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

inhibited the phosphorylation of p38 and JNK kinases along with its enhancing effect on MKP-1 expression; and when MKP-1 was down-regulated by siRNA, aurothiomalate's ability to inhibit p38 and JNK phosphorylation, and COX-2 expression reduced significantly.
Conclusions: The results provide a novel mechanism for the anti-inflammatory action of aurothiomalate through increased MKP-1 expression and reduced p38 and JNK MAP-kinase activation, and COX-2 expression. The results propose MKP-1 as a promising novel target for the development of disease modifying drugs for RA and OA.
*Murine H4 chondrocyte cell line was kindly provided by Peter van der Kraan, Laboratory of Experimental Rheumatology, University Medical Center Nijmegen, Netherlands.

109 IDENTIFICATION OF AGGRECANASES-MEDIATED AGGRECAN FRAGMENT CIRCULATING IN HUMAN PLASMA FROM PATIENTS SUFFERING FROM RHEUMATOID ARTHRITIS (RA)

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Purpose: Aggrecan, the main proteoglycan of articular cartilage, is composed of a core protein containing 3 globular domains G1, G2 and G3. The interglobular domain between G1 and G2 is the primary site for enzyme attacks of the molecule, matrix metalloproteinases (MMPs) and aggrecanases, generating fragments with specific N-terminal ends. The aim of this study was to identify aggrecan fragments circulating in human serum/plasma with specific antibodies corresponding to neoepitope generated by aggrecanases.
Methods: Pools of serum from controls (n = 22; mean age: 62 yrs ±2.7) and patients suffering from osteoarthritis (n = 19; mean age: 64±4) and plasma from patients suffering from RA (n = 20; 10 men and 10 women, mean age: 61.7 yrs ±3.9, DAS > 4.8) were investigated with affinity purification and Western blots using two monoclonal antibodies. First, monoclonal antibody BC-3 recognizing a neoepitope generated by aggrecanases in position 374-ARGSV. Secondly, a monoclonal antibody F-78 was used recognizing a repetitive epitope exposed at least twice on G1 and G2 domains.
Results: In Western blot, BC-3 recognized a band migrating at 45 kDa in RA patients only. The strong reactivity was abolished by pre-incubation with the peptide corresponding to BC-3 epitope. To further investigate the distribution of ARGSV-carrying neoepitopes in RA plasma, we subjected the plasma to affinity purification using either BC-3 or F-78. Again, the major reactivity was confined to a 45 kDa band using either BC-3 or F-78 as detecting antibody in western blots of the eluted material.
Conclusions: We have identified an aggrecan fragment of MW 45 kDa generated by aggrecanase cleavage with a N-terminal neoepitope 374-ARGSV and containing G2 domain suggesting that this fragment is a good candidate marker for a sandwich assay using monoclonal antibodies BC-3 and F-78. Apparently, this fragment of aggrecan is primarily found in circulation of RA patients and additional investigations are underway to determine the molecular nature of this fragment.

110 NEW BIOCHEMICAL MARKERS OF COLLAGEN TYPE II DEGRADATION

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Purpose: One hallmark of the progression of osteoarthritis and rheumatoid arthritis is the gradual destruction of articular cartilage in affected joints, in part mediated the matrix metalloproteases (MMPs) and aggrecanases. Collagen type II is found almost exclusively in cartilage tissue where it constitutes over 60% of the dry weight. Degradation of collagen type II is for the major part mediated by MMPs. Because of the abundance of collagen type II in cartilage and its tissue specificity, MMP generated fragments of type II collagen (neo-epitopes) are highly interesting as cartilage degradation markers. The aim of this study was to develop new biochemical enzyme immuno assays (EIA) of MMP cleaved collagen type II fragments to be used as markers of cartilage degradation.
Methods: Two neoepitopic sites representing ubiquitous MMP-generated fragments of collagen type II identified from cleavage of collagen type II were selected. The two neoepitopes corresponded to cleavage between amino acids 845–846 (neoepitope I) and 872–873 (neoepitope II).
Peptides reflecting the neoepitopic sites (^{846}LTGPAG and RDGAAG^{872}) were synthesized and used for immunization of balb/C mice and rabbits. Competitive EIA assays were established using biotinylated synthetic peptide as coater and the immune sera as primary antibody. The assays were used to profile the proteolytic events in the well characterized *ex vivo* articular cartilage explant model. The cartilage explants included catabolically stimulated TNFα (tumor necrosis factor alpha) and oncostatin M cultures, non-stimulated cultures, and metabolically inactive cultures.
Results: In catabolically stimulated bovine cartilage explant cultures a low concentration of neoepitope I of below 50 ng/ml was found in samples collected day 3, 6, and day 11. However after prolonged catabolic stimulation of 22 days a more than 20-fold dramatic increase was observed reaching a level higher than 1000 ng/ml. In comparison the control culture of non-stimulated explant culture media had the same low level below 50 ng/ml on day 3, 6, and 11, and reached only 100 ng/ml at day 22. The metabolically inactive culture remained below 50 ng/ml during the complete culture period. A similar pattern of a dramatic increase in the 22-day catabolically stimulated culture was observed in human articular cartilage explant cultures. The pattern of other marker of neopitope II mirrored that of neoepitope I.
Conclusions: Two new immunoassays of neoepitope markers of collagen type II degradation were established. Measurement of these neoepitopes in both the bovine and human *ex vivo* articular cartilage explant model demonstrated that the two markers reflect late cartilage degradation. Further studies are needed to investigate whether these fragments are of clinical relevance.

111 CHANGES IN SERUM CARTILAGE OLIGOMERIC MATRIX PROTEIN CONCENTRATION AFTER 12 WEEKS IMPACT OR NON-IMPACT EXERCISES

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Purpose: The objective of the present study was to investigate the changes of serum level of cartilage oligomeric matrix protein (COMP) during a 30-min exercise after 12-weeks of regular impact or non-impact exercise.
Methods: Blood samples were drawn from 33 healthy sedanter males immediately before and after and 0.5 h after a 30-min walking exercise on a motor-driven treadmill at 5 km/h speed. Serum COMP concentrations were determined using a commercial enzyme-linked immunosorbent assay (AnaMar Medical, Göteborg, Sweden). After first measurements participants were randomly and equally assigned to impact group (running, n = 11) non-impact group (swimming, n = 11), and control conditions (n = 11). All exercise groups participated in sessions of 40 minutes per day, 3 days per week for 12 weeks. Each session started with a 5 min. of warm-up, continuing with main set for 30 min. at their individual target heart rate zone which was determined according to the ACSMs' guidelines (60−70% of heart rate reserve) and lasted with a 5 min. cool down period. The target heart rate reserve (HRR) was determined by Karvonen formula (Bompa, 1994). Participants in running and cycling groups partake sessions in the human performance laboratory one by one, three times a week throughout their weekly Schedule (throughout Mondays, Wednesdays and Fridays) which was determined by the instructor while swimming group perform exercise sessions in indoor swimming pool. The heart rate of the subjects was monitored with Heart rate monitors (Polar Vantage NV Heart Rate Monitor) during exercise sessions. Throughout the 12-week period, the control group was told not to participate in any organized or structured exercise and continue their daily life activities. At the end of the 12-weeks intervention period, post tests were applied with the same procedure of pre-tests. The experimental protocol was approved by local ethics committee. All subjects signed a consent form after being fully informed of the study's methods, possible side effects and the purpose. Serum COMP concentrations within the groups of running, swimming and control were compared using separate repeated measures of analysis of variance (repeated-ANOVA). Main effect was compared for multiple comparisons by Bonferroni confidence interval adjustment. Statistical computation was performed using the SPSS. The level of statistical significance was accepted as $p < 0.05$.
Results: In the pre-tests measurements, significant change between the serum COMP concentrations were found only at $P < 0.1$ level. Immediately after the walking exercise, serum COMP concentrations were increased significantly in running ($P = 0.084$) and control groups ($P = 0.064$). In the