CHARACTERIZATION AND EXPRESSION OF INSULIN-LIKE GROWTH FACTOR BINDING PROTEINS IN HEALING TENDON LESIONS.

Introduction: Degenerative tendinopathy is a common and debilitating injury affecting elite and recreational athletes. Insulin-like growth factor 1 (IGF-I) is produced in a variety of tissues, including tendon, and is known to have beneficial effects on tendon healing. The IGF binding proteins (IGFBPs) bind IGF-I in the serum and tissues and modulate the bioavailability and activity of IGF-I. The pattern of expression of the IGFBPs in normal or healing tendon have not been described. Defining the expression of IGFBPs in normal and healing tendon is key to developing an understanding of the role of the IGF/IGFBP axis in the healing process. We hypothesize that the IGFBPs will exhibit a unique pattern of expression in normal and healing tendon that will correlate with tissue levels of IGF-I. The purpose of this study was to define the temporal expression of the IGFBPs in healing tendon, and to correlate the changes in expression of these binding proteins with those of IGF-I using a collagenase-induced model of flexor tendinitis in the horse.

Methods: Collagenase-induced lesions were created in the tensile region of both flexor digitorum superficialis (FDS) tendons of 12 adult horses. Horses were divided into 4 groups and were euthanatized at 1, 2, 4, and 8 weeks post-injection. All protocols involving live animals were approved by the IACUC. At the time of euthanasia, FDS tendons were harvested under RNase free conditions, and a portion of each tendon was fixed in 4% paraformaldehyde at 4°C for histology. IGF-I message and protein levels in the tissues were measured by TaqMan® PCR and radioimmunoassay respectively. Message expression for the IGFBPs was quantitated using TaqMan® probes with [3H]-IGF-I and western ligand blots probed with antibodies specific to the individual IGFBP-proteins. Data were analyzed by Kruskall-Wallis non-parametric ANOVA with the appropriate post-hoc comparison for differences between groups. Significance was set at p ≤ 0.05.

Results: Discrete core lesions of moderate severity were created in all tendons following collagenase injection. Message expression for IGF-I increased slowly following injury, peaked at 4 weeks and remained elevated throughout the course of the study (Fig 1). Tissue levels of IGF-I protein initially decreased in the 2 weeks following injury, peaked at 4 weeks, and remained elevated at 8 weeks (Fig 1).

Expression of the IGFBPs was universally low in normal tendon. Only one IGFBP (apparent MW 32) was detectable in the normal tendon; however, IGFBPs of apparent MWs of 24, 32, and 38-42 kDa were expressed in healing tendon (Fig 2a). Western immunoblots confirmed these bands to correlate with IGFBP-2, -3, and -4 respectively. IGFBPs -2 and -3 increased immediately following tendonitis induction and peaked at 2 weeks, remaining elevated throughout the course of the study. IGFBP-4 was slower to increase and peaked at 4 weeks (Fig 2b). The response of IGFBP-2 was most robust.

Expression of mRNA for IGFBP-2, -3, and -4 increased following injury and remained decreased throughout the course of the study (Fig 3). Levels of mRNA for IGFBP-6 were 10 fold higher than the other binding proteins.

Discussion: Expression of mRNA for IGF-I began increasing immediately following injury and continued increasing until 4 weeks. Protein levels for IGF-I initially lagged behind the message expression as expected. The initial decrease in tissue levels of IGF-I protein may have been due to physical damage to the cells and extracellular matrix or increased receptor binding and utilization of IGF-I. Peak protein levels of IGF-I correlated with the peak of mRNA expression for IGF-I at 4 weeks and confirmed translation of the new message into protein. The pattern of message expression for the IGFBPs was unique and varied in both the normal and healing tendon. The absence of message for IGFBP-1 in tendon is consistent with findings in other tissues.

The decrease in expression of IGFBP-5 and -6 is of particular interest for future investigations. Expression of IGFBP-6 on the ligand blots may not have been apparent due to the use of IGF-I only as the ligand. IGFBP-6 has a much higher affinity for IGF-II than IGF-I and may become apparent if [125I]-IGF-II is used as the ligand. The changes in message expression for the IGFBPs correlates with the increase in message expression for IGF-I. The peaks in protein expression for BP-2, -3, and -4 are well correlated with the increases in message expression for these molecules and with the changes in IGF-I expression. Levels of IGF-I and IGFBPs were low in normal tendon but increased immediately and in a coordinated manner in response to injury.


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