Altered expression of heat shock proteins in Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is a genetic disorder characterized by severe muscle weakness and wasting. The eventual treatment for DMD will be gene- and/or cell-based therapies, but these techniques are far from perfected and affected boys will continue to die prematurely. Therefore, 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 (HSPs) are a group of proteins induced by cellular stress and are implicated in cellular protection. We have demonstrated the therapeutic potential of HSP72 induction for ameliorating the dystrophic pathology in the *mdx* and *dko* murine models of DMD (Gehrig *et al.*, 2012). The role of other HSPs in the regulation of dystrophic muscle structure and function is less clear. Of specific interest are HSP10, HSP27, HSP90 and HSP110, HSP10 and HSP27 that have been shown to protect muscle from damage (Sharp *et al.*, 2006; Kayani *et al.*, 2010). Increased HSP110 expression was observed in muscles of *mdx* mice (Doran *et al.*, 2006), and our preliminary data show reduced HSP90 expression in human DMD patients. This study investigated the protein and mRNA expression of HSP10, HSP27, HSP90 and HSP110 in *tibialis anterior* (TA) and diaphragm muscle from 4 week old and 10 week old C57BL/10, *mdx* and *dko* mice. We tested the hypothesis that TA and diaphragm muscles from dystrophic *mdx* and *dko* mice would exhibit reduced mRNA and protein expression of HSP10, HSP27 and HSP90 and increased expression of HSP110 compared with C57BL/10 controls, and that these changes will be more apparent in muscles from 10 week old mice.

All experiments were approved by the Animal Ethics Committee of The University of Melbourne and conducted in accordance with the code of practice stipulated by the National Health and Medical Research Council (Australia). Real-time polymerase chain reaction (RT-PCR) analyses showed that in the TA muscle of 4-week-old *dko* mice the expression of HSP27 and HSP90 was increased compared with C57BL/10, but this was normalized by 10 weeks of age ($P<0.05$). Compared with C57BL/10 mice, the expression of HSP27 was increased in the diaphragm of 4-week-old *dko* mice but was normalized by 10 weeks of age ($P<0.05$). Lastly, the expression of HSP10, HSP90 and HSP110 was altered in the diaphragm of 10-week-old *dko* mice, with HSP90 and HSP110 showing a slight decrease and HSP10 being substantially increased compared with C57BL/10 ($P<0.05$).

These findings demonstrate altered mRNA expression of HSP10, HSP27 HSP90 and HSP110 in dystrophic skeletal muscle. The dramatic increase in HSP10 expression in dystrophic muscle in particular, holds promise as a potential therapeutic target. Whether similar differences in protein expression exist will be assessed with western blotting. Investigating the expression of this protein family will help to elucidate their role in muscular dystrophy as well as their modulation as a potential therapy for ameliorating the dystrophic pathology.

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Supported by the NHMRC (project grant 485022)