Histology profile as primary readout in validation of drug mechanism of action and efficacy in inherited muscular dystrophies

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Duchenne muscular distrophy (DMD) is a lethal wasting X-linked myopathy, due to the absence of the protein dystrophin. This defect disrupts the dystrophin-glycoprotein complex, and consequently the link between intracellular cytoskeleton of myofibers and the extracellular matrix. As a consequence, dystrophic fibers are susceptible to contraction-induced damage and undergo continuous rounds of degeneration/regeneration also accompanied by chronic muscle inflammation. Due to the high regenerative potential and plasticity of muscle tissue, these cycles occur with specific time frame, leading to clear histological pictures according to the pathology phase. In fact at later stages muscle fibers eventually lose the ability to regenerate and are replaced with fibrous and fatty tissue (Deconinck and Dan, 2007). Other than its undoubted diagnostic values, histopathology analysis is gaining an increasing importance in pre-clinical studies of DMD, for its high potential as main readout to validate drug efficacy before moving best candidates into clinical settings (Willmann et al., 2012). We presently performed a detailed morphometric analysis to validate the mechanism of action and efficacy of apocynin (38 mg/kg/daily orally) and taurine (1 g/Kg/daily orally) differently targeting oxidative stress in muscles of mdx mice in which the pathology was unmasked by a chronic exercise protocol. The treatment started at mouse age 4-5 weeks, soon after the appearance of pathology signs, and lasted about 4 weeks, then covering the first acute phase of degeneration, regeneration and inflammation. The morphometric analysis was performed on two muscle type: diaphragm (DIA), in which histopathology progressively increase due to continuous respiratory activity and gastrocnemius (GC), a hind-limb muscle, in which pathology is acutely aggravated by exercise. Middle belly transversal section of DIA or GC, were processed and stained with Haematoxylin and Eosin (H&E) to allow measurements by using Image J computer program. The morphometric measurements were performed on typical pathology signs by measuring extension of inflammatory infiltrates, early regeneration signs, area covered by fibrotic and adipose tissue, as well as number of normal myocites or of mature centronucleated-ones, a typical sign of degeneration-regeneration events. Immunohistochemistry was also used to assess in GC the number of positive nuclei to activated NF-kB (phosphorylated p65 antibodies). The results show that both apocynin and taurine reduced the total damaged area in DIA of mdx mice subjected to exercise. The effect was statistical significant with apocynin, the percent area of damage being 18$\pm$ 3,9% (n=5) vs 34$\pm$ 5,6% of vehicle-treated mdx (n=5) ($p<0.05$). Furthermore, both apocynin and taurine significantly reduced the total damaged area in GC of mdx mice. In particular the percent of damaged area were 16,2$\pm$ 4,2% with apocynin (n=5) and 16+ 2,7% with taurine (n=5) vs 52,2$\pm$ 3% of untreated mdx (n=5) ($p<0.0001$ vs mdx-exer for both drug). A more detailed morphometric measurements show that the number of normal myocites in GC of drug treated mice is significantly higher with apocynin (17,8$\pm$ 0,6) and taurine (18,3$\pm$ 1,5) vs untreated mdx (9,5$\pm$ 1,4) ($p<0.0001$). In contrast, in the DIA of drug treated mice the number of normal fibres was not statistically different with respect to mdx exer. The immunohistochemistry assessment evidenced that nuclei positive for activated NF-kB are significantly reduced in GC of both apocynin (-10,5%) and taurine (-51,3%) treated mdx mice with respect to untreated ones. The results show a clear improvement of the histopathology indices related to inflammation induced damage as a consequence of drug treatment, supporting the therapeutic interest of pharmacological intervention of drugs able to target oxidative stress with various mechanisms for DMD.

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Willmann et al. (2012). Neuromuscul Disord. 22(1):43-9