EFFECT OF A LONG TERM TREATMENT WITH METFORMIN ON IN VIVO AND EX VIVO PATHOLOGY SIGNS IN EXERCISED AND NON-EXERCISED DYSTROPHIC MDX MICE

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We have recently shown that in the mdx mouse, the most widely used animal model for Duchenne muscular dystrophy (DMD), the protocol of chronic treadmill exercise leads to a failing mechanical-metabolic coupling. In particular, genes of protective pathways (SIRT1/PGC-1α, PPARγ, adiponectin, follistatin, MIH, Bnip3) are severely down-regulated and likely unable to contrast the elevated expression of damage-related genes (NADPH oxidase 2, TGF-β1, TNFα, c-Src tyrosine kinase), then accounting for muscle damage and dysfunction [1, 2]. In line with this, various studies showed that metabolic modulators lead to beneficial effects on pathology-related signs in mdx mice. The aim of our study was to evaluate the effects of a chronic treatment with metformin, recently tested in DMD boys (NCT01995032.clinicaltrials.gov), able to indirectly stimulate AMP-activated protein kinase (AMPK) by modulating mitochondrial activity and cellular energetic state. Mdx mice were treated with metformin hydrochloride (200mg/kg/d) for 20 weeks, in parallel or not with a long protocol of exercise, as a modulator of metabolism. A validated multidisciplinary in vivo and ex vivo approach was used to assess the impact of drug treatment on primary readouts. In vivo, metformin improved normalized forelimb strength in both sedentary and exercised mdx mice from T8 to T16, with respect to their untreated counterparts, while no protection was exerted by the drug on exercise performance. Ex vivo, a trend toward increase of diaphragm twitch and tetanic forces was found in metformin-treated sedentary mdx mice (recovery score, r.s., ~28%); in exercised mdx mice, the treatment significantly ameliorated these indices with respect to untreated ones (r.s. ~40%). By contrast, no positive effect was observed on either isometric or eccentric contractile properties in mdx EDL muscles. Surprisingly, gene expression analysis did not reveal any significant modulation by metformin treatment of pathways involved in metabolic adaptation to exercise or autophagy in gastrocnemius (GC) muscles from dystrophic mice. Similarly, a slight and not significant increase of the pAMPK/AMPK ratio was found by western blot in tibialis anterior muscles from treated mdx mice, either exercised or not, compared to untreated ones. However, metformin ameliorated the histopathology of GC muscle, reducing the area of total damage (considered as the sum of necrosis, infiltration, and non-muscle area) in both exercised and sedentary animals, while only the latter showed the presence of areas in regeneration. In parallel, the spectrophotometric analysis of biochemical markers of muscle damage revealed that metformin slightly reduced high plasma levels of lactate dehydrogenase in both non-exercised and exercised mdx mice (r.s. 56% and 23%, respectively), while a slight reduction of creatine kinase was only observed in metformin-treated exercised animals (r.s. 22%). In the more severe mechanical-metabolic condition induced by chronic exercise in mdx mice [1, 2], metformin was able to lower the high plasma levels of matrix metalloproteinase-9, a biomarker associated with disease progression [3], as well as of the pro-fibrotic marker TGF-β1 in GC muscles (r.s. 43% and 106%, respectively). In addition, the mechanical threshold of EDL myofibers, an index

of calcium handling during contraction, showed a significant shift toward more positive potential values after metformin treatment in exercised mice, versus the values of wild type mice. Our study shows controversial evidences about the therapeutic interest of metformin for DMD, disclosing the need to conduct further experiments to elucidate the mechanisms which may be responsible of the limited drug efficacy on some important disease-related readouts in mdx mice. This is of importance in consideration of ongoing clinical trials with metfromin in DMD patients (Supported by PRIN-MIUR n°20108YB5W3_004 and DPP-NL).

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