Src tyrosine kinase as a drug target in Duchenne muscular dystrophy: effects of two different formulations of dasatinib in exercised *mdx* mouse

Brigida Boccanegra[1], Paola Mantuano[1], Francesca Sanarica[1], Elena Conte[1], Giulia Maria Camerino[1], Annalisa Cutrignelli[1], Nunzio Denora[1], Antonietta Mele[1], Michela De Bellis[1], Annamaria De Luca[1]

[1]Department of Pharmacy & Drug Sciences, University of Bari "Aldo Moro", Bari, Italy.

cSrc Tyrosine Kinase (TK) is a redox-sensitive protein overexpressed in dystrophin-deficient muscles and can be overactive due to excessive production of reactive oxygen species; cSrc-TK may contribute to β-dystroglycan (β-DG) degradation and to the impairment of mechanical-metabolic coupling, with consequent reinforcement of damaging signaling. Thus, cSrc-TK seems a potential drug target in Duchenne muscular dystrophy (DMD). We performed a proof-of concept preclinical study in treadmill-exercised *mdx* mice by *in vivo* subcutaneous administration of dasatinib, a competitive inhibitor of cSrc-TK (5 mg/kg, 3 times/week), already available for clinical use as antitumor drug. Although well tolerated, the treatment had no significant efficacy on pathology-related *in vivo* and *ex vivo* indices of muscle force. However, an increase in the expression of non-phosphorylated β-DG was found. In order to overcome possible pharmacokinetic issues, we tested the effects of a novel oral formulation of dasatinib in exercised *mdx* mice, also suitable for paediatric use. The drug was included in a complex with hydroxypropyl-β-cyclodextrin (HP-β-CD) and administered in drinking water to exercised *mdx* mice at two different doses (10 mg/kg and 20 mg/kg) for 12 weeks. The outcomes were evaluated by means of *in vivo* and *ex vivo* approaches on dystrophy-related endpoints. Dasatinib significantly increased maximal forelimb grip strength in treated animals, with values of 0.187 ± 0.006 kg for *mdx* + dasatinib 10 mg/kg and 0.188 ± 0.005 kg for *mdx* + dasatinib 20 mg/kg versus 0.156 ± 0.01 kg of untreated mice ($p < 0.0002$; n = 8/group). In parallel, *in vivo* torque force of plantar flexor muscles of treated *mdx* mice showed a clear trend toward increase at low frequencies of stimulation (+55% for *mdx* + dasatinib 10 mg/kg and + 40% for *mdx* + dasatinib 20 mg/kg versus untreated mice at 30 Hz). An ultrasonography assessment of diaphragm function showed an increased contractile amplitude in treated animals with respect to the untreated ones, which was more evident in *mdx* + dasatinib 10 mg/kg. The treatment also decreased plasma levels of creatine kinase in treated animals (recovery scores: 44% and 31% in *mdx* + dasatinib 10 mg/kg and *mdx* + dasatinib 20 mg/kg, respectively) and of lactate dehydrogenase (recovery score: 24% in *mdx* + dasatinib 10 mg/kg). Our preliminary results support the interest in the new oral formulation of dasatinib with HP-β-CD for the treatment of DMD. Further histological and biochemical analysis are ongoing along with the pharmacokinetic assessment of this novel formulation (supported by NL-DPP).

E-mail dell'autore che concorre ai premi: brigida.boccanegra@uniba.it