Muscle-specific heat shock protein 72 (HSP72) overexpression improves muscle structure and function in dystrophic *mdx* mice

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Duchenne muscular dystrophy (DMD) is the most severe of the muscular dystrophies, affecting 1 in 3,500 live male births. Affected patients generally die in their twenties, with respiratory and/or cardiac failure ultimately causing death in most cases (Finsterer, 2006). Absence of the dystrophin protein results in muscle fibre fragility, whereby contractions result in membrane tears and Ca^{2+} influx. Coupled with abnormalities in intracellular Ca^{2+} handling, this results in an elevated cytosolic [Ca^{2+}], resulting in the subsequent activation of degenerative pathways. Chronic muscle fibre degeneration and increasingly ineffective regeneration results in fibrotic tissue infiltration leading to major functional impairments in DMD patients. Heat shock protein 72 (HSP72) has been shown to protect contractile function and improve calcium handling dynamics under conditions of stress in cardiac muscle (Kim *et al.*, 2006). We tested the hypothesis that HSP72 overexpression would ameliorate the dystrophic pathology and thus preserve muscle function in *mdx* dystrophic mice.

Female *mdx* mice were crossed with male mice expressing a rat inducible HSP72 transgene under the control of a chicken β-actin promoter, which limited transgene expression to skeletal and cardiac muscle (and brain) tissue (Marber *et al.*, 1995). F_1 generation males were mated with female *mdx* mice to yield an equal proportion of *mdx*^{HSP72} and *mdx* littermate controls. Mice (25-30 week old) were anaesthetised (60 mg/kg sodium pentobarbitone), and the functional properties of diaphragm muscle strips were measured *in vitro* as described previously (Lynch *et al.*, 1997). Mice were killed by diaphragm and cardiac excision while still anaesthetized deeply. Diaphragm muscle strips were also frozen for subsequent histological analysis. Blood was sampled to measure serum creatine kinase (CK) 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.*) for assessment of damaged and necrotic muscle fibres.

HSP72 protein expression was elevated significantly in the muscles of *mdx*^{HSP72} compared with *mdx* littermate control mice. HSP72 overexpression improved specific (normalised) force in isolated diaphragm muscle strips ($p < 0.05$), reduced collagen infiltration ($p < 0.05$) and reduced minimal Ferets variance coefficient (used as an index of the severity of the pathology; $p < 0.05$). Serum CK levels were significantly lower in *mdx*^{HSP72} compared with *mdx* littermate controls ($p < 0.05$), which was further supported by a reduction in EBD-positive fibres indicating fewer damaged and/or necrotic fibres ($p < 0.05$).

Overexpression of HSP72 improved the dystrophic skeletal muscle pathology in *mdx* mice, especially in the severely affected diaphragm muscle. Further research is required to determine the therapeutic potential of this novel approach for DMD and related conditions.

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