formation), TRACP (osteoclast number), and GAG release (cartilage turnover). Overall cell viability was monitored using the dye Alamar Blue. Passive release from metabolically inactive femur heads was measured as background.
Results: Stimulation of the femur heads with RANKL, PTH, Il-1α and OSM + TNF-α led to an increase in CTX-I release. Adding GM6001 to OSM + TNF-α abrogated the release of CTX-I. Femur heads stimulated with Il-1α, OSM + TNF-α and OSM + TNF-α + GM6001 induced an increase in sGAG release. CTX-II release was increased by RANKL, PTH, Il-1α, OSM + TNF-α and IGF-I. CTX-II release from the OSM + TNF-α condition was abrogated when treated with GM6001. The osteoclast marker TRACP increased when stimulated by RANKL, PTH, Il-1α, OSM + TNF-α, OSM + TNF-α + GM6001. PIINP release was reduced when stimulating with Il-1α, OSM + TNF-α, OSM + TNF-α + GM6001, whereas PTH and IGF-I increased PIINP release.
Conclusions: We have established a whole tissue model for osteoarthritis consisting of both cartilage and bone, and which is highly responsive to both catabolic and anabolic stimulation. This is useful for testing potential treatments for OA interfering with more than one aspect of the pathological situation. Further it allows for investigating interactions between cartilage and bone cell types.
Hopefully, future treatments for OA may be better identified after the establishment of such a system for drug screening.

106 LOCALIZATION OF MMP- AND AGGRECANASE-GENERATED NEO-EPITOPES IN OA ARTICULAR CARTILAGE

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Purpose: MMP- and aggrecanase-mediated degradation of the cartilage matrix, and aggrecan in particular, have been extensively studied in cartilage explants as these two families of proteases are the most important proteolytic enzymes involved in cartilage turnover. Catabolic stimulated bovine explants cultures have been reported to release aggrecan-fragments carrying aggrecanase-mediated neo-epitopes (e.g. the amino acid sequence ^{374}ARGSV...) in the early phases, while the release into the supernatant of fragments expressing the MMP-mediated neo-epitopes (such as ^{342}FFGVG...) is delayed until the later stages. We therefore wanted to investigate if this separation in time was reflected in the differential localization of the aggrecan fragments in the cartilage tissue. The overall aim of the study was to determine the localization of aggrecan-related neo-epitopes in human sections of articular cartilage.
Methods: Human OA articular cartilage was obtained from knee replacement surgery. Full depth biopsies were isolated from areas proximal to lesions, followed by fixing in paraformaldehyde, decalcification and paraffin embedding. Sequential cartilage section were immunohistochemically stained for presence of MMP and aggrecanase-mediated neo-epitopes using monoclonal antibody AF28, BC-3 and 1H11 recognizing the ^{342}FFGVG..., the ^{374}ARGSV... and the NITEGE^{373}, respectively.
Results: IHC staining of the cartilage sections aggrecanase-mediated aggrecan fragments in the proximity of the chondrocytes in the upper zones. However, aggrecan fragments generated by aggrecanases – BC-3 and 1H11 – were found at the interface between the upper zone and the superficial layer. Since the superficial layer is still present at this part of the biopsies (low degree of erosion) it could depict the presence of aggrecanases-mediated fragments at earlier stages of disease. In contrast, MMP-mediated fragments were mainly observed in the upper-mid zone where the superficial layer was lost (high degree of erosion), specifically in the surrounding of clusters.
Conclusions: Our immunehistochemistry results support earlier reports, that the release of aggrecan fragments into the supernatant of the catabolic stimulated explants cultures showed a bi-phasic pattern with aggrecanase-mediated release at early time points and MMP-mediated release of aggrecan fragments at the later stages. We speculate that MMP and aggrecanases activity is related to disease states more than to specific sites. Furthermore that MMP-mediated degradation is related to areas with high cellular activity (e.g. clusters). The molecular mechanism and sequence of events is still unclear, but current study gives some direction to which path to follow.

107 WHOLE BLOOD LEAD (Pb) LEVELS AND PUTATIVE OSTEOARTHRITIS BIOMARKERS IN AFRICAN AMERICAN AND CAUCASIAN MEN: THE JOHNSTON COUNTY OSTEOARTHRITIS PROJECT

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Purpose: Lead (Pb) is a heavy metal that affects many aspects of bone including osteoclasts, osteoblasts, and calcium and vitamin D metabolism, and may have effects on cartilage as well. We have reported associations between whole blood Pb levels and radiographic OA severity in men and women, and with selected OA biomarkers in women. The purpose of this analysis was to examine associations between putative OA biomarkers and blood Pb levels in African American and white men.
Methods: A total of 333 men in the Johnston County OA Project Metals Exposure Sub-study (mean age 64.8 (10.7) years, 38.4% African American) had available demographic and clinical data and whole blood, serum, or urine samples for whole blood Pb and biomarkers assessments. Whole blood Pb was measured by inductively coupled plasma mass spectrometry at the Inorganic Toxicology laboratory, Division of Laboratory Sciences, National Center for Environmental Health, CDC, Atlanta, Georgia. Urine C-telopeptide fragments of type II collagen (CTX-II), cross linked N telopeptide of type I collagen (NTX-I), serum hyaluronic acid (HA), cartilage oligometric matrix protein (COMP), and type II procollagen (CPII) were measured by commercially available kits. Natural logarithm (ln) transformation was used to produce distributions close to normality for blood Pb and biomarkers. Spearman correlation coefficients were calculated between ln Pb and ln of each biomarker. Analysis of covariance models were used to examine associations between blood Pb levels and the 5 chosen biomarkers with ln transformed biomarkers as outcomes, adjusting for age, race, and BMI. Effect modification between ln Pb and race were examined, with significance defined by p-values <0.1 for interaction terms.
Results: Median Pb levels were 2.2 ug/dL (0.5−25.1) and were higher in African American men than white men ($p < 0.0001$). In bivariate associations, ln Pb was correlated with ln CTX-II ($r = 0.12$, $p = 0.035$) and ln COMP ($r = 0.20$, $p < 0.0002$), but not with ln NTX-I, ln HA, or ln CPII ($r = 0.02$, 0.0008, and 0.13, respectively, $p > 0.13$). In adjusted models, ln Pb was associated with mean ln CTX-II ($p = 0.024$) and COMP ($p = 0.0001$). There were no notable race and ln Pb interactions.
Conclusions: Mean blood Pb levels were associated with urine CTX-II and serum COMP, but not serum HA or CPII in both African American and white men. These data suggest that Pb may have an effect on type II collagen and non-collagenous matrix proteins. Potential effects of Pb in the pathogenesis of OA, then, are likely to be related to alterations in these factors, but less likely to effects on synovial inflammation.

108 AUROTHIOMALATE INHIBITS COX-2 EXPRESSION AND PGE_2 PRODUCTION IN CHONDROCYTES BY INCREASING MKP-1 EXPRESSION AND DECREASING p38 AND JNK PHOSPHORYLATION

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Purpose: Disease-modifying anti-rheumatic drugs (DMARDs) suppress inflammation, and retard cartilage degradation and bone erosion in arthritis. The molecular mechanisms of action of many traditional DMARDs are not known in detail. Inducible prostaglandin synthase (cyclooxygenase-2, COX-2) is highly expressed in OA and RA cartilage and it produces high amounts of proinflammatory prostanoids in the joint. The signaling mechanisms involved in the up-regulation of COX-2 in chondrocytes are not known in detail. In the present study we investigated the effects of DMARDs on mitogen-activated protein kinase (MAPK) pathways and MAP kinase phosphatase-1 (MKP-1) in immortalized H4 chondrocytes*.
Methods: PGE_2 production was measured by RIA, protein expression was measured by Western blot and mRNA expression was measured by quantitative PCR.
Results: We investigated the effects of traditional DMARDs on MKP-1 expression in chondrocytes. Unlike the other tested compounds (cyclosporin A, hydroxychloroquine, leflunomide, its active metabolite A771726, methotrexate and sulfasalazine), aurothiomalate was found to enhance MKP-1 expression. Aurothiomalate inhibited Il-1β-induced COX-2 expression and prostaglandin E_2 (PGE_2) production by destabilizing COX-2 mRNA as did p38 MAPK inhibitor SB203580. Interestingly, aurothiomalate