# Searching for orphan drugs for the therapy of Duchenne Muscular Dystrophy: potential role of Src tyrosine kinase inhibitors

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Duchenne muscular dystrophy (DMD) is a severe rare disease caused by mutations in the dystrophin gene. Being DMD an unmet clinical need, the identification of small molecules targeting key events of pathological cascade would be potentially useful for all patients, irrespective to gene mutation. Our previous findings in the mdx mouse, the most widely used animal model of DMD, showed that the redox-sensitive protein cSrc Tyrosine kinase (TK) is overexpressed in dystrophin-deficient muscles and likely overactive due to unbalanced production of reactive oxygen species [1]. Being cSrc TK involved in inflammation, autophagy and stability of dystrophin-linked proteins, such as β-dystroglycan, its pharmacological inhibition seems a feasible strategy to ameliorate the dystrophinopathy. We focused on two small molecules acting as Src TK inhibitors, PP2 and dasatinib, having an inhibitory potency in the low nanomolar range. We first tested the effect of PP2 (5 mg/kg, three times a week; s.c.) in the standardized model of chronically exercised mdx mice [2]. The treatment started at 4-5 weeks of age and lasted 5 weeks; the outcome was evaluated by a multidisciplinary approach on pathology-related in vivo and ex vivo endpoints. PP2 did not modify the normal growth of the mice and no significant differences were found in the normalized mass of vital organs. In vivo, PP2 improved forelimb strength, with a 48% recovery score, and slightly prevented the decline in running performance, observed in vehicle-treated mdx mice. No protection was observed on torque force and on ex vivo contraction parameters of extensor digitorum longus muscle. Furthermore, no effect was observed on plasma creatine kinase and lactate dehydrogenase. However, PP2 decreased by 40% the plasma level of matrix metallopeptidase 9, a biomarker of dystrophic pathology. In parallel PP2 ameliorated histopathology with a reduction of the total area of damage and of presence of inflammatory cells in mdx gastrocnemius (GC) muscle. In addition, a significant decrease in centronucleated myocytes, an index of cyclic degeneration-regeneration event, in favor of normal myocytes was observed in PP2 treated GC muscles Then, the results show that PP2, although not toxic, exerts a modest beneficial effects on pathological signs of dystrophic mdx mouse. The limited efficacy may be related to a muscle specific cellular toxicity of Src TK inhibitors able to impact the myogenic program of satellite cell involved in the regenerative process. We therefore conducted in vitro studies to evaluate the effects of this class of drugs on viability of undifferentiated proliferating $C_2C_{12}$ cells both in normal growth conditions and in the presence of oxidative stress induced by hydrogen peroxide ($H_2O_2$). We tested increasing concentrations of PP2 (0.1-300 μM) and dasatinib (0.1-150 μM). Dasatinib and to a lesser extent by PP2, causes cytotoxicity in $C_2C_{12}$ cells at relatively high concentrations, suggesting that Src TK is marginally involved in satellite cell viability. In fact, both PP2 and dasatinib at concentrations closer to their IC50 for Src TK inhibition lacked cytotoxicity. Furthermore, dasatinib exerted a protective effect against cytotoxicity induced by 1 mM of $H_2O_2$ at the concentrations of 1 and 0.1 μM. These in vitro data suggest that the cytotoxic action of oxidative agents in satellite cells is in part mediated by activation of Src TK. Studies are ongoing in order to assess pharmacokinetic profile of PP2 in plasma and muscles of treated mdx mice as well as to improve water solubility and oral bioavailability of Src TK inhibitors. Our results support the interest for further studies with PP2 and dasatinib as pharmacological agent for the treatment of DMD and open the way to longer pre-clinical trials in order to better evaluate the interest of Src TK inhibitors as therapeutics in DMD. (Supported by SIF-MSD).

1. Camerino et al., Hum Mol Genet. 23:5720-32,2014.
2. De Luca, Acta Myol. 31:40-7, 2012.