Local insulin-like growth factor binding proteins are required for successful skeletal muscle regeneration after injury

S.M. Gehrig, J.D. Schertzer and G.S. Lynch, Basic and Clinical Myology Laboratory, Department of Physiology, The University of Melbourne, VIC 3010, Australia.

Although exogenous administration of insulin-like growth factor I (IGF-I) to mice can hasten muscle fibre regeneration after injury (Schertzer & Lynch, 2006), the levels and actions of IGF-I are modulated tightly by IGF binding proteins (IGFBPs) at both the systemic and local tissue level (Duan & Xu, 2005). Administration of an IGF aptamer to mice inhibits the systemic actions of IGFBPs, elevating 'free' endogenous IGF-I, a strategy which was also found to hasten muscle fibre regeneration (Schertzer *et al*., 2007). We tested the hypothesis that local IGFBPs are required for successful skeletal muscle repair after injury, and that specific inhibition of IGFBP-2 would compromise skeletal 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 was injected with the myotoxin, notexin, to cause complete degeneration of all fibres and thus initiate spontaneous muscle fibre regeneration. Muscles were harvested at 3, 5, 7, 10, 14, 21 and 28 days post-injury to examine transcript expression of the six IGFBPs. In separate groups of mice, the TA muscles were injected with an IGF aptamer (100µg in DMSO, NBI-31772; Calbiochem) or IGFBP-2 antibody (200 µg/ml; R & D Systems) 3 days post-notexin injection. Muscle structure and function were evaluated at 10, 14 and 21 days post-injury. To examine muscle function, mice were anaesthetised (60 mg/kg, sodium pentobarbital), and the right TA muscle was surgically exposed and the distal tendon attached to the lever arm of a force transducer, with the knee and foot immobilised. The TA muscle and surrounding limb was immersed in mineral oil at 37°C and maximal isometric force (P_o) determined at optimal muscle length *in situ*.

The various IGFBP transcripts were differentially expressed during muscle regeneration, indicating that IGFBPs have different roles during the various phases of muscle regeneration. Inhibiting IGFBPs with the IGF aptamer inhibited functional recovery (P_o), and reduced viable muscle tissue at 10 and 14 days post-injury ($p < 0.05$). Inhibiting IGFBP-2 during the early stages of regeneration, with an IGFBP-2 antibody, affected the regenerative process. Structural and functional recovery during regeneration were compromised ($p < 0.05$, main effect) after IGFBP-2 antibody injection. These data indicate an inadequate functional redundancy where other IGFBPs were unable to compensate for the lack of IGFBP-2.

Duan C & Xu Q. (2005) *General and Comparative Endocrinology*, **142:** 44-52.
Schertzer JD & Lynch GS. (2006) *Gene Therapy*, **13:** 1657-64.
Schertzer JD, Gehrig SM, Ryall JG & Lynch GS. (2007) *American Journal of Pathology*, **171:** 1180-88.

Supported by the Muscular Dystrophy Association (USA)