Introduction: Cyclooxygenase (COX) and COX-derived Prostaglandin E2 (PGE2) is one of the main catabolic factors involved in the progression of OA [1-3]. COX-1 - COX-3 catalyze the initial step in the prostaglandin biosynthesis, the conversion of arachidonic acid into PGH2 [4], which is subsequently converted to PGE2. Although COX-1 and -2 share similar structural and biochemical properties, they are encoded on separate chromosomes and exhibit distinct physiologic functions. Traditionally, COX-1 was thought to serve a homeostatic function, required for tissues to maintain a basal level of PGs, and COX-2, the “inducible” isoform is upregulated by diverse stimuli including cytokines, mitogens, growth factors [5]. To examine the clinical relevance of PGE2 in cartilage degeneration we determined the modulated endogenous level of PGE2 production in knee joint synovial fluids collected from patients of OA and rheumatoid arthritis (RA) compared to normal. We also evaluated the catabolic action of PGE2 by assessing the production of proteoglycan (PG) and MMP-13 after stimulation with PGE2 using human adult articular chondrocytes.

Materials and Methods: Human synovial fluid was aspirated within 24 hr of death from the knee joints of asymptomatic human organ donors with no history of joint diseases (the Gift of Hope Ortan & Tissue Donor Network). Synovial fluid was also obtained with appropriate consent from OA and RA patients of the Rush University (Rheumatology) who were undergoing diagnostic or therapeutic arthrocentesis. Human articular chondrocytes were isolated from ankle or knee cartilages and were cultured under diagnostic or therapeutic arthrocentesis. Human articular chondrocytes were isolated from ankle or knee cartilages and were cultured using a modification of the previously described method [6-8]. Level of PGE2 was measured by ELISA (R&D System). Immunoblotting was performed by loading equal amount of total protein in the conditioned medium or cell lysates by protein assay (Pierce) on 10% SDS-PAGE gels. PG production was assessed by Dimethylmethane blue (DMMB) assay and normalized by DNA assay for as described previously [7]. PGE2 and Inhibitors of COX-1 (Piroxicam), COX-2 (DUP 697), and COX-3 (Acetaminophen) were purchased from Calbiochem and Tocris. Analysis of variance was performed using StatView 5.0 software. P values <0.05 were considered significant.

Results: The endogenous level of PGE2 was increased in OA and RA synovial fluids (Fig. 1). The presence of PGE2 showed no significant induction of MMP-13. Nevertheless, incubation with inhibitors of COX-2 and COX-3 inhibited the level of MMP-13 production (Fig. 2). Similar inhibitory results by COX inhibitors were observed after cells were stimulated by IL-1beta. The presence of PGE2 reduced PG production in a dose-dependent manner by human adult articular chondrocytes cultured in alginate beads for 21 days (Fig. 3). Importantly, the presence of COX-2 and COX-3-specific inhibitors completely blocked the suppressive effect of PG production mediated by IL-1 beta. Similar results were observed when the PG production was inhibited by bFGF in which the co-incubation with inhibitors of COX-2 and COX-3 antagonized the catabolic action mediated by bFGF.

Discussion: Mechanical injury or compression of articular cartilage stimulates production of PGE2 (9). Our studies suggest that PGE2-generated signaling pathway plays a role in cartilage degradation by suppressing PG production. Whether PGE2 inhibits PG synthesis is currently under investigation.

We did not observe the significant induction of MMP-13 after stimulation with PGE2. Inhibitors of COX-2 and COX-3, on the other hand, significantly modulated the production of MMP-13 and PG in the presence of pro-inflammatory cytokines and growth factor such as IL-1 beta and bFGF. These results suggest that other prostaglandin(s) derived by COX-pathway may be involved in the production of MMP-13 in human adult articular chondrocytes.

Blocking the COX-3 pathway potently inhibited MMP-13 production whereas the inhibition of the COX-2 cascades strongly promoted PG production. Identification of a precise common target molecule of COX-2 and COX-3 could lead to novel therapeutic approaches for cartilage degenerative diseases such as OA.


Acknowledgements: This work was funded by NIH RO1 (AR053220), NIH training grant 2T32-AR-007590, Arthritis Foundation, National Arthritis Foundation. Thanks to the Gift of Hope Organ and Tissue Donor Network and Drs. Margulis A, Block JA, Schmid TM for donor tissues & synovial fluids. IL-1β and bFGF are kindly provided by Amgen and NCI, respectively.