Finally, indomethacin increased the cell death induced by Ro+IL-1β; however, apoptosis induced by TNF-α+Ro was not modified by indomethacin.
Conclusions: These results confirm that TNFα and IL-1β differently regulate apoptosis machinery in human chondrocytes in this cell death model and that the differential effect of these cytokines is PGE_2 independent. Indomethacin potenciate the effect of IL-1β on cell death and this may be explanation for the reported effect of indomethacin in joint destruction progression.

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RESVERATROL, THE ANTIOXIDANT OF RED WINE, PREVENTS IL-1 MEDIATED MITOCHONDRIAL DYSFUNCTION AND ATP DEPLETION: ROLE OF PGE2 AS A CAUSE OF CELLULAR ENERGY DEPLETION

M. Dave, **M. Attur**, G. Palmer, J. Patel, H. Al-Mussawir, S.B. Abramson
NYU-Hospital for Joint Diseases, New York, NY

Purpose: Apoptosis of articular chondrocytes is considered a major pathogenic feature of osteoarthritis. Studies suggest that decreased mitochondrial function and ATP generation are key initiating events in this process. In the present study, we investigated the effects of resveratrol (RSV) on mitochondrial function and apoptosis in OA chondrocytes and the potential role of the inflammatory eicosanoid, PGE2.
Methods: Chondrocytes were isolated from patients undergoing knee replacement surgery. Assays were performed to measure resveratrol effects on PGE2 synthesis, COX activity, MMP production and proteoglycan synthesis. Apoptotic effects were determined by measuring mitochondrial membrane potential, ATP levels, annexin staining and cytochrome c release.
Results: We have previously demonstrated anti-inflammatory effects of RSV on both the spontaneous and IL-1-mediated production of PGE2 via inhibition of COX-2 expression and activity in OA chondrocytes; this effect was associated with marked inhibition of IL-1 effects on proteoglycan synthesis and matrix metalloproteinase expression at RSV concentrations of 5 - 10 uM. To investigate whether RSV can also protect OA chondrocytes from IL-1 - induced apoptosis, we examined apoptosis, mitochondrial membrane potential (MMP) and ATP generation following IL-1 stimulation with or without RSV pretreatment. Using a mitochondrial dye (JC1), MMP changes were tracked by fluorescence microscopy. Exposure of chondrocytes to IL-1 (10 ng/ml) caused MMP depolarization observed as a shift from red to green fluorescence. RSV pretreatment (10 uM) blocked this shift, restoring MMP to near control (unstimulated) levels. Further evidence of IL-1 or PGE2 alone, induced loss of mitochondrial function was demonstrated by decreased ATP levels (44% and 65% of untreated controls, respectively) and also observed decreased mitochondrial biomass (50% and 34% of control, respectively), using a mitotracker dye. Restoration of both ATP levels (111% of control, for IL-1; 68% for PGE2) and mitochondrial biomass (176% of control, for IL-1; 173% for PGE2) were again observed following RSV pretreatment. To determine whether these protective effects of RSV prevented apoptosis, annexin V was assessed using FACS analysis of IL-1 treated OA chondrocytes. IL-1 stimulation resulted in a high “apoptotic index” of 39 (compared to 6.4 for unstimulated cells) determined as the ratio of the annexin, Propidium iodide (PI) positive cell population divided by the total number of annexin positive cells. RSV pretreatment restored the apoptotic index from 39 to 16 in IL-1 treated cultures, confirming its anti-apoptotic capability. Importantly, add-back of PGE2 (10 um) to RSV-treated cultures ameliorated its anti-apoptotic effect.
Conclusions: IL-1 induced mitochondrial dysfunction and apoptosis in OA chondrocytes is mediated in part by induction of PGE2 and can be reversed by resveratrol. Therefore, blocking of PGE2 mediated effects on mitochondrial function could represent a previously unappreciated strategy for disease modification in OA.

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INFLUENCE OF OP-1 and IGF-1 ON CARTILAGE SUBJECTED TO COMBINED MECHANICAL INJURY AND CO-CULTURE WITH JOINT CAPSULE

C.A. Wheeler, A.R. Perez, A.J. Grodzinsky
MIT, Cambridge, MA

Purpose: Progression to osteoarthritis after joint injury involves multiple tissue interactions. The objective of this study was to test whether OP-1 and IGF-1 protect cartilage and joint capsule (JC) following injury, using an in vitro model incorporating mechanically injured cartilage co-cultured with JC.
Methods: Tissue Harvest: Bovine calf cartilage disks were harvested from the patello-femoral groove and equilibrated for 2 days under free-swell (FS) conditions in serum-free DMEM supplemented with 1% ITS. JC was excised adjacent to the femoral condyles and equilibrated for two days. Injury and Time Course: At time = 0, disks were allocated into one of 8 conditions; (1) FS, (2) normal cartilage co-cultured with JC (Co), (3) cartilage mechanically injured (INJ, 50% strain at 1mm/sec), and (4) injured cartilage co-cultured with joint capsule (INJ+Co), (5-8) with or without growth factors (100ng/ml OP-1 + 300ng/ml IGF-1) throughout culture. Gene Expression: Cartilage disks and JC were flash frozen 2, 8, 24, 48, and 72 hours after injury; RNA was extracted, reverse-transcribed, and mRNA levels of 48 cartilage relevant genes measured using qPCR and normalized to 18s. K-means-Clustering analysis was performed to determine gene co-expression patterns. Protein Biosynthesis: 35S-sulfate and 3H-proline incorporation (for proteoglycans and total protein synthesis) were assessed in the last 24 hours of a 1, 4, 8, 12, or 16 day culture post injury. Statistics: Significance was determined using the Wilcoxon-Sign ranked Test at the $p < 0.05$ level.
Results: Cartilage Gene Expression: Key ECM molecules (aggrecan, collagens) were significantly down-regulated in the presence of Co, INJ, and INJ+Co. Proteinases (MMP-1,3,9,13; ADAMTS-1,4,5) and TIMP-1,2,3 were maximally expressed versus controls by INJ+Co. GF failed to rescue ECM down-regulation, while up-regulating proteinases transcripts. iNOS and caspace-3 were maximally expressed under Co alone; iNOS reached values of 2000x FS. Clustering analysis revealed 5 distinct gene expression profiles. Group-1 (ECM molecules) was down-regulated with Co, INJ, and INJ+Co. Group-2 (iNOS, Cas-3) was up-regulated by Co. Groups-3,5 (proteases) were maximally expressed by INJ+Co. Group-4 (oxidation genes) was responsive to GF treatment. Joint Capsule Gene Expression: Basil levels of JC expression showed significantly higher levels of protease abundance compared to normal cartilage. All genes in JC (except ADAMTS-5) were stimulated by GF treatment. Chondrocyte Biosynthesis: 35S-sulfate and 3H-proline incorporation was significantly reduced by Co and INJ+Co. GF treatment slightly increased 35S-sulfate incorporation for all conditions.
Conclusions: GF treatment stimulated most of the genes measured, suggesting that the growth factor combination upregulated general remodeling rather than just anabolic stimulation of ECM. Aggrecan and collagen transcript levels were severely down regulated under each injury stimuli, and GF treatment failed to protect such decreases in transcript levels. GF was able to increase protein synthesis (ongoing studies using immunohistochemistry are to identify specific anabolic and catabolic events). The joint capsule expressed proteases at levels orders of magnitude greater than non-injured cartilage, suggesting the probability of cartilage-JC communication. GF down-regulated ADAMTS-5