Pharmacological evaluation of NADPH oxidase involvement in pathophysiology of mdx mouse, an animal model of muscular dystrophy

A. Cozzoli[1] , R.F. Capogrosso [1], A. Massari [1], P. Mantuano [1], M. Cannone [1], A. Giustino [2], G.M. Camerino[1], A. De Luca[1]

[1]Unit of Pharmacology, Department of Pharmacy and Drug Sciences, University of Bari "A. Moro", Italy;
[2]Department of Biomedical Sciences and Human Oncology, University of Bari, Medical School, Italy.

Oxidative stress, caused by reactive oxygen species (ROS), has been implicated on disease progression and chronic inflammation in Duchenne Muscular Dystrophy (DMD). NADPH oxidase 2 (NOX2) is currently considered to be a major source of ROS and it is over-expressed in skeletal muscle and heart of mdx mice, the most widely used model for DMD. Its activation in mdx myofibers via stretch-sensitive pathways has also been shown (Whitehead et al., 2010; Khairallah et al., 2012). Consequently, drugs able to reduce ROS production by inhibition of NOX-2 are potential treatment for muscular dystrophy. In line with this view, we have recently shown that enalapril, by inhibiting the production of angiotensin II (Ang II), one of the main endogenous activator of NOX, reduces the signs of oxidative stress and the percentage of p65-NF-kB positive nuclei in the mdx muscles. In this frame we also observed, in the myofibers of mdx mice treated with enalapril, a dose-dependent restoration of macroscopic chloride conductance (gCl), a sensitive biomarker of inflammation in skeletal muscle (Cozzoli et al., 2011). The aim of the present study was to investigate the involvement of NOX-2 dependent-ROS production in relation to the aberrant mechano-transduction occurring in dystrophic muscle. RT-PCR experiments confirmed a higher expression of β-tubulin and NOX2 ($gp91^{phox}$) mRNA in gastrocnemius (GC) muscle of mdx mice. Interestingly, this increased expression was maintained in GC muscles of mdx mice that underwent a standard chronic (1-2 months) exercise protocol on treadmill. Then, we tested the effect of a chronic treatment with apocynin (38 mg/kg in drinking water/day for 5-9 weeks), a natural compound able to directly inhibit NOX-2, on exercised mdx mice (De Luca et al., 2003). Treatment started at 4-5 weeks of age and the outcome was evaluated by a multidisciplinary approach on pathology-related *in vivo* and *ex vivo* endpoints. *In vivo*, apocynin significantly increased mouse strength, with normalized forelimb force values of 6.4 ± 0.16 (n=9) vs. 5.6 ± 0.19 (n=10; $p<0.05$) of untreated mice, but did not improve exercise performance. Furthermore, no effect was observed on plasma creatine kinase and lactate dehydrogenase. However, the treatment with apocynin counteracted the exercise-induced impairment of total membrane conductance (gm), which is mainly sustained by the reduction of gCl, in extensor digitorum longus (EDL) muscle fibers, gm being 2536 ±105 $\mu S/cm^2$ (n =37) vs. 1886 ± 92 $\mu S/cm^2$ (n = 42, $p <0.0001$) of untreated mdx myofibers. Then the recovery score for this parameter, considering the value of 2607 ± 23 $\mu S/cm^2$ (n =19) of wild-type C57BL10 myofibers, was 90% in apocynin-treated myofibers. This latter effect prompted us to investigate the possibility that the channels underlying macroscopic gCl could be target of the redox-dependent NOX actions in skeletal muscle. Parallel experiments on EDL muscle fibers of C57BL10 mouse showed that Ang II decreases gCl in a concentration-dependent manner (IC50 = 60nM) and this effect was fully contrasted by the prior incubation of apocynin (10 μM) or with a known anti-oxidant N-acetyl cysteine (5 mM). Markers of oxidative stress and inflammation, RT-PCR, histo-morphology are currently under evaluation. However, this preliminary data support the hypothesis that pharmacological targeting of NOX-2, providing protection from cross-talk between ROS production and inflammation, may represent a valuable approach in DMD. (Supported by DPP/NL and MIUR-PRIN n° 20108YB5W3).

Whitehead et al. (2010). PLoS One, 5(12):e15354.
Khairallah et al. (2012). Sci Signal.5(236):ra56.
Cozzoli et al. (2011). Pharmacol Res. 64(5):482-92.
De Luca et al. (2003) J Pharmacol Exp Ther.304(1):453-63.