Conclusions: These data indicate that HA, irrespective of its molecular size, inhibits the production of mRNA for MMP-1 and -3 in monolayer chondrocyte culture. However, only the 1.6 and 3.0 MDa HA inhibited mRNA and protein production for MMP-13. This MW dependence on MMP inhibition is consistent with the ability of higher MW HA to bind to multiple CD44 receptors on the cell surface thereby resulting in a more robust down regulation of MMP. Furthermore, MMP inhibition at transcriptional level and absence of HA interaction with IL-1 receptor binding suggests that HA modulates MMP production indirectly from the IL-1 receptor. In addition, 0.1, 0.72 and 3.0 MDa HA prevented proteoglycan loss in bovine explant cultures. These in vitro culture systems suggest that high MW HA is potentially more chondroprotective in the treatment of osteoarthritic joints.

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ESTROGENS INHIBIT INTERLEUKIN-1β-MEDIATED NITRIC OXIDE SYNTHASE EXPRESSION IN ARTICULAR CHONDROCYTES THROUGH NF-κB IMPAIRMENT

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Purpose: To investigate the presence and functionality of estrogen receptor alpha (ERα) in IL-1β-treated rabbit articular chondrocytes in culture. To determine the mechanisms of 17β estradiol (E2) effects on IL-1β-induced iNOS expression.

Methods: The presence and functionality of ERα were investigated by immunocytochemistry and transient expression of an E2-responsive reporter construct. iNOS expression and production were determined by transient expression of a chimeric iNOS promoter-luciferase construct and protein immunoblotting. NO production was determined by the Griess reaction. DNA binding activities of NF-κB and AP-1 were determined by EMSA-ELISA assays. Nuclear translocation of p65 was studied by immunocytochemistry.

Results: ERα was identified in the nucleus of chondrocytes. ERα efficiently transactivated a transiently expressed E2-responsive construct. Upon IL-1β treatment, ERα partially diffused from its nuclear localization into the cytoplasm and its transactivation ability was impaired. Nevertheless, E2, Tamoxifen and Rafaxifen efficiently inhibited IL-1β induced NO production (-34%, -31% and -36% respectively). E2 decreased IL-1β induced iNOS protein expression (-40%). Transient expression of an iNOS promoter construct strongly suggested that iNOS expression was inhibited at the transcriptional level and EMSA-ELISA assays showed that E2 reduced (-60%) the IL-1β induced p65 DNA binding capacity. Finally, the p65 nuclear translocation induced by IL-1β was also strongly decreased by E2.

Conclusions: Our data support a reciprocal antagonism between estrogens and IL-1β ultimately resulting in the decrease of cytokine-dependent NO production through transcriptional inhibition of iNOS expression. This effect was correlated with selective inhibition of p65 DNA binding and nuclear translocation.

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NFKAPPAB AND JNK PATHWAYS MEDIATE THE IL-1β DOWN-REGULATION OF TGFβ TYPE II RECEPTOR

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Purpose: We have previously shown that Interleukin-1β (IL-1β), which plays crucial role in osteoarthritic cartilage breakdown, impairs TGFβ signaling through TGFβ down-regulation and secondarily Smad7 up-regulation. These effect could explain the reduced responsiveness of OA chondrocytes to TGFβ and degradative process. The aim of this present study was to investigate the mechanism of IL-1β induced down-regulation of TGFβ in Human Articular Chondrocytes (HAC).

Methods: mRNA and protein half-lives were determined, using transcription and translation inhibitors. Then, the protein degradation mechanism and signalling pathways implicated were investigated, using specific inhibitors (proteasome, MAPK, ...). Transient transfections of human TGFβ promoter constructs were performed to delineate the DNA sequences that mediate IL-1β transcriptional effect. Experiments were also done to overexpress or inhibit transcription factors involved in that regulation.

Results: IL-1β decreases TGFβ protein half-life and reduces gene transcriptional activity. The effect on TGFβ gene expression is mediated through the core promoter (-47/-15 bp) that contains putative binding site for Sp1, and involves both NF-κB and JNK pathways. Experiments of gain/loss of function for p65, Sp1 and Sp3 indicate that these transcription factors cooperate to induce TGFβ repression. Furthermore, IL-1β effects are dependent of acetylation and methylation processes.

Conclusions: These findings enlighten the regulatory process of IL-1β on TGFβ expression. Understanding the molecular basis for IL-1β reduction of TGFβ expression provides new insight into molecular mechanisms of OA and may facilitate identification of novel approaches for its treatment.

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FLUID PHASE OF BONE MARROW - POSSIBLE COMPONENT OF JOINT LUBRICATION?

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Purpose: Cartilage lubrication and intensity of free-radical processes in joint tissues play a crucial role in the joint functioning. Mechanisms which provide adequate biological lubrication and maintenance of physiological level of free-radical processes in the joints are not established yet. We have studied some biochemical and lubricating properties of bone marrow fat in the assumption of its participation in joint lubrication.

Methods: Stability of cruciate ligaments to cyclic loadings was tested for using a tribometer and a "polyethylene-stainless steel" or "cartilage-on-cartilage" friction pair.

Results: In experiments on preparations of human knee joints it was revealed that cyclic loading on ligamentous structures leads to the occurrence of an oily transudate in a joint cavity. We
believe that this transudate is fluid phase of bone marrow and includes lipids (bone fat) from the internal volume of bones. It was established, that bone fat contains mainly triglycerides (98%), phospholipids (1.2%), small amount of cholesterol and high contents of natural antioxidants - 2-4 mM. Using "polyethylene-metal" friction pair friction coefficient was measured in present of some biological fluids, including oily transudate and bone fat. The addition of the oily transudate to the friction pair reduced friction coefficient 2-fold in comparison with effect of inflammatory effusion. The most notable effect was observed with the bone fat - friction coefficient decreases by 3.4 times. It was also revealed, that introduction of the bone fat to synovial fluid essentially improved its lubricating properties. In model experiment on the sliding of a rabbit cartilage on a cartilage the influence of bone fat under mechanical wear of articular cartilage was studied. During experiment the friction coefficient has grown from value 0.07 up to value 0.51. After the introduction of bone fat the friction coefficient was reduced and remained at a near-normal level.

Conclusions: These results give the basis to believe about possible existence of arthro-medullary connection which participates in the functioning of natural joints. The ligaments can play an important role as a transport channels for the intrinsic joint lubricant. Presence of antioxidant-bearing bone fat in a joint cavity can improve considerably the sliding of the articular cartilages, inhibits formation of harmful reactive oxygen species and thus promotes normal functioning of joints.

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CYTOKINE-INDUCED BOVINE ARTICULAR CARTILAGE DEGRADATION: EFFECTS OF EIGHT ANTI-ARTHRITIS DRUGS ON CARTILAGE DESTRUCTION

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Purpose: One of the key events in cartilage destruction in joint diseases such as osteoarthritis is the degradation of the cartilage collagen network. To support the development of novel therapies for these diseases relevant preclinical models are needed to study mechanisms of collagen degradation and synthesis. Ideally these processes are studied in cartilage of human origin. However, due to the limited availability of human tissues, research is often performed using for example bovine nasal cartilage and/or isolated chondrocytes that are cultured in 3 dimensional matrices. These methods are clearly useful, but would benefit from an intermediate model that fills the gap to the human cartilage explants. Therefore the aim of the current study was to setup a bovine model for cytokine-induced articular cartilage degradation and characterize the model using a variety of compounds targeting different disease mechanisms relevant to arthritis.

Methods: Full thickness articular cartilage punches (4.5 mm) from the metacarpophalangeal joints of 6 months old calves were cultured for 3 weeks in 96-well plates in the presence or absence of a cytokine cocktail consisting of 10 ng/ml IL-1α, 10 ng/ml TNF-α and 10 ng/ml OSM. Culture medium was refreshed weekly. After three weeks the cartilage and culture medium were analyzed for weight changes, water content, DNA content, glycosaminoglycans (GAG), hydroxyproline (Hyp), damaged collagen molecules, MMP activity, C-telopeptide fragments of Type II collagen (CTX-II) and cartilage oligomeric matrix protein (COMP). Diclofenac (10 μM), Dexamethasone (0.5 μM), Pioglitazone (1 μM), Remicade (10 μg/ml), Risedronate (100 μM), Galardin (10 μM) and A77-1726 (active metabolite of Leflunomide; 100 μM) were tested for their effect on cartilage degradation. The experiment was performed on cartilage from three different donors and with each test condition cultured in eight-fold for each donor.

Results: Stimulation of the cartilage explants with a cytokine cocktail resulted in changes that are also seen in human OA. The weight of the explants was decreased compared to untreated controls (24%, p < 0.01). Degradation of proteoglycans was significantly increased by the cytokine treatment (47%, p < 0.01) and could already be observed within 1 week after initiation of the study. Collagen degradation (measured as Hyp and CTX-II) was also significantly elevated, but this was seen only after three weeks of culture. The collagen release was accompanied by an increase in the amount of denatured collagen molecules within the tissue (17% versus 11% in control cartilage; NS). In accordance with the increased collagen damage, the water content of the tissue was also increased (from 76% to 83%, p < 0.01) which correlated with the collagen damage as well (r = 0.88, p < 0.01). The level of active MMPs increased 40-fold upon stimulation and correlated well with the collagen damage (p = 0.99, p < 0.01). COMP release during the first week of culture showed a trend towards up regulation during the first week of culture for all three donors, this was however not significant. A series of test compound was used to characterize the response of the model to intervention, see table for details.

Conclusions: Stimulation of bovine articular cartilage explants with a cocktail of IL-1α, TNF-α and OSM results in clear and consistent changes in the cartilage, highly reminiscent of cartilage destruction during arthritis. The processes can be modulated by one or more of the compounds tested, indicating that this model for articular cartilage destruction is sensitive to treatment.

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STRUCTURAL DOMAINS WITHIN CHONDROITIN SULPHATE/DERMATAN SULPHATE GLYCOSAMINOGLYCANS

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Purpose: Chondroitin sulphate (CS) is a complex carbohydrate polymer with variable sulphation which impacts function. Shank