Stimulating the Notch signalling pathway does not improve muscle regeneration after myotoxic injury in dystrophic mice

J.E. Church,[1] J. Trieu,[1] A.P. Russell[2] and G.S. Lynch,[1] [1]Basic and Clinical Myology Laboratory, Department of Physiology, The University of Melbourne, VIC 3010, Australia and [2]School of Exercise and Nutrition Sciences, Deakin University, 221 Burwood Highway, Burwood, VIC 3125, Australia.

Duchenne muscular dystrophy (DMD) is the most severe of the muscular dystrophies, affecting 1 in 3,500 live male births, and characterised by chronic muscle fibre degeneration and increasingly ineffective regeneration which results in fibrotic tissue infiltration and leads to major functional impairments. The Notch signalling pathway is a central regulator of development, and participates in regulation of myogenesis both in the embryo and postnatally following injury (Conboy *et al.*, 2002). Stimulation of the Notch signalling pathway has previously been shown to improve muscle regeneration in aged animals (Conboy & Rando, 2003). The present study sought to test the hypothesis that stimulation of the Notch signalling pathway would have a similarly beneficial effect on muscle regeneration in dystrophic mice.

Male C57BL/10 or *mdx* dystrophic mice (8-9 weeks) were used in these experiments. Briefly, mice were anaesthetised (ketamine 80 mg/kg and xylazine 10 mg/kg; *i.p.*) and the *tibialis anterior* (TA) muscle of the right hindlimb was injected with Notexin (1 μg/ml, *i.m.*) to cause complete muscle fibre degeneration. After 3 days, the mice were given a single intramuscular injection of either saline, a Notch antibody (to activate Notch signalling), a Jagged-Fc fusion protein (to inhibit Notch signalling), or their respective controls (Hamster IgG as a control for the Notch antibody and human Fc protein as a control for Jagged-Fc). Mice were then allowed to recover for a further 4 or 11 days (corresponding to 7 and 14 days post-injury). At these times, mice were anaesthetised (60 mg/kg, sodium pentobarbital, *i.p.*) and TA muscle function was assessed *in situ* using methods described previously (Gehrig *et al.*, 2010). Mice were killed at the end of the experiment by cardiac excision while still anaesthetized deeply.

We found that neither activation nor inhibition of Notch signalling at 3 days post-injury had any significant effect on force production by the regenerating muscles of either BL/10 or *mdx* mice when measured at 7 or 14 days post-injury. We then examined the effect of a single intramuscular injection of the Notch antibody (an activator of the signalling pathway) on muscle function in the *dko* mouse (a mouse model of muscular dystrophy with a more severe phenotype matching that of DMD patients). When muscle function was assessed 3 days after injection, force production was reduced significantly in the antibody-treated mouse compared with control ($p<0.05$).

These results suggest that, contrary to our hypothesis, activating the Notch signalling pathway in injured muscles of C57BL/10 or *mdx* mice does not improve muscle regeneration, and that activating the pathway in a severely dystrophic but otherwise uninjured *dko* mouse actually reduces muscle function. These findings reflect the complex and progressive nature of the muscle wasting in muscular dystrophy, and indicate that the continuous cycles and heterogeneous nature of the degeneration and regeneration of muscle fibres in DMD will be a significant barrier for therapeutic modulation of developmental signalling pathways.

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