THE LOCALIZATION OF CONNECTIVE TISSUE GROWTH FACTOR IN A CYCLICALLY LOADED TENDON AT THE EPICONDYLE IN AN IN VIVO MODEL OF TENDINOSIS

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INTRODUCTION:

Connective Tissue Growth Factor (CTGF) has recently been investigated in wound healing and scar formation studies. Its role in the pathology of tendon overuse injuries has not yet been investigated. CTGF is a cysteine-rich secretory protein and belongs to a six member family who are known to be involved in fundamental biological processes such as cell proliferation, attachment, migration, differentiation, wound healing and angiogenesis as well as in the development of several pathologic conditions including fibrosis and tumorigenesis (4). CTGF has been identified as a potent inducer of extracellular matrix synthesis (2) and is up-regulated by tensile stresses (3, 7).

Soft tissue injuries are common in athletes and workers whose job functions require repetitive and forceful hand activities. The mechanism leading to tendinosis is not well known but likely involves microtears (6) and an inflammatory response (1). The purpose of this study was to evaluate the effects of *in vivo* cyclical digit loading on CTGF-positive staining cell density in the Flexor Digitorum Profundus (FDP) tendon at the epicondyle.

METHODS:

Nine female, New Zealand White rabbits weighing 3.49 kg (± 0.30) were used. Under general anesthesia, the FDP muscle on one limb was electrically stimulated to cyclically contract for 2 hours per day, 3 days a week, for 14 weeks (80h of cumulative loading). The contralateral limb, although restrained in the same manner as the loaded limb, did not receive a stimulus and served as the control. This study was approved by the University's Committee on Animal Research.

After inducing anesthesia with isoflurane, the rabbit was placed in a supine position with the forearms loosely secured to supports. A muscle stimulation needle (33G) was inserted subcutaneously in the middle forearm region over the central region of the FDP muscle and the needle tip was pushed back through the skin. A needle was similarly inserted in the contralateral limb, but was not stimulated. A fingertip glove was slipped over digit 3 and connected to a load cell to measure flexion force of the finger about the metacarpophalangeal joint. The muscle was stimulated (Grass) with a train of pulses at 1Hz, with a train duration of 200ms and a pulse rate of 100 pulses/s. The stimulation voltage was adjusted [6-12V] to maintain a peak fingertip force of 0.42N (15% of P_o). After two hours of repetitive loading, the stimulation electrode and fingertip glove were removed.

At 14wks, the animals were euthanized and the epicondyles were dissected with the FDP tendon and muscle attached. Samples were fixed, decalcified and sectioned. Serial sections were analyzed by immunohistochemistry for the presence of CTGF using a mouse monoclonal antibody directed against CTGF (10µg/ml) and then incubated with a biotinylated horse anti-mouse 2° antibody. Sections were stained with the Vectastain ABC system and developed with DAB substrate. Six areas of interest were digitally photographed at 200x: 3 areas along the enthesis (classified as inner, center and outer) and 3 corresponding areas 1500µm distal to the enthesis. The inner area is that part of the tendon that contacts the bone. Positive staining cells were manually counted in each region (200x400µm^2) and normalized by the area observed (Figure 1). Tissue preparation and cell counting was performed blinded to loading status. A mixed model repeated measures ANOVA was used to analyze differences in tear measures by region (inner enthesis, outer enthesis, inner distal or outer distal) and by limb loading status (loaded or unloaded). Post hoc analysis was performed using the Tukey method for multiple comparisons.

RESULTS:

Examinations of the wrist, forearm and elbow revealed no limping, reduction in gross claw flexion strength, or skin breaks. Mean rabbit weight at the end of the study was 3.89 kg (± 0.19). The average number of cells in the loaded tendons ranged from 584 to 778 cells/mm^2 and 397 to 570 cells/mm^2 in the unloaded tendons. The limb by region interaction term was not significant ($p = 0.48$). Loaded limbs had significantly greater CTGF staining cells than the unloaded limbs ($p < 0.0001$), regardless of region. Based on the Tukey follow-up tests, there were also regional variations; the outer region of the tendon distal to the enthesis had a lower CTGF cell staining density than the inner ($p = 0.018$) and outer ($p = 0.008$) regions at the enthesis. (Figure 2).

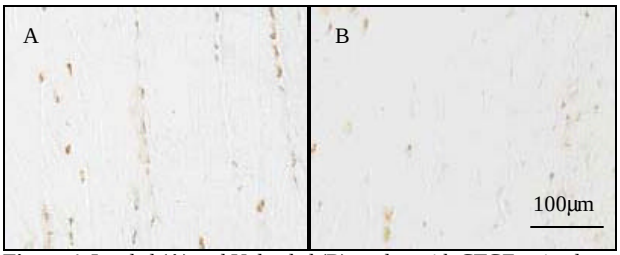

Figure 1. Loaded (A) and Unloaded (B) tendon with CTGF stained cells.

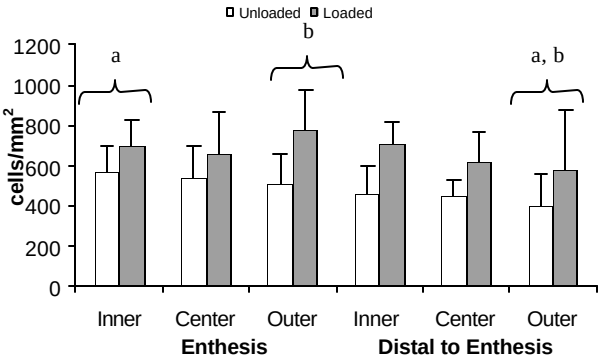

Figure 2. Positive staining cells were manually counted and normalized to tendon area.

DISCUSSION:

In this epicondylosis model, repetitive *in vivo* loading of the digit causes an increase in CTGF-staining cell density in the tendon compared to the nonloaded, control limbs. In addition, the cell density in both limbs varies by region. The regional differences in CTGF cell density may be due to a differential strain distribution within the FDP tendon as it is loaded. Recent studies (3, 4) have shown that CTGF is up-regulated under tensile stresses. In our model, the outer region of the loaded tendon at the enthesis has the greatest CTGF cell density. This may be due to higher tensile loading in this location and may be a region at higher risk for injury. This region also has an increased density of tissue microtears and VEGF producing cells (5, 6).

CTGF has recently been identified to play a role in wound healing and scar formation. The function it plays in the development of tendinopathy is unclear. The limitations of this study included the biomechanical exposure pattern, the effects of histologic preparation, and the use of rabbits in the model. The load applied to the finger was well within the physiologic range of the muscle and the number of repetitions and the duration of loading are less than that experienced by athletes and workers who perform repeated tasks. The rabbit forearm biomechanics and anatomy are not identical to the human; therefore, generalization of these findings to humans should be carried out with caution.

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