Investigating the effect of Heat Shock Protein 72 Induction on the Dystrophic Pathology of mdx and dko Mice

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Duchenne muscular dystrophy (DMD) is a genetic disorder characterised by severe muscle weakness and wasting. The eventual cure for DMD will come from gene- and/or cell-based therapies, but these techniques are far from perfected and there is currently no effective treatment. There is an urgent need for the development of pharmacological strategies that can preserve the structure and function of dystrophic muscle until gene- and cell-based therapies become viable options.

Heat shock proteins (HSP's) are a family of proteins induced by cellular stress and are implicated in cellular protection. We recently demonstrated the therapeutic potential of HSP72 induction, via BGP-15 treatment, to ameliorate the dystrophic pathology of young *dko* and *mdx* mice; two models of DMD (Gehrig *et al.*, 2012). These studies were initiated in young (3-4 week old) mice but in most cases DMD is diagnosed well into the disease progression, when functional impairments are already evident. To fully elucidate the clinical potential of BGP-15 treatment of DMD, we need to determine whether BGP-15 treatment can improve the established pathology in older dystrophic mice. Due to the progressive nature of DMD, we hypothesised that BGP-15 treatment of older mice would reduce the severity of the dystrophic pathology but to a lesser extent than in young mice.

All experiments were approved by the Animal Ethics Committee of The University of Melbourne and conducted in accordance with the codes of practice stipulated by the National Health and Medical Research Council (Australia). In order to test this hypothesis, older *dko* mice (8 weeks of age) and older *mdx* mice (20 weeks of age), were administered BGP-15 (4 mg/kg) or vehicle control daily via oral gavage. After 4 weeks, rotarod performance and contractile properties of *tibialis anterior* (TA) muscles *in situ* and diaphragm muscle strips *in vitro* were determined. Using procedures we have described in detail previously (Gehrig *et al.*, 2012), animals were anaesthetised deeply with sodium pentobarbitone (Nembutal, 60 mg/kg, *i.p.*) prior to assessment of muscle contractile properties and later killed by cardiac excision while still anaesthetised deeply.

BGP-15 treatment improved rotarod performance in *mdx* but not *dko* mice. There was no effect of BGP-15 treatment on body mass or the contractile properties of diaphragm muscle strips in either *dko* or *mdx* mice. However, BGP-15 increased TA muscle mass in *dko* mice and preliminary results indicate that this was accompanied by functional improvements in TA muscles, despite no change in muscle architecture, as determined from H&E staining of muscle cross sections.

These results support the therapeutic potential of BGP-15 treatment of established dystrophic pathology, despite the effects being less profound than those in young dystrophic mice. Further functional and biochemical analyses will elucidate the extent of late treatment with BGP-15 in the dystrophic pathology. This research will assist in determining an optimal treatment window for administration of BGP-15 for clinical translation.

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