Induction of heat shock protein 72 improves the cardiomyopathy and reduces fibrosis in severely dystrophic dko mice

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Duchenne muscular dystrophy (DMD) is an X-linked disorder affecting 1 in 3500-6000 live male births, characterized by severe and progressive muscle wasting and weakness. Patients are wheelchair-bound early in adolescence and eventual respiratory and/or cardiac muscle failure leads to premature death. Currently there is no cure for DMD and effective treatments are needed urgently. Induction of heat shock protein (Hsp) 72 was shown to be highly efficacious for improving the dystrophic pathology in young dystrophin/utrophin 'double knockout' (*dko*) mouse, a severe model of DMD (Gehrig *et al*, 2012). Hsp72 induction was achieved via administration of BGP-15, a pharmacological co-inducer of Hsp72. Previous studies using BGP-15 in dystrophic mice were focused on skeletal muscle effects and did not investigate the cardiomyopathy in these mice. Additionally, these studies were conducted in 3-4 week old *dko* mice, an age when the pathology is less severe. Therefore in order to better understand the therapeutic potential of BGP-15 administration for DMD, the aims of this study were: 1) to investigate the effect of BGP-15 treatment on *dko* mice when the dystrophic pathology was already well established, i.e. as a later stage intervention; and 2) to investigate the effect of BGP-15 on the hearts of dystrophic mice, when administered as both an early and a later stage intervention.

All experiments were approved by the Animal Ethics Committee of The University of Melbourne and conducted in accordance with the Australian code of practice for the care and use of animals for scientific purposes (NHMRC). In order to test Aim 1, 8 week old *dko* mice were administered BGP-15 in 0.9% saline (15 mg/kg) daily via oral gavage for 4 weeks, with age-matched vehicle control *dko* mice and wild type control C57BL/10 mice receiving an equivalent volume of 0.9% saline. *In situ* assessment of the contractile properties of *tibialis anterior* (TA) muscles and *in vitro* assessment of diaphragm muscle strips were performed using procedures described previously (Murphy *et al.*, 2011). Mice were anaesthetized deeply with sodium pentobarbitone (Nembutal, 60 mg/kg, *i.p.*) prior to the assessment of muscle contractility and then killed by cardiac excision while still anaesthetized deeply. Analysis of fibrotic infiltration was assessed via Masson's Trichrome staining of TA and diaphragm muscle cross sections. To test Aim 2, 3-4 week old and 8 week old *dko* mice were treated with BGP-15 for 4-5 weeks, with similar age-matched control groups for comparisons. Mice were anaesthetized deeply with sodium pentobarbitone (Nembutal, 60 mg/kg, *i.p.*) and killed *via* cardiac excision.

Later stage administration of BGP-15 reduced fibrosis in the TA muscles of *dko* mice ($P < 0.05$), relative to controls, while fibrosis was unchanged in the diaphragm. BGP-15 treatment did not improve contractile properties of the TA muscles or diaphragm muscle strips. Early- and later-stage administration of BGP-15 reduced fibrosis in hearts of BGP-15 treated *dko* mice relative to control mice, with both groups showing reductions of up to 6% ($P < 0.05$). Whether this small reduction in fibrosis translates to functional improvements in the heart is currently being investigated using echocardiography.

Our findings reveal that while the impact of BGP-15 is less profound when administered later in the disease progression, it is still effective in ameliorating important aspects of the dystrophic pathology. This is the first study to demonstrate the capacity of BGP-15 to improve the cardiac pathology in dystrophic mice, broadening its potential clinical application for DMD and related muscle diseases.

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