REPURPOSING METFORMIN IN DUCHENNE MUSCULAR DYSTROPHY: A LONG-TERM PRECLINICAL EVALUATION IN THE DYSTROPHIC MDX MOUSE MODEL

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Introduction: In recent years, the pharmacological stimulation of AMP-activated protein kinase (AMPK) via metabolic enhancers has emerged as a potential therapeutic strategy for Duchenne muscular dystrophy (DMD), a lethal X-linked muscle-wasting disorder, primarily due to the absence of protein dystrophin in striated muscle fibers. Here, we focused on metformin, a biguanide with a long-standing evidence base for efficacy and safety as anti-diabetic drug, known to indirectly activate AMPK via inhibition of mitochondrial respiratory chain complex I. Recently, metformin has been proposed in clinical trials on DMD patients, in combination with amino acids sources of nitric oxide (NO) for a potential synergic action on muscle metabolism. Nonetheless, preclinical data concretely supporting the efficacy of metformin by itself on disease progression are still poor. Moreover, the role of metformin in regulating skeletal muscle metabolism, as well as the molecular mechanisms behind its cellular actions, still appear controversial. In light of these considerations, we questioned about the actual therapeutic potential of metformin as a metabolic enhancer on dystrophic muscle in mdx mice and humans.

Materials and methods: We investigated the effects of a long-term oral treatment with metformin in the exercised mdx mouse, a widely-used model characterized by a severe mechanical-metabolic maladaptation, which more closely resembles DMD patients' condition. Metformin was formulated in drinking water and administered to mice at a dose of 200mg/kg/day, for 20 weeks. A validated multidisciplinary *in vivo* and *ex vivo* approach was used to assess the impact of drug treatment on disease-related primary readouts.

Results: We disclosed the ability of metformin to markedly ameliorate histopathology of mdx gastrocnemius (GC) muscle by significantly reducing the total area of damage and, specifically, non-muscle area, compared to vehicle. This was accompanied by a significant decrease in pro-fibrotic transforming growth factor-β1levels, measured by ELISA in GC muscle, with a recovery score – r.s. – of 106% toward wild type value. In addition, we found a notable decrease of matrix metalloproteinase-9plasma levels (r.s. 43%), also measured by ELISA. In parallel, metformin partially counteracted mdx mice *in vivo* weakness, detected by forelimb grip strength test. Ex vivo, the drug was able to significantly increase specific twitch and tetanic forces of isolated diaphragm, compared to vehicle. This positive effect was not observed on contractile parameters of extensor digitorum longus muscle, although this latter was partially protected by the treatment with respect to mechanical threshold, an electrophysiological index of excitation-contraction coupling. However, these improvements did not seem to be related to a protective action of metformin on dystrophic muscle metabolism. This was shown by the limited effect on the pAMPK/AMPK ratio measured by western blot and on the expression of genes involved in mechanical-metabolic response measured by qRT-PCR, as well as by the lack of fast-to-slow fiber type shift revealed by SDH staining on tibialis anterior muscle. Importantly, we obtained similar results in the milder phenotype of sedentary mdx mice. The limited efficacy could be related to the inability of metformin to increase the low muscle levels of L-arginine, L-citrulline and taurine in either non-exercised or exercised mdx mice, as shown by HPLC analysis, suggesting the need of a synergic action on metabolic pathways for an efficient genetic or epigenetic effect of metformin.

Conclusions: These findings encourage the need to improve our knowledge on alternative, metabolism-independent mechanisms of action of metformin, in order to better address the repurposing of this drug for the treatment of DMD. In addition, the results further support the use of metformin in combination with metabolic enhancers, such as NO precursors, for a synergistic action (Supported by PRIN-MIUR n° 20108YB5W3_004and by DPP NL 2015).