PENTOXIFYLLINE AMELIORATES CALCIUM HOMEOSTASIS AND IMPROVES REGENERATION IN SKELETAL MUSCLE OF DYSTROPHIC MDX MICE: A COMPARISON WITH THE EFFECTS OF A COMBINED TREATMENT PREDNISOLONE-TAURINE

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The phosphodiesterases inhibitor pentoxifylline gained attention for the treatment of Duchenne muscular dystrophy for its wide anti-inflammatory, anti-ischemic and anti-fibrotic action. To better understand its therapeutic potential in DMD, a 4-8 weeks treatment was performed in treadmill-exercised mdx mice (50 mg/kg/day i.p.). *In vivo*, the treatment fully prevented the exercise-induced weakness, the normalized fore-limb strength increment after 4 weeks of exercise being 1.10 ± 0.12 vs. 0.26 ± 0.02 of untreated mice ($p<0.0001$ by ANOVA). *Ex vivo*, pentoxifylline restored the mechanical threshold, an electrophysiological index of calcium homeostasis, in extensor digitorum longus (EDL) muscle fibers. In parallel, fura-2 imaging experiments showed a significant reduction of resting cytosolic calcium and of sarcolemmal calcium permeability toward the values of wild-type myofibers. Patch-clamp recordings on flexor digitorum brevis (FDB) muscle fibers confirmed a decrease of open probability and a normalization of occurrence of the abnormally active voltage-insensitive cation channels of dystrophic fibers by pentoxifylline. The treatment also counteracted the typical impairment of chloride channel conductance (gCl) in mdx diaphragm fibers. Immunohistochemistry and ELISA experiments showed a significant increase of area in active regeneration in treated diaphragm and gastrocnemious muscle, along with a modest decrease in non-muscle area and of the pro-fibrotic cytokine TGF-β1. Plasma creatine kinase (CK) was reduced by 40% in treated animals. The effects of pentoxifylline were compared with those exerted by a potentially clinical relevant drug association. To this aim, based on their efficacy on inflammatory pathways and calcium homeostasis (1), we performed a combined treatment with α-methyl-prednisolone (1mg/kg/day i.p.), used in DMD patients, and the safe aminoacid taurine (1g/kg/day per os). The associated treatment was largely effective in preserving fore limb strength and in ameliorating mechanical threshold in EDL muscle and produced a pentoxifylline-like reduction of calcium channel activity in FDB myofibers. However, almost no effects were observed on plasma CK and histology profile of gastrocnemious muscle. Thus, both treatments may exert a beneficial effect on muscle function by ameliorating calcium homeostasis, while the enhanced regeneration may reside in the pentoxifylline ability to induce a cyclic nucleotide-dependent satellite cells activation (Telethon GGP05130).

1) De Luca A., Pierno S., Liantonio A., Cetrone M., Camerino C., Fraysse B., Mirabella M., Servidei S., Ruegg U.T., Conte Camerino D. (2003) J. Pharmacol. Exp. Ther. 304:453-463.