Evaluation of the efficacy of a long-term treatment wih metformin on functional, histological and biochemical disease-related endpoints in exercised dystrophic mdx mice.

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The progressive degeneration and myofibers fragility during contractile activity in Duchenne muscular dystrophy (DMD) are caused by the complex cascade of events triggered by the absence of dystrophin, a protein with a key role for a proper mechano-transduction (Hoffman and Dressman, 2001). We have recently shown that in the mdx mouse, the most widely used animal model for DMD, the protocol of chronic treadmill exercise leads to a failing mechanical-metabolic coupling. In particular, genes of protective metabolic pathways such as SIRT1/PGC-1Î±, Pparγ, adiponectin, Bnip-3 are severely down-regulated and are likely unable to contrast the high-expression of damage-related genes (NADPH-oxidase 2, TGF-β1, TNFÎ±, c-Src tyrosine kinase), then accounting for muscle damage and dysfunction (Camerino et al., 2014). In line with this, several recent studies showed that metabolic modulators lead to beneficial effects on pathology-related signs in the mdx mouse. The aim of our study was to evaluate the effects of chronic treatment with metformin hydrochloride, currently in clinical trial in DMD boys (NCT01995032.clinicaltrials.gov), able to indirectly activate AMPK by modulating mitochondrial activity and cellular energetic state. Metformin (200 mg/kg/day, *per os*) was administered to mdx mice for 20 weeks, in parallel with a standard protocol of exercise in order to exacerbate metabolic sufferance. A multidisciplinary approach, in vivo and ex vivo, was used according to standard operating procedures to assess the impact of drug treatment on primary readouts. In vivo, metformin significantly increased dystrophic mice strength, which showed higher normalized forelimb force values with respect to untreated animals (5.66 ± 0.16, n=7 vs. 4.66 ± 0.01, n=6; $p<0.001$). The drug did not significantly ameliorate twitch and tetanic force values of extensor digitorum longus (EDL) muscle, while a slight, although not significant increase, was observed in diaphragm twitch and tetanic forces (+54% and +35% vs. untreated mdx mice, respectively). No protection was exerted by the treatment on force drop of dystrophic EDL muscle during eccentric contraction. Interestingly, metformin ameliorated histopathology of mdx gastrocnemius (GC) muscle, with a significative reduction of the percentage of both total damage area and fibrosis, paralleled by a clear trend of reduction of pro-fibrotic marker TGF-β1, measured in GC samples by ELISA assay. Moreover, the drug treatment induced a marked decrease of plasma levels of matrix metalloprotease-9, biomarker associated with dystrophic pathology progression. This was accompanied by a slight reduction of plasma levels of creatine kinase and lactate dehydrogenase, biochemical markers of muscle damage. Overall, these results show a partial efficacy of metformin on pathology signs of chronically exercised mdx mice. Molecular biology studies are currently ongoing in order to gain insight into the overall efficacy and mechanism of action of metformin in dystrophic muscle (Supported by PRIN-MIUR n°20108YB5W3_004).

References:

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