Tissue Stretch Decreases Procollagen-1 and TGF-β1 in Mouse Subcutaneous Fascia

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BACKGROUND Increased collagen and transforming growth factor beta 1 (TGF-β1) production are known to play an important role in connective tissue remodeling responses to injury, and in the production of excessive scarring and fibrosis. Although mechanical forces are known to influence connective tissue remodeling *in vivo*, their effect on TGF-β1 and collagen production in injured tissues are not well understood. We hypothesize that tissue stretch will attenuate TGF-β1-mediated collagen deposition in response to injury which may have implications for the therapeutic mechanisms of treatments that utilize tissue stretch.

METHODS Two different models were used to examine the effect of stretch on procollagen-1 and TGF-β1 protein levels in injured tissue:

1) *in vivo* mouse microinjury model: a 5 mm incision was performed under anesthesia in the middle of the back at the level of the scapula. A microsurgery blade was introduced along the subcutaneous fascia plane and the attachments between the fascia and subcutaneous muscle were cut over a 1.5 cm x 1.5 cm area on one side of the back lateral to the midline, with the other side of the back serving as the control. Mice where then randomized to either stretch or no stretch. In the stretch group mice underwent stretching (approximately 30% strain) of the trunk for 10 minutes (mice suspended by the tail with the forelimbs touching a slightly inclined surface) twice a day for 7 days. All mice were sacrificed by decapitation at day 7. Subcutaneous fascia of the back was dissected, fixed and immunohistochemically stained for procollagen-1 (a marker for newly formed collagen).

2) mouse subcutaneous fascia explant model: tissue was excised and kept in organ culture (DMEM culture media) for 4 days. One day after excision, fascia samples were either stretched (20% strain) for 10 min or not stretched. Aliquots of DMEM culture media were assayed by ELISA for TGF-β1 protein on days 0, 1 and 3 post stretch (or no stretch).

RESULTS In the *in vivo* microinjury model a significant difference in fascia procollagen-1 between injury and no-injury was observed in the absence of stretch ($p < 0.008$). However, when injury was followed by tissue stretch, no significant difference was found between injury and no-injury ($p=0.27$). In the *ex vivo* model, both stretched and unstretched fascia samples showed increased TGF-β1 protein levels in the media from day 0 to day 3. However, at day 3, TGF-β1 protein levels were significantly lower in stretched vs. unstretched fascia (repeated measures ANOVA, $p<0.01$).

CONCLUSION These experiments suggest that short term tissue stretch modulates TGF-β1-mediated tissue remodeling by attenuating procollagen-1 production. These results may be potentially relevant to the therapeutic mechanisms of treatments applying tissue stretch (eg. massage, Rolfing, yoga, acupuncture).

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