DISPARATE EFFICACY OF COLLAGEN HYDROLYSATE AND GLUCOSAMINE ON THE EXTRACELLULAR MATRIX METABOLISM OF ARTICULAR CHONDROCYTES

M. Schunck1, C.H. Schulze2, S. Oessler1
1Collagen Research Institute, Kiel, Germany, 2Department of General Surgery and Traumatology, Klinikum Elmshorn, Elmshorn, Germany

Purpose: Glucosamine has been used in the treatment of osteoarthritis (OA) for several years. More recently, collagen fragments were shown to provide a positive effect on joint health. The objective of this study was to investigate the efficacy of a specific type of collagen hydrolysate (CH) on the biosynthesis of ECM macromolecules in comparison with glucosamine sulfate (GS) and glucosamine hydrochloride (GH) in a chondrocyte culture model.

Methods: Primary porcine articular chondrocytes and human femoral head chondrocytes were cultured under reduced oxygen conditions. The culture medium was supplemented with various concentrations of CH. In parallel experiments chondrocytes were treated with either GS or GH in various concentrations up to 2.5 mM. At the end of the culture period the amount of cell-associated proteoglycans (PGs) and the extent of PG synthesis were quantified by measuring 35S-sulfate incorporation and by colorimetric assay. The amount of aggrecan accumulated in the ECM was determined by Western Blotting. In addition, type II collagen biosynthesis was quantified by means of ELISA technique. The results were confirmed by immunocytochemical detection of type II collagen and by analyzing the incorporation of [14C]-proline into matrix proteins.

Results: Supplementation of the culture medium with CH resulted in a statistically significant (p<0.05) increase of PG synthesis. The amount of cell-associated PGs was almost doubled after CH treatment, compared with the control cells. Administration of CH was also associated with increased aggrecan expression and a statistically significant (p<0.05) 1.5-fold increase of type II collagen biosynthesis. In contrast, the administration of GS or GH had no stimulatory effect on the type II collagen biosynthesis of the chondrocytes. Moreover, although slight differences could be observed between GS and GH, supplementation of glucosamine had no significant effect on the amount of cell-associated PGs, or total PG synthesis, compared to the controls.

Conclusions: These results indicate a stimulatory effect of CH on the synthesis of PG and type II collagen. In contrast, GS and GH failed to stimulate the synthesis extracellular matrix (ECM) macromolecules by chondrocytes. These data suggest that CH may help reduce degenerative changes of the ECM by stimulating anabolic processes in cartilage tissue.

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DERMATAN SULFATE REMOVAL DOES NOT CHANGE INDENTATION PROPERTIES OF ARTICULAR CARTILAGE

J. Lewis, M. Hall, D. Krawczak, N. Simha
University of Minnesota, Minneapolis, MN

Purpose: Decorin is attached to the Type II collagen fibrils in articular cartilage. It has been hypothesized that the dermatan sulfate (DS) attached to the decorin core protein projects out from the protein and forms a bond with adjacent fibrils, either directly to another DS chain or through an intermediary molecule, thus serving as a gluing molecule between the collagen molecules. As such, decorin would serve as a structural molecule, carry load in cartilage, and its damage would lead to change in mechanical properties. The purpose of this study was to test this hypothesis by digesting DS in bovine patellar cartilage with chondroitinase B (cB) and testing the cartilage by indentation before and after digestion.

Methods: Articular cartilage from bovine patella was tested in indentation before and after digesting with cB. For comparison, to validate our methods, we also tested cartilage before and after digestion with chondroitinase ACI (cAC), an agent known to alter indentation properties. In both cases, controls were cartilage specimens treated in the same way in buffer, without the enzyme. Digestion was with 0.1U/ml for 24 hrs at 37°C with gentle agitation. Indentation was with a 2 mm diameter flat ended non-porous cylinder on an ELF 3100 (Bose, Inc., Minneapolis, MN). Indentation was with a series of 7 step-hold displacements. Step loads were applied in approximately 0.1 sec; hold times were 300 sec. Instantaneous modulus, equilibrium modulus, and relaxation function were determined for each test. 5 indents for
each condition were performed on 4 test/control pairs for both cB and cAC. To verify that the cB removed a significant amount of the DS, specimens were cut into 14 sections, 7 in a top half layer and 7 in a bottom half layer, and Western blot analysis for the decorin protein performed on each section. For comparison, cAC specimens were cut in a similar fashion and total proteoglycans assayed by the DMB assay.

**Results:** cB had no detectable effect on rapid modulus, equilibrium modulus, or relaxation function in indentation. Conversely, cAC caused reduction in equilibrium modulus and relaxation function, consistent with published work of others. Approximately one-half of the DS was removed from the top layer. Assay of the cAC specimens for total proteoglycan showed similar digestion patterns, suggesting that the two enzymes acted similarly.

**Conclusions:** Removal of DS from the cartilage did not affect the viscoelastic properties of the cartilage, indicating that decorin is not a structural molecule in cartilage in indentation.

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**ACTIVATION OF C-JUN N-TERMINAL KINASE (JNK) IN ARTICULAR CHONDROCYTES BY MERCURY AND LEAD**

J.B. Collins, J.W. Neal, R.D. Graff

**UNC School of Medicine, Chapel Hill, NC**

**Purpose:** There is growing recognition that environmental factors may play a significant role in determining the health of musculoskeletal tissues, including cartilage. Many of the cellular mechanisms that contribute to cartilage catabolism such as oxidative stress, altered growth factor signaling, matrix mineralization and activation of metalloproteinases can be initiated by heavy metal toxicants. The aim of this study was to determine if heavy metal toxicants activate chondrocyte mitogen-activated protein kinases (MAPKs) that can then lead to altered cartilage homeostasis.

**Methods:** Chondrocytes were enzymatically isolated from commercially obtained 4–6 month-old porcine knee joints (approved by the UNC IACUC) by sequential digestion in pronase and collagenase, and grown in high-density monolayer culture (105 cells/cm2). Cultures were serum-starved overnight and subsequently treated with organic methylmercury (MeHg), inorganic mercuric chloride (HgCl2), or lead acetate (PbAc), for up to 60 minutes at varying concentrations prior to cell extraction with ice-cold lysis buffer. Additional cultured were treated with IL-1.

**Results:** PFE at the concentrations used was found to be non-toxic and remarkably effective in inhibiting IL-1β-induced human chondrocyte apoptosis. PFE also blocked the gelatinolytic activities of recombinant human MMPs as well as MMP activity recovered from SF of OA patients. Quantitative zymography also showed that in IL-1β-stimulated human chondrocytes cultures pretreated with PFE the substrate degrading activity of MMP-9 and MMP-13 was significantly lower (p < 0.05). Western immunoblotting showed that IL-1β-induced protein expression of MMP-9 and MMP-13 was inhibited when human chondrocytes were pre-treated with PFE. Quantitative RT-PCR showed that PFE blocked MMP-9 and MMP-13 mRNA expression. Furthermore, PFE inhibited IL-1β-induced MMP-9 and -13 gene promoter activity (p < 0.005) in transfected human chondrocytes indicating that PFE was acting at the level of transcription. Histochemical and biochemical analysis of human cartilage explants showed that the proteoglycan and Type-II collagen content loss was significantly less (p < 0.05) in cartilage explants when PFE was present during IL-1β stimulation or when activated recombinant MMPs were exogenously added to explant cultures pretreated with PFE. Chondrocytes cultured in the presence of PFE also showed increased proteoglycan and collagen synthesis. PFE also blocked the IL-1β-induced loss and inhibition of matrix synthesis.

**Conclusions:** Our results indicated that PFE blocked the IL-1β-induced human cartilage degradation and MMP-9 and MMP-13