Cross-talk between inflammation and aberrant mechano-transduction in degenerative primary myopathies: validation of druggable targets via animal models and pharmacological studies

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Inflammation of skeletal muscle is pivotal in repairing mechanisms after injury and adaptation to exercise. In some degenerative muscle disorders, such as muscular dystrophies and idiopathic inflammatory myopathies, the establishment of a chronic inflammatory state creates a non-favorable environment for regeneration and repair, contributing to damage and loss of muscle function. The causal and temporal cascade of signals through which mechano-transduction modulates metabolic adaptive responses and damage/repair in skeletal muscle, is still unclear. The present study was aimed at clarifying these events by two approaches. First we focused on Duchenne Muscular Dystrophy (DMD), a severe genetic disease caused by the absence of sub-sarcolemmal cytoskeletal protein dystrophin (Hoffman and Dressman, 2001). We hypothesized that in DMD, the chronic inflammation is a direct consequence of the structural deficit via a failing mechanical-metabolic coupling. In fact we observed that, in the mdx mouse model, a standardized exercise protocol worsens the pathology by leading to an unbalance between protective metabolic pathways (Sirt1/Pgc-1α, Pparγ, Bnip-3) and damaging inflammation-related (NADPH-oxidase, TGFβ, TNFα, c-Src) ones (De Luca et al., 2005; Camerino et al., 2014). We presently validated this hypothesis by a pharmacological approach, by comparing compounds able to activate Pgc-1α pathways (resveratrol; 100 mg/kg/day *i.p*) or reduce oxidative stress (taurine, 1g/kg/day *per os* and apocynin, 38 mg/kg/day *per os*) upon 4 week-treatment in exercised mdx mice. All the compounds enhanced *in vivo* mouse force, with the following order of potency: resveratrol>taurine>apocynin. Resveratrol also significantly decreased plasma levels of creatine kinase and lactate dehydrogenase and improved resistance to exercise. The three drugs significantly reduced the production of superoxide anion, assessed in tibialis anterior muscle by dihydroethidium staining, up to 80%; taurine and apocynin also led to an improvement of functional biomarkers of oxidative stress, such as membrane ionic conductance of *extensor digitorum longus* (EDL) fibers. In parallel the compounds markedly reduced activated nuclear factor-kB, as shown by a decrease of positive fibers in gastrocnemius muscle. Minimal if any effect was observed on force of isolated diaphragm and EDL muscle. The results validate the working hypothesis and corroborate the primary interest of drugs which counteract inflammation and oxidative stress in DMD. The second approach was to use a novel animal model of idiopathic autoimmune polymyositis (PM) to verify the hypothesis that in inflammatory myopathies, conversely to muscular dystrophies, inflammation is the triggering event that causes metabolic dysfunction, oxidative stress and mechanical impairment. A double-transgenic mouse model (H^+T^+) with a tetracycline-conditional muscle-specific upregulation of major histocompatibility complex I (Nagaraju et al., 2000) has been recently introduced in our laboratory. Our colony is expanding and newborn mice are regularly genotyped by PCR. The H^+T^+ genotype has occurred in 15% (7 of 49 mice) of the live-born offspring from 10 successful matings, of which 28% are females. These latter, since two months after transgene induction by doxycycline withdrawal, showed both a reduced *in vivo* normalized forelimb force (28%), and a lower resistance to treadmill exercise (25%) compared with controls, further reduced four months after the transgene induction by 35% and 52%, respectively. Ex vivo functional and molecular biology studies are currently ongoing to identify disease-related parameters and potential drug targets (Supported by MIUR-PRIN n°20108YB5W3_004).

References:

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