (p&lt;0,017 vs controls), and without statistical difference of those parameters in comparison of BPC treated animals and healthy animals. BPC 157 treated animals demonstrated practically no difference in muscle fibers diameters compared to healthy animals, while control group revealed significantly shorter diameter, being only 70% of healthy animals values. Besides, cross sections of control muscles revealed many smaller muscle fibers with centralized nuclei, while morphologically there were also no significant difference between BPC 157 treated animals and healthy ones.</p> <p>CONCLUSION: Pentadecapeptide BPC 157 prevented muscle atrophy and preserved muscle function after denervation.</p>

PW25-315	<p><b>MEDICAL FOOD IN MDX MICE: GENISTEIN AND FLAVOCOXID AMELIORATE MUSCLE PATHOLOGY AND FUNCTION.</b>  MESSINA S<sup>1</sup>, MAZZEO A<sup>1</sup>, BITTO A<sup>2</sup>, AGUENNOUZ M<sup>1</sup>, MIGLIORATO A<sup>1</sup>, DE PASQUALE MG<sup>1</sup>, SQUADRITO F<sup>2</sup>, VITA G<sup>1</sup>  (1) Department of Neuroscience, Psychiatry and Anaesthesiology, University of Messina, Messina, ITALY. (2) Department of Clinical and Experimental Medicine and Pharmacology, University of Messina, Messina, ITALY.</p>
To contact the author:: messinasonia@libero.it.	<p><b>Abstract</b></p> <p>Soy isoflavones have been reported to have antioxidant bioactivities, scavenging free radicals and increasing antioxidant protein expression, and also to inhibit the transcription factor NF-κB. We showed in previous studies that the inhibition of the transcription factor NF-κB through drugs with also antioxidant properties, have beneficial effects in <i>mdx</i> mice. The drugs used are not available for clinical studies. We tested whether genistein and flavocoxid, supplements with known antioxidant and antiinflammatory properties readily available for clinical use, could have a beneficial effect on muscle function, morphology and biochemical pattern in <i>mdx</i> mice. Five-week old <i>mdx</i> mice received for five weeks either genistein (2 mg/kg i.p. daily), flavocoxid (5 mg/kg i.p. daily) or vehicle. Flavonoids treatment 1) increased forelimb strength ( <math>p&lt;0.05</math>) and strength normalized to weight (<math>p&lt;0.05</math>) and decreased fatigue (<math>p&lt;0.05</math>); 2) reduced serum creatine-kinase levels (<math>p&lt;0.01</math>); 3) increased GPX activity and reduced markers of oxidative stress (<math>p&lt;0.05</math>); 4) blunted NF-κB DNA-binding activity (<math>p&lt;0.05</math>); 5) reduces muscle necrosis (<math>p&lt;0.01</math>) and enhances regeneration (<math>p&lt;0.05</math>) with an augmented number of myogenin-positive satellite cells and myonuclei, and of developmental myosin heavy chain-positive fibers. Our results suggest that these flavonoids might have a beneficial effect on muscle function and morphology in <i>mdx</i> mice. Further studies are needed to investigate the biochemical substrates of such encouraging preliminary results taking into account that these supplements could be easily introduced in the daily diet of patients with DMD.</p>

PW25-316

**IMIPRAMINE TREATMENT INCREASES THE MUSCLE STRENGTH OF MDX MICE**

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Several bioactive molecules, including antidepressants, efficiently prevent muscle necrosis in dystrophin-deficient *C. elegans*. We tested the effect of the antidepressant, Imipramine, on the muscle degeneration of *mdx* mice. The *mdx 5cv* strain was preferred to the classical *mdx Scsn* strain because it has almost no revertant fibers, and is easier to breed. Groups of 6-8 mice (sex matched) were fed from 2 to 6 weeks of age with a diet containing 10 and 40 mg/kg/day of Imipramine, and tested at 6 weeks. The analysis included both histological and functional assays. The histology was performed on the extensor digitorum longus (EDL), a fast-twitch muscle, and the diaphragm. Histological parameters (% centrally nucleated fibers, fiber size variance, necrosis index) were improved 20-40% in treated versus non-treated animals. Treated animals also performed significantly better in the wire test behavioral assay. Moreover, the analysis of mechanical properties showed that the force developed by the whole EDL muscle *in situ* was 25% higher in treated versus non-treated animals. Surprisingly, the force developed by the EDLs of Imipramine-treated *mdx* mice was also larger than that of wild-type mice. Finally, using Triton-skinned fibers, diaphragm muscle from treated mice exhibited 30% higher maximal tension than *mdx* mice. Thus, Imipramine treatment of young *mdx* mice reduces the first wave of muscle necrosis and improves muscle strength of fast-twitch muscles.

PW25-317	<p><b><u>EFFECT OF ANDROGEN THERAPY ON SKELETAL MUSCLE IN THE AGED MALE RAT MODEL (ANDROGEN DEPRIVATION)</u></b>          HOURDÉ C<sup>1</sup>, VIGNAUD A<sup>1</sup>, JAGERSCHMIDT C<sup>2</sup>, BUTLER-BROWNE G<sup>1</sup>, FERRY A<sup>1</sup>          (1) INSERM U787, UPMC, Institut de myologie, paris, FRANCE. (2) galapagos, romainville, FRANCE.</p>
To contact the author:: ferry@chups.jussieu.fr.	<p>We have analysed the effect of physiological doses of androgens administrated after orchidectomy on the skeletal muscle of male rats, as well as the relationships between muscle performance, hypertrophy as well as the Akt/mTOR signalling pathway which is involved in the control of anabolic and catabolic muscle metabolism. We studied the soleus muscles from intact adult rats (SHAM), orchidectomized rats treated during 3 months with vehicle (ORX), nandrolone (NAN) or dehydrotestosterone (DHT). Our results indicate that NAN and DHT treatments maintained highly androgen dependent tissues (levator ani muscle, seminal vesicle, prostate, bone) in a normal state after orchidectomy. We found that there is a trend for absolute and relative (normalized to muscle weight) maximal tetanic isometric forces and fatigue resistance to be reduced by orchidectomy. Whereas absolute and relative maximal tetanic isometric forces (respectively + 69% and + 49%) and fatigue resistance (+35%) in NAN rats were increased when compared to ORX rats. In contrast, DHT treatment did not improve muscle function. Interestingly, muscle weights, cross sectional area of muscle fibres, relative number of muscle fibres expressing slow myosin heavy chain and citrate synthase activity were identical in NAN, DHT, ORX and SHAM rats. Furthermore, phosphorylation levels of downstream targets of the Akt/mTOR signalling pathway, Akt, ribosomal protein S6 and eukaryotic initiation factor 4E-binding protein 1 were also similar in NAN, DHT, ORX and SHAM rats. In conclusion, physiological doses of androgens are sufficient to prevent virtually all the effects of orchidectomy in rats. Interestingly, their beneficial effects on muscle performance are not related to muscle fibre growth or activation of Akt/mTOR signalling pathway in the context of androgen deprivation.</p>

PW25-318	<p><b><u>RESTORATION OF THE CALCIUM SIGNALING IN MDX MOUSE DUODENUM MYOCYTES BY GENTAMYCIN SULFATE TREATMENT AND DYSTROPHIN EXON SKIPPING.</u></b></p> <p>DABERTRAND F<sup>1</sup>, MACREZ N<sup>1</sup>, MIRONNEAU J<sup>2</sup>, HENAFF M<sup>1</sup>, MOREL JL<sup>1</sup>  (1) umr5228 CNRS universit� Bordeaux, talence, FRANCE. (2) retired, umr5017 CNRS universit� bordeaux, bordeaux, FRANCE.</p>
<p>To contact the author::  jl.morel@cnic.u-bordeaux1.fr.</p>	<p><b><u>RESTORATION OF THE CALCIUM SIGNALING IN MDX MOUSE DUODENUM MYOCYTES BY GENTAMYCIN SULFATE TREATMENT AND DYSTROPHIN EXON SKIPPING.</u></b></p> <p>We have previously described that the loss of dystrophin expression in duodenum myocytes from mdx mouse induces the decrease of ryanodine receptor subtype 2 (RYR2)-dependent calcium signalling. This effect is due to a lower level of RYR2 expression in mdx duodenum myocytes [Morel et al., 2004]. Both aminoglycoside treatment and dystrophin exon skipping are proposed as therapeutic ways for the Duchenne muscular dystrophy. After 14 days of gentamycin treatment, the activation of RYR2 by caffeine in mdx duodenum myocytes induced the same calcium responses than those observed in wild-type mice and an increase of the expression of RYR2. In addition, mdx mice were treated by antisense oligo-2' O-Methyl (O-Me) nucleotides directed against the dystrophin exon23 (asDYS) to induce exon skipping and the expression of truncated dystrophin. The Cy5 labelling of asDYS enables to follow its presence in duodenum myocytes. As observed in gentamycin treated mdx mice, the treatment with asDYS was able to increase the RYR2 expression and restore the calcium signals encoded by RYR activation. These results suggest that gentamycin as well as antisense strategy could be used to restore the Ca<sup>2+</sup> signalling in smooth muscle from mdx mice. Both of these therapeutic ways have benefits and inconvenience but are possibly usable to restore the smooth muscle function for patients affected by Duchenne muscular dystrophy.</p>

PW25-319	<p><b>BENEFICIAL EFFECT OF MOLSIDOMINE ON MDX MICE MUSCLE MEMBRANE EVIDENCED BY IN SITU MALDI PROFILING</b>  BENABDELLAH F<sup>1</sup>, TAHALLAH N<sup>1</sup>, TOUBOUL D<sup>1</sup>, YU H<sup>2</sup>, BRUNELLE A<sup>1</sup>, LAPRÉVOTE O<sup>1</sup>, DE LA PORTE S<sup>2</sup>  (1) Institut de Chimie des Substances Naturelles, Laboratoire de Spectrométrie de Masse, CNRS - UPR 2301, Gif-sur-Yvette, FRANCE. (2) Institut de Neurobiologie Alfred Fessard - FRC2118, Laboratoire de Neurobiologie Cellulaire et Moléculaire, CNRS -UPR 9040, Gif-sur-Yvette, FRANCE.</p>
To contact the author:: sporte@nbcn.cnrs-gif.fr.	<p>Duchenne muscular dystrophy (DMD) is the most common genetic disorder characterized by the lack of dystrophin, a sub-sarcolemmal protein necessary for normal muscle functions. Among therapeutic approaches, the upregulation of utrophin to replace defectious dystrophin is developed. Previous studies demonstrate an increase of utrophin labelling, a decrease of necrotic surface, a diminution of creatine kinase release in the serum, and an improvement of isometric strength as consequences of improved NO production via the injection of L-arginine, the substrat for NO synthase or of molsidomine, an NO donor commercialized as Corvasal®. While histology indicates that molsidomine can reverse the degenerating process induced in <i>mdx</i> mice, the mechanism of action on the muscle structure is not really known. We examine the part of the phospholipid composition in membrane myofibers by addressing the phospholipid intensity ratio PC34:2(PC C16:0/C18:2)/PC34:1(PC C16:0/C18:1). This ratio has been demonstrated to be different in normal and dystrophic mice models by <i>in situ</i> MALDI-MS profiling. This variation was recently confirmed by cluster-TOF-SIMS (Time-of-flight Secondary Ion Mass Spectrometry) imaging on human dystrophic tissue sections. On the other hand, cell cultures were performed and the same intensity ratio inversion was shown during the differentiation from myoblasts to myotubes. It was thus suggested that this ratio could be a marker of the regenerating process of muscle cell lines. After the treatment of <i>mdx</i> mice with molsidomine, we evidenced by MALDI-TOF MS profiling a restoration of a membrane lipid composition equivalent to those of wild-type mice. This restoration was associated with an increase of the regeneration process in the mice. This indicates that NO donors restore a normal membrane structure mainly via a regeneration process although the eventuality of a simple compensation of the membrane structure by incorporation of specific class of fatty acids cannot be excluded.</p>