Local insulin-like growth factor binding proteins are essential for successful skeletal muscle regeneration

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Increasing insulin-like growth factor-I (IGF-I) levels in skeletal muscle hastens muscle fibre regeneration after injury (Schertzer & Lynch, 2006). The actions of IGF-I are modulated strongly by six IGF binding proteins (IGFBPs) at both the systemic and local tissue level (Duan & Xu, 2005). Treating mice with an IGF aptamer that inhibits the systemic actions of the IGFBPs and elevates 'free' endogenous IGF-I can similarly hasten muscle fibre regeneration (Schertzer *et al*., 2007). Since IGFBPs also have effects on skeletal muscle independent of IGF-I, we tested the hypothesis that local IGFBPs are required for successful skeletal muscle repair after injury, and that muscle-specific inhibition of IGFBPs would compromise muscle fibre regeneration.

Twelve-week old C57BL/6 mice were anaesthetised (100 mg/kg ketamine/ 10 mg/kg xylazine), and the tibialis anterior (TA) muscle of the right hindlimb injected with the myotoxin, Notexin, to cause complete degeneration of all fibres and initiate spontaneous muscle fibre regeneration. Muscles were excised at different times post-injury to examine transcript expression of the IGFBPs. In separate groups of mice, the TA muscles were injected with an IGF aptamer (100 μg in DMSO, NBI-31772; Calbiochem), an IGFBP-2 antibody (200 μg/ml; R & D Systems), or their appropriate vehicle control at 3 days post-injury. Muscle function and histology were evaluated at 10, 14 and 21 days post-injury using methods described in detail previously (Schertzer *et al*., 2007). The effect of the IGF aptamer on myoblast proliferation *in vitro* was also determined. C2C12 cells were plated in 6-well plates at a density of 2.5×10^4 cells/ml in growth medium (DMEM, 10% FBS, 1% antibiotics) supplemented with varying concentrations of IGF aptamer (0.1-10 μM). After 48 hrs cells were trypsinised and counted using a haemocytometer.

The various IGFBP transcripts were differentially expressed during muscle regeneration, suggesting that IGFBPs play different roles during the various phases of regeneration. Inhibiting all six IGFBPs with the IGF aptamer suppressed functional recovery (P_o), and reduced the proportion of muscle fibres at 10 and 14 days post-injury ($p < 0.05$). The IGF aptamer also dose-dependently inhibited the proliferation of C2C12 cells *in vitro*. Specific inhibition of IGFBP-2 with a blocking/neutralising antibody during the early stages of regeneration also affected regeneration with structural and functional recovery being compromised ($p < 0.05$, main effect). These data indicate that the other IGFBPs were unable to compensate for the lack of IGFBP-2, highlighting the importance of this IGF binding protein in successful muscle repair after injury.

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Supported by the Muscular Dystrophy Association (USA)