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Conclusions: The methods developed in this study are based on general principles, and showed a significant correlation with a widely used grading protocol for a urine model of OA. The usefulness of these methods for other animal models, other grading protocols, and in treatment studies needs to be further demonstrated.

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INTERLEUKIN-1 ALPHA IN COMPARISON TO INTERLEUKIN-1 BETA IS A MORE POTENT STIMULATOR OF AGGRECANASE MEDIATED AGGRECAN DEGRADATION IN HUMAN ARTICULAR CARTILAGE

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Purpose: Aggrecan is the predominant proteoglycan in the extracellular matrix of articular cartilage. One of the early steps in initiation of cartilage degradation is loss of proteoglycans, which leads to further degradation of the collagen network. Interleukin-1 (IL-1) is a potent pro-inflammatory cytokine associated with the pathology of osteoarthritis (OA). However, controversy exists regarding the potency of IL-1alpha (IL-1α) compared to IL-1beta (IL-1β) on degradation of articular cartilage. Therefore, the aim of the present work was to assess the potential of IL-1α and IL-1β on human articular cartilage degradation, through quantifying MMP and aggrecanase activity with special emphasis on protease generated fragments of aggrecan.
Methods: Articular cartilage was obtained from OA patients undergoing total kne arthroplasty. Articular cartilage explants were cultured for 21 days with refreshment of medium every 3rd day in the presence of 100 ng/mL IL-1α or 100 ng/mL IL-1β to stimulate cartilage degradation. As negative control, explants were cultured without stimulation. Cartilage degradation was monitored by measuring the conditioned medium in 2 independent sandwich assays (1) 342-G2 assay, composed of the capturing antibody AF-28 against the ^{342}FFGVG neo-epitope generated by MMP-cleavage and the monoclonal antibody F-78 binding to both the Globular 1 (G1) and Globular 2 (G2) domain of aggrecan; (2) 374-G2 assay, made up by the capturing antibody BC-3 against the ^{374}ARGSVI neo-epitope created by aggrecanase cleavage and F-78.
Results: Both IL-1α or IL-1β stimulated aggrecanase mediated cartilage degradation as measured by the 374-G2 assay. However, IL-1α was approximately 200% more potent in inducing aggrecanase activity in human chondrocytes compared to IL-1β at equimolar concentrations. Neither IL-1α or IL-1β treatment of the human articular cartilage explants resulted in increased release of MMP generated fragments of aggrecan quantified by the 342-G2 assay.
Conclusions: Both IL-1α or IL-1β induce aggrecanases in human osteoarthritic cartilage. However, IL-1α seems to be approximately 200% more potent than IL-1β, suggesting important differences in cartilage metabolism induced by these two cytokines.

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ESTROGEN DEFICIENCY RESULTS IN INCREASED TYPE II COLLAGEN DEGRADATION AND DECREASED SYNTHESIS, WHICH LEAD TO INCREASED CARTILAGE EROSION

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Purpose: Cartilage degradation is a hallmark in osteoarthritis (OA) and loss of estrogen has been shown to be associated with increased prevalence of OA in postmenopausal women. We investigated the effect of estrogen on cartilage health in the ovariectomized (OVX) rat model by measuring collagen type II degradation and a novel markers of collagen type II formation.
Methods: *In vivo*, forty 7-month old Sprague-Dawley rats were divided into four groups. One group was subjected to sham operation, whereas the others to OVX, followed by treatment with either vehicle alone, 17-β-estradiol [0.25mg/pellet s.c. implanted] initiated instantly after surgery and continued until termination after 9 weeks. Serum was collected at baseline and week 4, 6 and 9 (termination), followed by quantification of telopeptides from collagen type II degradation(CTX-II) and fragments of pro-peptides of type II collagen (PIINP) by ELISA. Cartilage erosion was evaluated by histology through toluidine blue staining followed by microscopic measurement of the eroded articular cartilage surfaces of the four condyles.
Results: Cartilage degradation (CTX-II) was significantly elevated after week 2, $P<0.001$; 200%, and remained higher than sham operated-rats throughout the nine weeks. In contrast, PIINP levels in OVX rats were significantly lower, $P<0.05$; 50%, than sham-operated animals at week 6 and 9.
Treatment with estrogen to OVX rats resulted in a suppression of CTX-II release to below sham-operated animals, $P<0.01$. With regards to cartilage formation, estrogen treatment of OVX rats, restored PIINP levels to sham levels, $P<0.01$. Histological evaluation of cartilage erosions in estrogen deficient (OVX) animals compared to that of sham operated animals, displayed an 150% increase in cartilage erosion, $P<0.01$. This was completely restored to sham levels by estrogen replacement, $P<0.01$.
Conclusions: Loss of estrogen may result both in an increase in cartilage degradation and a decrease in cartilage formation. Consequently loss of estrogen results in increased cartilage erosion. This emphasises the importance of measuring both tissue formation and degradation when evaluating potential treatment for OA.

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A LONGITUDINAL ANALYSIS OF SERUM CYTOKINES IN THE HARTLEY GUINEA PIG MODEL OF OSTEOARTHRITIS

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Purpose: To examine the levels of a panel of cytokines in the serum of Hartley guinea pigs ranging from 3 weeks to 18 months of age in order to identify biochemical markers of osteoarthritis.
Methods: Forty six male Hartley guinea pigs were obtained from Charles River Laboratories and sacrificed at time points from 3 weeks to 18 months of age, at which time blood samples were obtained. Histological severity of knee OA was determined using a semi-quantitative grading scheme described previously, which assessed cartilage structure abnormalities and proteoglycan loss. Cytokines and chemokines in the serum were measured using the Bio-Plex Protein Array System with the Bio-Plex Mouse cytokine 18-Plex Panel. Cytokine concentrations were log transformed to meet assumptions of normalcy. To differentiate OA-related from purely age-related effects, we evaluated the association between cytokine serum concentrations (pg/ml) and total histological score for the cytokines that previously showed a significant difference between age-matched animals differing in OA susceptibility, namely the OA-prone Hartley strain and the OA-resistant Strain 13. Standard correlation analyses and multiple linear regression were performed using JMP Discovery software. Values were considered significant after adjustment for multiple testing using the Holm method.
Results: Histological OA presented at 4 months and significantly

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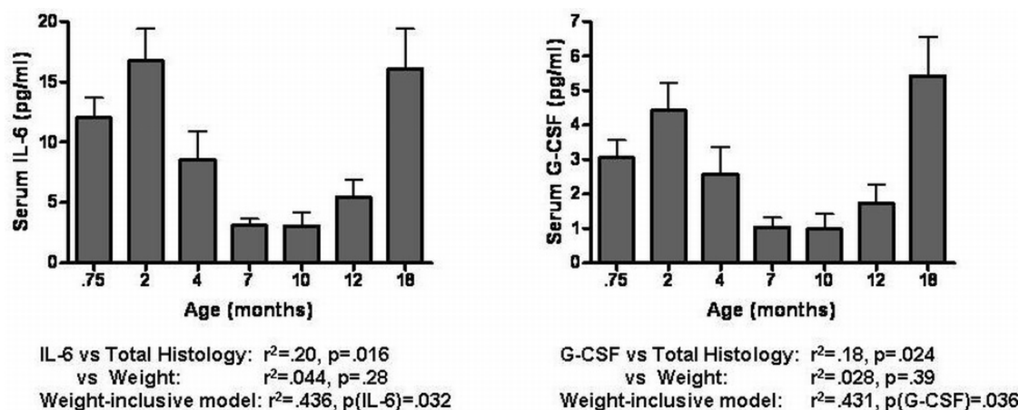

Fig. 1

worsened through 18 months. Despite the use of a kit designed for mouse sera, levels above detection limits were obtained for 16 of the 18 cytokines and chemokines measured (IL-1 alpha and IL-4 were undetectable). Serum concentrations of each of the measurable cytokines and chemokines displayed a similar bimodal distribution, with a first peak occurring at 2 months of age, a nadir at 7 months of age, and a significant increase from 7 to 18 months of age corresponding to the period of histological OA development in this model system (Fig. 1). Of the 11 cytokines associated with OA susceptibility in age-matched animals of two different strains, 7 showed a positive association with total histological severity: IL-6 (p=0.016), G-CSF (p=0.024), IL-2 (p=0.032), IL-12p70 (p=0.036), IL-5 (p=0.042), GM-CSF (p=0.044), and IL-17 (p=0.047). None of these were significantly associated with animal weight. After correcting for multiple comparisons, two correlations remained significant (IL-6 and G-CSF) and were evaluated further with multiple linear regression. The two resultant models, IL-6 and weight, and G-CSF and weight, explained 43-44% of the variance in total histological score respectively, with the cytokine and weight presenting significant p-values in both models. Utilizing the serum concentrations of both of these cytokines did not improve upon the predictive ability of the model.

Conclusions: The association of IL-6 and G-CSF with histological OA is independent of weight. Though the observed changes of these cytokine levels may be due to a correlation with age, it is unlikely given their pattern of age-related bimodal variance and the significant difference between Hartley and Strain 13 age-matched cohorts. IL-6 has been shown to play a role in inflammatory arthritis and synovial fluid levels are associated with synovitis and cartilage degeneration. G-CSF can induce changes in the production of inflammatory mediators such nitric oxide and PGE2 from cartilage chondrocytes and may therefore play a role in influencing cartilage metabolism in rheumatoid and osteoarthritis. These results warrant further investigation of these cytokines in the context of human OA.

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EVALUATION OF JOINT PATHOLOGY BY MEASURING CARTILAGE DEGRADATION PRODUCTS IN JOINT EXTRACTS

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Purpose: One model of Osteoarthritis (OA) is the anterior cruciate ligament transection (ACLT) model in rats. Protease degradation of collagens and proteoglycans in the articular cartilage is an integrated part in the development of OA, which most often is evaluated by histology through various staining techniques. We investigated whether simple extraction of proteins from knee joints could be a measurement of joint pathology. Thus, instead of only performing traditional histology, we investigated whether collagen type II degradation measured by specific ELISA technique, could be used as surrogated assessment for joint pathology, and thereby be a novel method of evaluating the entire pathology status of joints.

Methods: Twentyfour female Wistar rats (150-175g) were randomized into 3 groups and surgical OA was induced by ACLT in one knee joint, while the contralateral knee was sham operated. Urine and serum was collected at baseline and on day 7, 14, 21 and 28 after surgery. Groups of rats (n=8) were terminated 7, 14 and 28 days after surgery. ACLT- and sham-operated knee joints were either snap-frozen in liquid nitrogen, homogenized and extracted for proteins in an extraction buffer, or fixated in formaldehyde and decalcified in EDTA for histology and immunohistochemistry. The extraction buffer contained 50mM TrisHCl (pH 7.4), 0.1 NaCl, 0.1% Triton X-100 and protease inhibitors. Levels of C-terminal telopeptides of type II collagen (CTX-II) were measured in protein extracts from joints. Proteoglycan loss, cartilage erosion and osteophyte formation in the chondyle articular cartilage was evaluated by histology, and immunohistochemistry for the CTX-II epitope was performed.

Results: The articular cartilage and bone appearence in sham operated knees appeared histologically normal throughout the experiment. In ACLT-operated knee joints, degenerative changes in the surface articular cartilage evaluated by histology and immunohistochemistry were evident 7 days after surgery and seen throughout the study period. By extraction of proteins