CATABOLISM OF PROTEOGLYCANS IN YOUNG VERSUS MATURE TENDON

Introduction

Tendon is a dense, fibrous connective tissue in which the cells are surrounded by an extracellular matrix, composed primarily of type 1 collagen. A number of non-collagenous proteins are also present including the large proteoglycan (PG) aggrecan and the small PGs, decorin and biglycan (1). The predominant PG in purely tensional regions is decorin, whereas in morphologically distinct fibrocartilaginous regions (which are subjected to compressive and frictional forces in addition to tensional loads) aggrecan, decorin and biglycan are present at significantly increased levels (2). Turnover of tendon PGs in both compressed and tensional regions of tendon may predispose the tissue to mechanical disruption of the collagen matrix, an effect which may be exacerbated with age. In articular cartilage, aggrecan degradation can be attributed to proteolytic cleavage within the interglobular domain (IGD) of the molecule, with two major cleavage sites occurring between amino acid residues Asn 341-Phe342 and Glu 373-Ala374 (human sequence enumeration) (3). The former site is cleaved by many of the matrix metalloproteinases (MMPs 1,2,3,7,8,9,10,13,14) (4), whilst the latter cleavage is generated by the recently identified enzyme “aggrecanase” (5). In this study we have determined the relative contribution of these enzymes to the catabolism of tendon PGs in young versus mature bovine deep digital flexor tendon (DDFT).

Methods

Bovine DDFT explants were dissected under sterile conditions from young and mature bovine metacarpophalangeal joints (2-week-old and 18-month-old, respectively), from compressed and tensional regions. Explants were cultured in DMEM containing 10% FBS for 3 days and subsequently in serum-free DMEM for a further 4 days with or without the catabolic stimuli, all-trans retinoic acid (RA; 10^{-6}M), interleukin-1 (IL-1; 10 ng ml^{-1}) and tumour necrosis factor-α (TNF; 100 ng ml^{-1}) and the anabolic stimuli, insulin-like growth factor-1 (IGF; 50 ng ml^{-1}) and transforming growth factor-β (TGF; 2 ng ml^{-1}). PG degradation products released either into the culture medium or retained within the tendon matrix were deglycosylated with chondroitinase ABC, keratanase and keratanase II and separated using 4-12% gradient SDS-PAGE. Electrophoretically separated samples were transferred to nitrocellulose and immunolocated with a variety of monoclonal antibodies against constitutive “structural” epitopes and neoepitopes generated by aggrecanase and MMP-related catabolism of the aggrecan IGD, as well as epitopes in the small PGs, decorin and biglycan.

Results

Data indicated that there was no evidence of release of MMP-generated aggrecan metabolites into the medium nor accumulation within the tissue in young or mature cultures of DDFT under conditions of basal or stimulated PG catabolism. However, aggrecanase-generated fragments were released with both basal and stimulated PG turnover in compressed and tensional regions of young and mature cultures (Figure 1). This finding contrasts with that of normal articular cartilage, where aggrecanase-generated fragments are only released when PG turnover is stimulated by exposure to cytokines (6). An increase in the release of these fragments was observed in mature versus young tendon and expression of aggrecanase-1 and -2 activity was also observed using RT-PCR analysis. Western analysis with the glycosaminoglycan (GAG)-specific antibodies, in young tendon, revealed the presence of aggrecan-degradation products with 6-, 0- and 4- sulphation; however, in mature tendon no positive immunostaining for 4-sulphated chains could be detected. Furthermore, an increased release of 0-sulphated aggrecan-degradation products occurred in mature tendon. Western analysis also revealed extensive catabolism of the small PGs, particularly decorin, in young and mature tendon.

Discussion

This study demonstrates that aggrecan catabolism and its release from normal explant cultures of DDFT involves cleavage by aggrecanase and that the increased turnover of aggrecan in mature tendon may be attributed to an increase in the activity of the enzyme. The data also suggests that the active enzyme is present and constitutively expressed in tendon (i.e., it is involved in normal, everyday turnover). Cleavage of aggrecan by MMPs does not appear to play a significant role in normal DDFT-PG turnover or cytokine-induced catabolism. The data also shows that there is an alteration in sulphation pattern on the aggrecanase-degradation products in association with increasing age, suggesting that specific sulphotransferase activity is affected after maturation. The extensive catabolism of the small PGs suggests that there is a rapid turnover of these molecules in young and mature tendon, which may compromise the integrity of the collagen matrix.

References

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