Muscle-specific HSP72 over-expression improves muscle structure and function in mdx dystrophic mice

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The primary abnormality in Duchenne muscular dystrophy (DMD) is the complete absence of the membrane stabilizing protein, dystrophin, which results in a highly fragile sarcolemma. In dystrophic muscles, what would normally be considered routine contractions can result in membrane tears and Ca^{2+} influx. These repetitive injurious events, coupled with abnormalities in intracellular Ca^{2+} handling, result in an elevated cytoplasmic [Ca^{2+}] and subsequent activation of degenerative pathways. Chronic cycles of degeneration and increasingly ineffective regeneration results in fibrotic and other non-contractile tissue infiltration with major functional impairments. Heat shock protein 72 (HSP72), is a molecular chaperone protein with various cytoprotective functions such as preserving contractile function and improving Ca^{2+} handling dynamics under conditions of stress in cardiac muscle cells (Kim *et al.*, 2006). We tested the hypothesis that HSP72 overexpression would ameliorate the pathophysiology of muscular dystrophy in dystrophic *mdx* mice, a well-described model of DMD.

All experiments were approved by the Animal Ethics Committee of The University of Melbourne and conducted in accordance with the current code of practice as stipulated by the National Health and Medical Research Council. Female *mdx* mice were crossed with male mice expressing a rat inducible HSP72 transgene under the control of a chicken β-actin promoter (Marber *et al.*, 1995) which limited transgene expression to skeletal and cardiac muscle (and brain). F1 generation males were mated with female *mdx* mice to yield an equal proportion of *mdx*^{HSP72} and *mdx* littermate controls. 25-30 week old mice were used for all experiments. Mice were anaesthetized deeply with an intraperitoneal (*i.p.*) injection of Nembutal (60 mg/kg), and the functional properties of diaphragm muscle strips were measured *in vitro* as described previously (Gehrig *et al.*, 2008). Blood was sampled to measure serum creatine kinase levels, a myoplasmic protein commonly used as a measure of whole body muscle breakdown. In a separate group of mice, Evans blue dye (EBD) was injected (1% w/v, 10μl/g BM, *i.p.*) to assess damaged and necrotic muscle fibres. Mice were killed by cardiac excision while under deep anaesthesia.

HSP72 protein expression was elevated significantly in the muscles of *mdx*^{HSP72} compared with *mdx* littermate control mice. HSP72 overexpression improved specific (normalized) force of isolated diaphragm muscle strips ($P < 0.05$). There was reduced collagen infiltration ($P < 0.05$) and a reduction in the minimal Feret's variance coefficient (a measure of the dystrophic pathology; $P < 0.05$). Serum CK was also significantly lower in *mdx*^{HSP72} compared with *mdx* littermate controls ($P < 0.05$), a finding supported by the reduced number of EBD positive fibres indicating fewer damaged and/or necrotic fibres ($P < 0.05$).

These data show that transgenic overexpression of the HSP72 protein in the skeletal muscles of *mdx* mice improved the dystrophic pathology at the whole body level, and importantly within the diaphragm muscle. These results indicate that inducing HSP72 in muscular dystrophy is an important and novel therapeutic approach that may improve the dystrophic pathology and attenuate the disease progression.

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