Characterisation of Suppressor of Cytokine Signalling protein expression in regenerating mouse skeletal muscle after injury

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Muscle injury can result in a significant loss of function that can impact on quality of life. A better understanding of the signalling pathways controlling skeletal muscle is critical for devising therapies to enhance muscle regeneration and function after injury. One of the key regulators of skeletal muscle regeneration is the JAK/STAT (Janus kinase/hyperlink) pathway, which transduces the signal of cytokines and growth factors. This pathway is negatively regulated by the Suppressor of Cytokine Signalling (SOCS) family of proteins. In many tissues, SOCS regulate inflammation, proliferation, differentiation and growth; important events for successful muscle regeneration. The expression patterns and relative importance of SOCS proteins during muscle regeneration have yet to be properly characterised. The aim of this study was to characterise SOCS1, SOCS2 and SOCS3 mRNA expression during skeletal muscle regeneration after injury, and examine the expression of Leukemia Inhibitory Factor (LIF), a key growth factor regulating SOCS expression.

Male C57BL/6 mice (˜12 weeks old) were anaesthetised deeply (76 mg/kg Ketamine and 10 mg/kg Xylazine; *i.p.*) and the tibialis anterior (TA) muscle injected with the myotoxin Notexin (1.6 mg/kg). Muscle function was assessed *in situ* at 7 days (n = 8), 14 days (n = 8) and 21 days (n = 7) post-injury using methods described in detail elsewhere (Schertzer *et al.*, 2007). Before performing functional analysis mice were anaesthetised (60 mg/kg Sodium pentobarbitone; *i.p.*). SOCS1, SOCS2, SOCS3 and LIF mRNA expression were assessed using Real-Time RT-PCR and normalised against total cDNA content.

Specific (normalised) force of injured TA muscles was decreased by 31%, 60% and 79% of control uninjured values at 7, 14 and 21 days, respectively ($p < 0.001$). Fibre cross-sectional area in injured muscles was 52% and 70% of control uninjured values at 7 and 14 days, respectively ($p < 0.05$). increased The percentage of centrally nucleated fibres was increased in injured muscles by 15-, 7- and 5-fold of control uninjured values at 7, 14 and 21 days, respectively ($p < 0.001$). SOCS2 mRNA expression was 30% lower at 7 days post-injury compared with uninjured controls ($p < 0.05$), but recovered to control uninjured levels by 21 days post-injury. There was no effect of injury on SOCS1 or SOCS3 mRNA expression at any time after injury. LIF mRNA expression was 400% higher at 21 days-post injury compared with control uninjured values ($p < 0.01$).

These preliminary findings implicate SOCS2 and LIF in successful muscle regeneration since they appear to play important regulatory roles in the events controlling muscle repair after injury.

Schertzer JD, Gehrig SM, Ryall JG, Lynch GS. (2007) *American Journal of Pathology*, **171:** 1180-8.
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