Osteoarthritis and Cartilage Vol. 14, Supplement B S115

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 with 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.

P203
INHIBITION OF IL-1β-INDUCED HUMAN CARTILAGE DEGRADATION BY POMEGRANATE
M. Shukla1, K. Gupta1, K.A. Khan2, C.J. Malemud1, T.M. Haqqi1
1Case Western Reserve University, Cleveland, OH, 2Aligarh Muslim University, Aligarh, India

Purpose: Pomegranate fruit (Punica granatum L) is revered through the ages for its medicinal properties. Pomegranate fruit (PF) or its extract (PFE) is widely used in Asian and Mediterranean cultures for the treatment of inflammation, for pain in arthritides and other diseases. Osteoarthritis (OA) is the most common musculoskeletal disease among the aging population. Pro-inflammatory cytokine IL-1β plays a dominant role in OA pathogenesis which is characterized by degeneration of articular cartilage of the joints in hands, knees, spine and hips. We have evaluated the effects of a standardized PFE preparation on human OA cartilage and OA chondrocytes treated with IL-1β with a view to determining whether consumption of PF or PFE can be useful in preventing joint cartilage degradation in OA.

Methods: Human cartilage and synovial fluid (SF) were obtained at the time of hip arthroplasty. Cartilage explants were prepared by standard methods and chondrocytes were liberated by enzymatic dissociation from femoral heads. Cartilage explants or chondrocytes were treated with IL-1β (5 ng/ml) or IL-1β and PFE (10 - 200 μg/ml) using previously described methods. The effect of PFE on IL-1β-induced apoptosis in chondrocytes was determined by viability assays and Western blotting. Substrate-embedded enzymography and/or collagen degradation assays were used to characterize the MMP activity in conditioned media and SF. Western immunoblotting and immunoprecipitation was used to confirm the presence and/or interaction of specific proteins in the SF and conditioned media. Quantitative RT-PCR was employed to quantify MMP mRNA expression levels. Human chondrocyte nucleofection with reporter constructs was used to analyze the effect of PFE on IL-1β-induced gene promoter activity. Cartilage explants were stained with Safranin-O/Fast Green. Matrix proteoglycan and collagen degradation was determined in culture supernatant using metachromatic assays. The results were analyzed using the Student-t test where p<0.05 was considered significant.

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 lowered (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...