IN VIVO AND IN VITRO PRECLINICAL STUDIES TO ASSESS THE THERAPEUTIC POTENTIAL OF SRC-TK INHIBITORS IN DUCHENNE MUSCULAR DYSTROPHY

1)Sanarica F. 2)Mantuano P. 3)Cozzoli A. 4)Conte E. 5)Capogrosso RF. 6)Denora N. 7)Cutrignelli A. 8)Giustino A. 9)De bellis M. 10)Camerino GM. 11)De luca A.

University of Bari "Aldo Moro"

The progressive myofibers degeneration in Duchenne muscular dystrophy (DMD) is caused by the complex cascade of events triggered by the absence of the protein dystrophin [1]. cSrc Tyrosine Kinase (TK), a redox-sensitive protein, is overexpressed in dystrophin-deficient muscles and can be overactive due to excessive production of reactive oxygen species [2]. This event may contribute to beta-dystroglycan degradation and to reinforcement of damaging signaling in DMD [3]. Thus, the pharmacological inhibition of Src TK seems a feasible strategy to ameliorate the pathology. We presently focused on two small molecules acting as Src-TK inhibitors, PP2 and dasatinib. To assess the efficacy and any potential side effect of these two compounds, we performed a proof-of concept preclinical study in treadmill exercised mdx mice by in vivo administration of PP2 (5mg/kg, three times a week; s.c.) and dasatinib (5mg/kg, three times a week; s.c.). PP2 improved in vivo forelimb-strength and, slightly, the resistance to exercise, while no effect was observed on torque force. Ex vivo, no protection was observed on contraction parameters of EDL muscle of PP2-treated animals, although a trend of amelioration was observed on MMP-9 plasma levels, a zinc-metalloproteinase involved in degradation of extracellular matrix [4]. By contrast, dasatinib did not ameliorate neither in vivo nor ex vivo pathology-related parameters. Surprisingly, the histological profile of gastrocnemius muscle of treated mice was improved, with a reduction in the percentage of damaged area of 59% and 39% for mice treated with PP2 and dasatinib, respectively. Furthermore, the mechanism of action of the two compounds was validated by means of western blot analysis. In fact, both PP2 and dasatinib restored the level of beta-dystroglycan while reducing the expression of phosphorylated one in tibialis anterior muscle. The lack of efficacy, in spite of the validated mechanism of action, lead us to hypothesize PK issues (under evaluation) or the occurrence of a muscle specific cellular toxicity of the drug that can affect the myogenic program of satellite cell involved in the regenerative process. Thus, we conducted in vitro studies on a murine muscle satellite cell line (C2C12) to evaluate the effects of the drugs on cell viability and their potential protection against oxidative stress-induced cytotoxicity. We tested increasing concentrations of PP2 (0.1-300µM) and dasatinib (0.1-150µM). PP2 from 3µM, exerted a significant cytotoxic effect which was not concentration-dependent. By contrast, dasatinib showed a marked concentration-dependent decrease of cell viability. The cytotoxic effect of 300µM H_2O_2 was significantly counteracted by 0.1µM dasatinib and by 0.3-100µM PP2. Higher concentrations of dasatinib (0.5µM and 1µM) were necessary to exert a cytoprotective effect against 1mM H_2O_2, while no protection was observed with PP2. 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 of cSrc TK as drug target, however further studies with PP2 and dasatinib are necessary to establish the risk-benefit ratio as potential treatments for DMD (Supported by Duchenne Parent Project NL_DPP).

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