

Methods: Human knee articular cartilage with its subchondral bone was either obtained from a tissue bank or sampled during surgery for total knee joint replacement. Tissues were immediately processed for in situ hybridization and immunohistology. Fluorescence intensity was semi-quantified and values obtained for PH-20 were normalized by those obtained for cell DNA by using DAPI. The severity of the OA disease process was evaluated by using the histologic-histochemical grading system devised by Mankin.

Results: In normal cartilage PH-20 mRNA and PH-20 protein were only detected in few chondrocytes of the superficial cartilage layer as well as in few osteoclasts and osteoblasts of the subchondral bone. As the severity of the OA disease process increased, chondrocytes of middle and deeper cartilage layers expressed the enzyme both at the mRNA and protein levels. Scores of the Mankin's scale correlated significantly with the level of PH-20 expression as assessed by fluorescence intensity. The enzyme was also detected outside cartilage cells mostly in areas exhibiting erosions and in areas surrounding clefts. In specimens with moderate to severe OA lesions, one could also detect the enzyme signal in subchondral bone and in some connective tissues cells filling up the spaces between bone trabeculae.

Conclusions: Our data provide evidence that the PH-20 produced by chondrocytes might be implicated in the degradation of cartilage matrix during OA. Indeed, the enzyme not only degrades hyaluronan, the backbone of aggrecan aggregates and a backbone of the pericellular matrix, but also chondroitin sulfate chains. Our data also suggest that, in OA, other cell lines present in subchondral bone areas might contribute to the local production of PH-20.

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SELECTIVE INHIBITION OF AGGREGANASE PREVENTS AGGREGAN DEGRADATION AND PROMOTES INCORPORATION OF NEWLY SYNTHESIZED AGGREGAN INTO THE EXTRACELLULAR MATRIX OF HUMAN OSTEOARTHRITIC CARTILAGE

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Purpose: Depletion of aggrecan is one of the earliest changes observed in osteoarthritis (OA). Aggrecan is a chondroitin sulfate and keratan sulfate-bearing proteoglycan. Aggrecan cleavage by Aggrecanase results in the release of a large sulfated glycosaminoglycan (sGAG)-containing aggrecan fragments which diffuse out of the cartilage matrix. Inhibiting Aggrecanase activity is an attractive therapeutic target. The purpose of this study was to understand the effects of a selective Aggrecanase inhibitor on the fate of existing and newly synthesized proteoglycan in human osteoarthritic articular cartilage.

Methods: Human cartilage from total knee replacements harvested within 3 hours of surgery was used for this study (n=3 donors). Harvested cartilage was maintained in culture in the presence or absence of a selective Aggrecanase inhibitor. Total proteoglycan content of conditioned media or cartilage matrix was measured as sGAG by a colorimetric assay with dimethyl-methylene blue. Aggrecan released from the cartilage was further characterized using a monoclonal antibody (BC-3) that recognizes the neopeptide generated by Aggrecanase-mediated cleavage of aggrecan. Articular cartilage was cultured in the presence of [³⁵S]sulfate to enable localization and characterization of newly synthesized aggrecan.

Results: Human osteoarthritic articular cartilage from joints undergoing total knee replacement demonstrated significant, ongoing aggrecanase activity evidenced by release of aggrecan

cleaved at the "aggrecanase cleavage site" (G373-374A). Incubation in the presence of the selective Aggrecanase inhibitor reduced total proteoglycan loss. Cleavage of newly synthesized aggrecan was a significant component of the degradative process, and Aggrecanase inhibition resulted in increased incorporation of new proteoglycan into the cartilage matrix.

Conclusions: Aggrecanase activity is a characteristic of human osteoarthritis throughout the disease process. Inhibition of Aggrecanase activity reduced release of aggrecan degradation products from the articular cartilage matrix. Because newly synthesized aggrecan appeared to be especially susceptible to degradation by Aggrecanases, selective Aggrecanase inhibition resulted in a net increase in proteoglycan incorporation into the matrix of human osteoarthritic cartilage. These results indicate that inhibition of Aggrecanases will reduce aggrecan degradation in human osteoarthritic articular cartilage throughout the disease process, and may also result in a net increase in extracellular matrix proteoglycan.

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STIMULATING CARTILAGE OLIGOMERIC MATRIX PROTEIN PRODUCTION IN CARTILAGE REGENERATION

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Purpose: Cartilage Oligomeric Matrix Protein (COMP) is a protein present in the cartilage matrix. It binds to chondrocytes and collagen and is involved in interaction between chondrocytes and collagen and probably also in collagen fibrillogenesis. COMP is expressed more abundantly in OA cartilage than in normal healthy cartilage. The present study was designed to investigate the effect of growth factors on COMP deposition and the influence of COMP on collagen deposition and mechanical properties.

Methods: Bovine chondrocytes in alginate beads were cultured with or without 25 ng/ml IGF1, TGFbeta2 or FGF2. COMP gene expression was determined with quantitative RT-PCR. COMP protein production was determined with ELISA and Western blot (reducing and non-reducing). Human COMP (hCOMP) was also overexpressed in bovine chondrocytes using lentiviral transfection. hCOMP gene expression, COMP, collagen and proteoglycan deposition and mechanical properties were determined.

Results: Addition of TGFbeta2 resulted in more COMP gene expression than the control condition without growth factors or with addition of IGF1. The COMP deposition in alginate beads and excretion in the culture medium was also increased with addition of TGFbeta2. FGF2 resulted in less and partially degraded COMP. Lentiviral transfection with hCOMP resulted in elevated gene expression of hCOMP and increased COMP levels in the culture medium compared to untransfected cells. However, no difference was seen in the COMP deposition in the alginate bead. COMP overexpression also did not affect the deposition of collagen and proteoglycans or the mechanical properties.

Conclusions: It appears that COMP overexpression is not sufficient to increase extracellular COMP deposition in chondrocyte alginate culture. However, increased COMP deposition can be achieved by addition of TGFbeta, suggesting that modification of other factors such as collagen type IX or matrilins is also needed to increase COMP deposition in cartilage regeneration.