Pre-clinical evaluation of S48168/ARM210, a new Rycal® compound, on in vivo and ex vivo pathology-related signs of dystrophic mdx mouse

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Duchenne muscular dystrophy (DMD) is caused by X-linked mutations of the dystrophin gene which result in the absence of the protein dystrophin (Hoffman & Dressman, 2001). Dystrophin-lacking fibers suffer from a chronic Ca^{2+} overload, a key event accounting for crucial pathological events, such as myofiber necrosis and contractile dysfunction (Fraysse et al., 2004; Rolland et al., 2006). Studies in the mdx mouse, the most widely used model of DMD, have shown that pathology-related post-translational modifications of the Ryanodine receptor subtype 1 (RyR1) result in the dissociation of the stabilizing subunit calstabin-1, leading to "leaky" channels and contributing to the altered Ca^{2+} homeostasis (Bellinger et al., 2009). S48168, also known as ARM210, is a novel small molecule belonging to the Rycal class designed to selectively stabilize the RyR channel complex and reduce Ca^{2+} leak, by improving the rebinding with calstabin. We assessed the effects of 4 weeks oral administration of S48168 at two dose-levels (10 and 50 mg/kg/day) on treadmill-exercised mdx mice, starting at 4-5 weeks of age. Multidisciplinary in vivo and ex vivo approaches were used to evaluate drug-effect on disease-sensitive indices and clinically-relevant parameters and to further understand S48168's mechanism of action on dystrophic muscle. A functional improvement was observed in vivo in S48168-treated mdx mice, with a 50% recovery score of maximal forelimb force at 50 mg/kg. Both doses prevented the decline in running performance, observed in vehicle-treated mdx mice, as assessed in an exhaustion test. Ex vivo assessment showed a dose-dependent improvement of diaphragm specific force, with a significant 30% increase of maximal force (100-140Hz) at 50 mg/kg, while minor, if any, effects were observed in hind-limb extensor digitorum longus (EDL) muscle. However, consistently with the S48168 mechanism of action, the mechanical threshold of EDL myofibers, an index of calcium handling during contraction, was significantly shifted toward the wild type (wt) values at the highest dose. In parallel, the resting cytosolic calcium level in flexor digitorum brevis (FDB) myofibers was significantly reduced by 25% in the 50 mg/kg S48168-treated mice toward the wt values. In addition, the activity of calcium-dependent protease calpain in diaphragm samples was reduced after S48168 treatment at 10 mg/kg, with a 49% recovery score. S48168 did not lower CK or LDH plasma levels. However, the morphometric analysis showed a trend in reduction of damaged area in diaphragm and in gastrocnemius muscle of 50 mg/kg treated mdx mice. In addition the level of the pro-fibrotic cytokine transforming growth factor beta 1 (TGF-β1), was significantly lower in the diaphragm muscle treated with S48168 at 10 mg/kg with respect to vehicle-treated mice. TGF-β1 gene expression was not modified in gastrocnemiusmuscle. These results support S48168 as a potential therapy for DMD and opened the way to longer pre-clinical trials in order to better evaluate its interest for clinical trials in DMD (Supported by Servier, as part of a collaboration with ARMGO Pharma Inc.).

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