Introduction: Achilles tendinitis and rotator cuff disruption are debilitating injuries of the flexor tendons. Growth factors are potent proteins that can enhance local tissue responses and may play a role in improving the organized healing of these tendon injuries. Insulin-like growth factor I (IGF-I) is produced by endotenon fibroblasts and has been shown to improve healing in normal and damaged tendon explants by stimulating matrix synthesis and cell proliferation. The purpose of this study was to investigate the effects of IGF-I on in vivo tendon healing at the molecular, cellular and mechanical levels in a collagenase-induced model of flexor tendinitis.

Methods: Collagenase-induced lesions were created in the tibial region of the flexor digitorum superficialis tendon in both forelimbs of 8 horses using a commonly applied protocol. One limb of each horse was randomly assigned as the treated limb and the opposite limb was used as the untreated control. IGF-I (2 μg) was injected intratendinously every other day for 10 injections in the treated limb. An identical series of 0.9% NaCl injections was performed in the control limb. Both tendons were evaluated by limb circumference measurements and ultrasoundography prior to the study and weekly throughout the 60 day trial period. All protocols were approved by the IACUC. Following euthanasia, the tendons were harvested for mechanical testing, histology, immunohistochemistry, in situ hybridization, and biochemical assays. Mechanical data collected included tensile strain, serial load, and failure load. Load was normalized using tendon cross-sectional area (CSA) measured at the time of the final ultrasound examination. Parallel-embedded sections were examined by routine H & E and immunohistochemistry for collagen types I and III. Northern and in situ hybridizations were performed for collagen types I and III, and types I and III collagen were quantitated via CNBr cleavage and SDS-PAGE analysis. Total proteoglycan content was measured by the DMMB dye binding assay, total DNA content was measured fluorometrically and total hydroxyproline was measured by Pico-Tag rPHLC. Data were compared using a paired t-test. Significance was set at p < 0.05.

Results: Discrete tendinitis lesions of moderate severity were created in all tendons following collagenase injection. Local soft tissue swelling varied between horses but was less severe in the IGF-I treated limbs. No significant differences were identified between treated and control tendons for ultrasound cross-sectional area or limb circumference at any time point in the study. Significant reductions in the size of the tendon core lesion of the treated limbs were detected on days 28 and 56 (p=0.02). By 60 days after the first injection, the core lesions of both the treated and control limbs had gained sufficient strength such that only 2/16 (12.5%) failed at the lesion during mechanical testing. Six tendons (37.5%) failed proximal to the lesion and 5 (31.2%) failed distal to the lesion. There was no correlation between treated and control limbs and the location of tendon failure. The tendons sustained loads of 6,062 to 12,144 Newtons. No significant differences in ultimate failure loads or failure loads normalized by CSA were identified between treated and control tendons. The IGF-I treated tendons showed a strong trend toward an increase in stiffness compared to the saline treated group (p=0.08) (Table 1). Figure 1: In situ hybridization to type I procollagen mRNA of control and IGF-I treated tendons. Signal was stronger in the IGF-I treated (B) than in the control (A) limbs. Light field illumination.

Discussion: The increase in cell proliferation within the tendon tissue, though not statistically significant, has been previously reported and indicates one mechanism by which IGF-I can improve the intrinsic healing capacity of damaged tendon. compared to the control limbs. IGF-I resulted in greater reductions in lesion size on ultrasound, increased organization of tenocytes along lines of tension and increased type I procollagen expression. Other specific tendon matrix changes may have been masked in this longer-term study. In short term explant cultures, IGF-I was shown to increase collagen production; however, with increased time in culture, these differences diminished. The ratio of type I to type III collagen may be more significant in the later stages of healing. The increased stiffness of the IGF-I treated tendons indicates a tissue property more similar to normal tendon. Based on this data, the use of intratendinous IGF-I has potential for the improved treatment of debilitating tendon injuries.

References:

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