Assessment of TRPC1 and TRPC3 as potential therapeutic targets for DMD treatment in complement to rAAV-microDystrophin gene therapy

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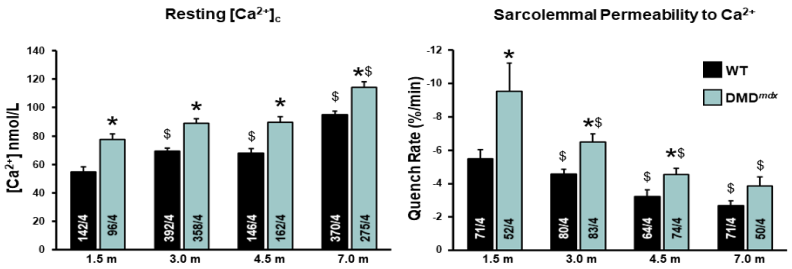
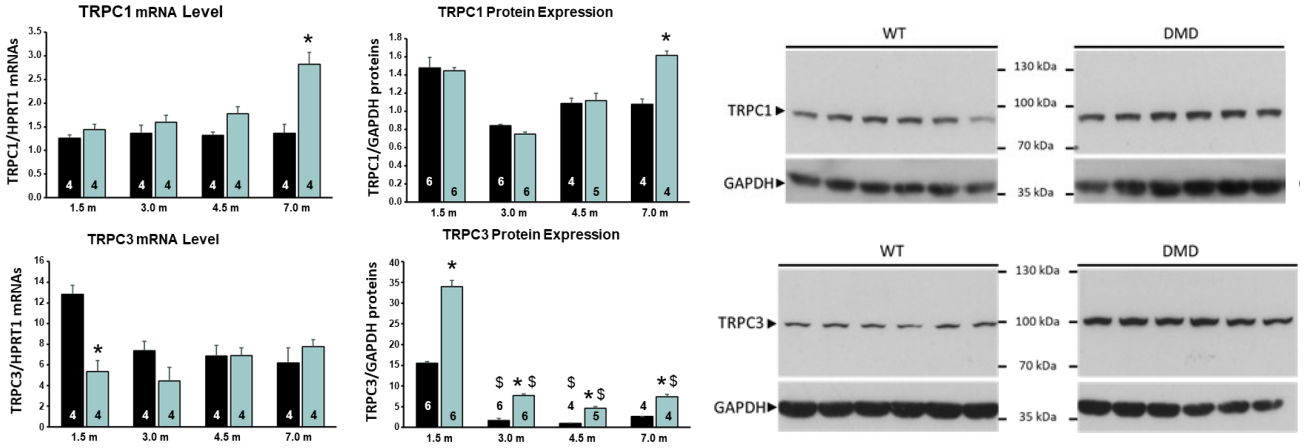
Duchenne Muscular Dystrophy (DMD) is a lethal and genetic disease caused by the lack of dystrophin expression, for which there is no curative treatment. Clinical trials are ongoing using rAAV-microdystrophin (rAAV-MD) gene therapy that compensates the lack of full-length dystrophin by the expression of a smaller but functional protein. The aim being a shift from DMD toward a milder dystrophinopathy, the Becker muscular dystrophy (BMD). However, BMD patients still exhibit muscle decline and die before the age of 60. In DMD, patients, as in animal models, muscle cell necrosis is triggered by an increased Ca2+ influx (SPCa). This may rely on a deregulation of the activity and/or expression of some ion channels, in particular, the Transient Receptor Potential channels (TRP). In this study, we evaluated the involvement of TRPC1 and TRPC3 Ca2+ channels in the pathogenesis of DMD in skeletal muscle from the DMDmdx rat, an animal model that faithfully mimics human DMD disease.

Evolution of resting calcium homeostasis in EDL muscle fibers from WT and DMD^{mdx} rats during post-natal development

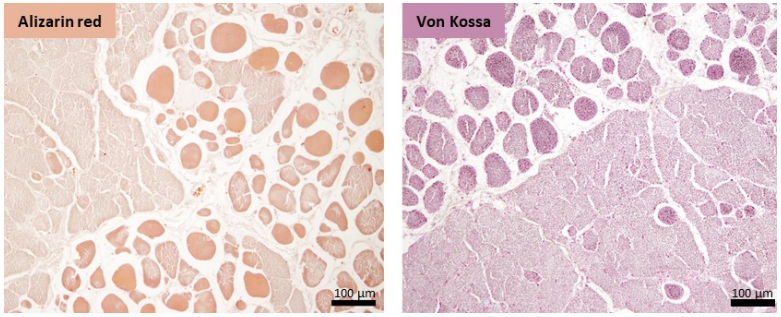


For each graphics : bars represents mean value ± SEM
*: significantly different from mean value measured in age-matched WT animals. $: significantly different from mean value measured in animals from the same genotype of 1.5 months of age. P<0.05, Two-way ANOVA and Fisher-LSD post-hoc test for pairwise comparisons

Evolution of TRPC1 and TRPC3 mRNA and protein levels in EDL muscles from WT and DMDmdx rats during post-natal development

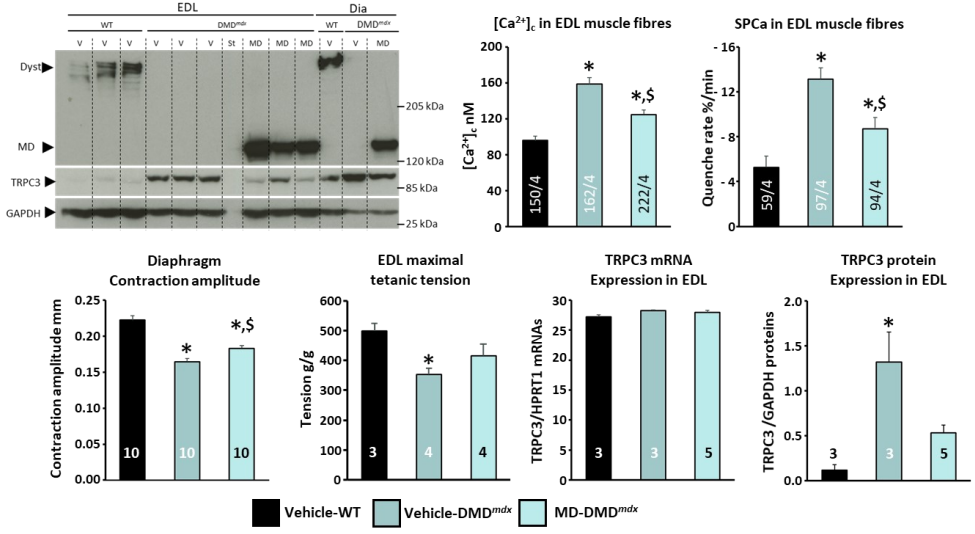


Histopathological evaluation of skeletal muscles from DMD^{mdx} rats died after hyperthermia syndrom



After specific staining for calcium (left panel: alizarin red and right panel: von Kossa), we observed differences of dye affinity between normal appearing fibers and round hypercontracted fibers that unlighted disruption of calcium concentrations between intracellular and extracellular compartments. Note the severe endomysial edema typical of hyperthermia syndrom

Alterations of calcium homeostasis, TRCP3 mRNA and protein expressions, and skeletal muscle contraction before and after AAV2/8-MD treatment in DMD^{mdx} rats



We demonstrated early increases in [Ca2+]c and SPCa in EDL muscle fibers. This was accompanied by an increase of TRPC3 protein level. TRPC1 mRNA and protein levels increased only 7 months after birth. MD expression via rAAV-MD injection induced only partial prevention of alterations in Ca2+ homeostasis, muscle strength and TRPC3 overexpression. TRPC3 thus represents a good therapeutic target for the development of a treatment for DMD, as a complement or alternative to rAAV-MD gene therapy.

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