# Oxidative stress and Duchenne muscular dystrophy: in vivo and in vitro preclinical evaluation of inhibition of Src Tyrosin Kinase and NADPH Oxidase.

F. Sanarica[1], A. Cozzoli[1], R.F. Capogrosso[1], P. Mantuano[1], M. De Bellis[1], A. De Luca[1].

[1]Section of Pharmacology, Department of Pharmacy and Drug Sciences, University of Bari "A. Moro", Italy.

Duchenne muscular dystrophy (DMD) is a severe X-linked pathology due to mutations of the dystrophin gene that cause the absence of the protein dystrophin. Similar gene defect leads to dystrophic conditions in animals such as the mdx mouse, widely used for pre-clinical studies [1]. Dystrophin is a sub-sarcolemmal component of the dystrophin-glycoprotein complex (DGC) and ensures a physical linkage between the intra-cellular cytoskeleton and the extracellular matrix [2]. The absence of dystrophin and the consequent disassembling of DGC cause an improper transduction of mechanical stimulus that leads to a complex cascade of pathological events causing muscle wasting [3]. Among these events, we focused on oxidative stress that is an intrinsic and early sign of myofiber distress. The main source of reactive oxygen species (ROS) is the NADPH oxidase (NOX), whose expression and activity are increased in both skeletal muscle and heart of dystrophic subjects [4]. ROS contribute to pathology progression reinforcing various pathways [5] via modulation of redox-sensitive targets. Among them non-receptor tyrosine kinases of the Src family (Src TKs) are of particular interest. Importantly, cSrc TK is overexpressed in dystrophin-deficient muscles and can be overactive due to the excessive production of ROS [4]. Src TKs play a critical role in ROS-mediated cellular signaling and in increasing oxidative stress by activation of NOX, in an auto-reinforcing loop. In addition, cSrc TK is involved in phosphorylation and degradation of beta-dystroglycan contributing to the loss of DGC in the dystrophin-deficient myofibers. Thus, the pharmacological inhibition of either Src-TKs or NOX seems a feasible strategy to ameliorate the pathology. Presently we focused on dasatinib, a Src TKs inhibitor already clinically available as anti-tumor drug and apocynin, a natural compound that inhibits NOX. First, we evaluated the effects of dasatinib (5mg/kg, three times a week; s.c.) and apocynin (38 mg/kg, daily per os) by means of a 5-week proof-of-concept preclinical study in treadmill exercised mdx mice. The outcome was evaluated in vivo and ex vivo on functional, histological and biochemical parameters. Apocynin ameliorated in vivo mouse fore limb force and ex vivo the extensor digitorum longus muscle force of exercised mdx mice, and markedly reduced the muscle production of ROS and the activation of NF-kB. Histopathology of apocynin-treated gastrocnemius muscle was also improved, while the high plasma levels of creatine kinase and lactate dehydrogenase were not counteracted. Differently from apocynin, dasatinib, although well tolerated, had no efficacy on pathology-related in vivo and ex vivo parameters. This may be related to a muscle specific cellular toxicity of the drug that can affect the myogenic program of satellite cell involved in the regenerative process. We therefore conducted in vitro studies to test dasatinib on undifferentiated murine muscle satellite cell line ($C_2C_{12}$) to evaluate the drug effects on cell viability and its potential protection against oxidative stress-induced cytotoxicity. Dasatinib (0.1-150μM) showed a concentration-dependent decrease of cell viability from the concentration of 5μM onward. We used two cytotoxic concentrations of $H_2O_2$ (300μM and 1mM) to evaluate potential cytoprotective effects of dasatinib. Cytotoxic effect of 300μM $H_2O_2$ was significantly reduced by 0.1μM dasatinib, while higher concentrations (0.5μM and 1μM) showed cytoprotection against 1mM $H_2O_2$. According with these data, we can postulate that the cytotoxic action of oxidative agents in satellite cells is in part mediated by activation of Src TKs. Our results support the interest to target NOX for a safe and efficacious control of pathology-related events, while more studies are necessary to better establish the therapeutic potential of Src TKs inhibitors in DMD (Supported by Duchenne Parent Project NL_DPP).