

## Poster Abstract

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### **Transient upregulation of certain smooth muscle-associated proteins in osteoprogenitor cells of early, soft fracture callus: implications for the fracture repair process**

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The essential contractile protein alpha smooth muscle actin ( $\alpha$ SMA) is abundant in osteoprogenitor cells of early soft fracture callus. We have also recently shown rat rib fracture callus displays smooth muscle(SM)-like tonic contractility *ex vivo* when stimulated by the  $\alpha_1$  adrenergic receptor (AR) agonist phenylephrine.

If such calluses are SM-like and capable of contraction, we hypothesized they could possibly relax via an established SM mechanism,  $\beta_2$ AR activation via the  $\beta_2$ AR agonist terbutaline. We also aimed to further define the nature of callus by exploring the possible presence and location of  $\beta_2$ ARs,  $\alpha_1$ ARs and various SM proteins.

Under anaesthesia male rats had one rib fractured and resulting calluses were removed 7, 14 and 21 days later for examination. An isometric force transducer quantified 7 day callus force in response to  $10^{-6}$ M terbutaline. Calluses were analysed using RT-PCR and immunohistochemistry.

There was no evidence of callus relaxation with addition of terbutaline ( $n = 5, p > 0.18$ ). Gene expression of  $\alpha_1$ ARs,  $\alpha$ SMA, SM myosin, calponin, h-caldesmon and smoothelin was up-regulated in callus; peaking at 7 days and returning towards unfractured levels by 21 days post-fracture. Data suggests there was no upregulation of  $\beta_2$ AR gene expression in calluses. Immunohistochemistry supported the RT-PCR findings that osteoprogenitor cells of early, soft callus possess  $\alpha_1$ ARs but not  $\beta_2$ ARs.

Increases in expression of certain SM-associated proteins in early callus provide further evidence of this tissue's SM-like nature. The presence of  $\alpha_1$ ARs and not  $\beta_2$ ARs in osteoprogenitor cells of early, soft callus implicates that contraction (rather than relaxation) is the preferential mechanism to create static tension in this callus. Static strain is known to stimulate SM cell differentiation into an osteoblastic phenotype. Therefore, contraction of early, soft callus is hypothesized to be an important aspect of the fracture repair process.