Metformin has partial efficacy on in vivo and ex vivo pathology signs in dystrophic mdx mice undergoing a long protocol of treadmill exercise

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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 in mechano-transduction (Hoffman & 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 Sirt-1/Pgc1-α, Ppar-γ, 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, TNF-α, c-Src tyrosine kinase), then accounting for muscle damage and dysfunction (Camerino et al., 2014). In line with this, the activation of PGC1-α and AMP kinase, key modulators of muscle metabolism, leads to beneficial effects on pathology-related signs of mdx mouse (Handschin et al., 2007; Ljubicic et al., 2012). The aim of this study was to evaluate the effect of chronic treatment with metformin due to its ability to indirectly activate AMPK, by modulating mitochondrial activity and cellular energetic state; metformin is currently in clinical trial in DMD boys (NCT01995032.clinicaltrials.gov) in spite it has not been extensively tested pre-clinically in mdx mice. For the present experimental design, mdx mice of 4-5 weeks of age underwent a long protocol of exercise (24-28 weeks) to exacerbate metabolic sufferance and were in parallel treated with metformin (200 mg/kg/days orally). A multidisciplinary approach in vivo and ex vivo was used, according to standard operating procedures, to assess the impact of drug treatment on both primary and secondary readouts. In vivo, metformin significantly increased mouse strength, with normalized forelimb force values of 5.66 ± 0.16 (n=7) vs. 4.66 ± 0.01 (n=6; $p<0.001$) of untreated mice, but did not improve exercise performance. Contractile parameters of extensor digitorum longus (EDL) and diaphragm (DIA) were determined ex vivo. Twitch and tetanic force values of EDL muscle were only minimally improved with metformin treatment. However the effect of metformin is more evident on DIA muscle with a significative increase of twitch and tetanic force of 54% and 35%, respectively, with respect to untreated mdx mice. Moreover we did not observe any protection of the drug on EDL muscles of mdx mice by the damage induced by eccentric contraction recordings. Furthermore, no effect of metformin was observed on the exercise-induced impairment of macroscopic membrane conductance (gm) of EDL mdx muscle, a sensitive electrophysiological biomarker of the disease. Metformin did not lower CK or LDH plasma level. Then the present results open question about the interest of metformin for DMD. Further histological and molecular biology analysis will help to gain a better insight in its overall efficacy and mechanism of action in dystrophic muscle. (Supported by Prin-MIUR n°20108YB5W3_004).

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