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subsequently treated with tumour necrosis factor-α (TNF-α) in combination with Actinomycin D to induce apoptosis. Apoptotic chondrocytes in cartilage sections were identified using an indirect immunohistochemical staining technique to detect expression of active caspase-3. Haematoxylin and eosin/safranin-o stained sections were used to score cellularity and structural differences between samples.

Results: Prior to culture, (mean±standard deviation) chondrocyte viability was 80.7% (3.5). The extent of chondrocyte apoptosis induced by TNF-α/Actinomycin D varied markedly according to the joint type that the cartilage was sampled from. For MCP joints, the extent of overall chondrocyte apoptosis was significantly higher ($P < 0.001$) in TNF-α/Actinomycin D-stimulated explants (26.7%, 10.3) than that observed in unstimulated control samples (9.6%, 7.5). Chondrocytes from PIP and DIP joint cartilage did not respond significantly to apoptotic stimulation ($P > 0.05$); apoptosis in both control and stimulated explants was virtually identical. Significant variations in cellularity and thickness were evident between cartilages of different joint types. Cartilage from DIP and PIP joints was significantly thicker than that of the MCP joint ($P < 0.001$ and $P < 0.05$ respectively). Moreover, MCP joint AC was significantly more cellular than both PIP and DIP joints ($P < 0.001$).

Conclusions: Data in this study demonstrate that chondrocytes from three equine joint types with varying prevalence's of OA differ significantly in terms of susceptibility to apoptosis induction. This may provide a possible explanation for the joint-specific nature of the disease. Joint-type dependent differences in cartilage thickness and cellularity may also have contributory roles.

172 THE CO-RELEASING MOLECULE CORM-2 IS A NOVEL STRATEGY TO COUNTERACT OXIDATIVE STRESS AND INFLAMMATION IN OSTEOARTHRITIC CHONDROCYTES

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Purpose: The production of reactive oxygen species and inflammatory mediators may contribute to the disregulation of cartilage homeostasis in osteoarthritis (OA). We have shown recently the protective effects of the CO-releasing molecule (CO-RM) tricarbonyldichlororuthenium (II) dimer (CORM-2) against OA cartilage degradation, with down-regulation of catabolic enzymes and enhancement of glycosaminoglycan synthesis. The objective of the present study was to investigate whether CORM-2 is able to inhibit the production of oxidative stress and the inflammatory response induced by interleukin 1β (Il-1β) in primary human OA chondrocytes.

Methods: Cartilage specimens were obtained from 17 patients with diagnosis of advanced OA undergoing total knee joint replacement. Chondrocytes were isolated by digestion with collagenase and used in primary culture. Cells were stimulated with Il-1β (100 U/ml) for different times in the presence or absence of CORM-2 (50, 100 or 150 μM). Protein expression was investigated by Western blot and immunofluorescence. Cytokine levels were determined by ELISA and prostaglandin E_2 (PGE_2) by RIA. Gene expression was measured by real-time quantitative PCR. Oxidative stress was assessed by laser scanning cytometry analysis. Nuclear factor-κB (NF-κB) and hypoxia inducible factor-1α (HIF-1α) DNA binding was quantitated by ELISA in nuclear extracts.

Results: CORM-2 significantly decreased Il-1β-stimulated reactive oxygen species production in OA chondrocytes. The inhibition of oxidative stress was related to the down-regulation of gp91-phox. In addition, CORM-2 inhibited tumor necrosis factor-α levels but enhanced Il-1 receptor antagonist production. Our studies have shown that CORM-2 is able to control the enhanced synthesis of PGE_2 in OA chondrocytes treated with Il-1β, which can be mainly dependent on the reduction in microsomal PGE synthase-1 gene expression with minor effects on cyclooxygenase-2. Il-1β strongly stimulated the binding of HIF-1α to its consensus sequence, whereas in chondrocytes treated with CORM-2, a concentration-dependent inhibition was observed. This agent also inhibited NF-κB-DNA binding.

Conclusions: Results from the present study establish the presence of antioxidative and antiinflammatory mechanisms in the beneficial effects elicited by CORM-2 in OA chondrocytes and cartilage. Our data suggest that inhibition of NF-κB and HIF-1α activation may play a role in the inhibitory effects of CORM-2 on the production of mediators relevant in OA.

173 ROSEMARY EXTRACT INHIBITS GLYCOSAMINOGLYCAN AND COLLAGEN DEGRADATION IN BOVINE ARTICULAR CARTILAGE EXPLANTS

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Purpose: The objective of the study was to investigate the effects of rosemary, an herb frequently used in Mediterranean cuisine, and of carnosol, one of its main antioxidant, on cartilage degradation after catabolic stimulation.

Methods: In a first set of experiments ($n = 3$), S35-labelled bovine articular cartilage explants were cultured for 3 days with Il-β in the presence or absence of various doses of rosemary extract or carnosol (50, 25, 10, 5 μg/ml for both). Cell viability was assessed by the release of lactate deshydrogenase. Glycosaminoglycan degradation was measured by quantifying the amount of S35 released in the culture media. In a second set of experiment ($n = 1$), bovine articular cartilage explants were cultured for 21 days with oncostatin and TNF-α in the presence or absence of various doses of rosemary extract (100, 50, 10 μg/ml) or carnosol (3.31, 1.66, 0.33 μg/ml corresponding to 10, 5 and 1 μM). Cell viability was assessed by the AlamarBlue assay. MMP mediated collagen and glycosaminoglycan degradation were assessed by measuring the amounts of CTX-II and ^{342}FFGVG epitopes released in the culture media over the 21 days of culture. Aggrecanase mediated aggrecan degradation was assessed by measuring the amount of ^{374}ARGSV epitope released in the culture media over the 9 first days of culture.

Results: In the first set of experiments, rosemary extract, at doses ranging from 50 down to 5 μg/ml, and carnosol, at the doses of 10 and 5 μg/ml, inhibited IL1-β induced S35 release without affecting cell viability. At the highest doses of 50 and 25 μg/ml, carnosol decreased cell viability. In the second set of experiment, rosemary extract at the lowest tested dose (10 μg/ml) almost completely abolished the MMP mediated degradation of aggrecan and of type II collagen induced by oncostatin and TNF-α without affecting the aggreganase mediated aggrecan degradation. At this low dose, rosemary extract did not affect cell viability contrary to the 2 highest tested doses (50 and 100 μg/ml). Conversely, the 2 lowest tested doses of carnosol (1.66, 0.33 μg/ml) decreased the aggrecanase mediated aggrecan degradation witouth affecting the MMP mediated degradation of aggrecan and type II collagen. The highest tested dose of carnosol (3.31 g/ml) decreased cell viability.

Conclusions: Taken together, these results indicate that rosemary extract slow down cartilage degeneration in vitro by inhibiting MMP activity. Preliminary observations suggest that the effects of rosemary extract on cartilage degeneration are not solely mediated by its content in carnosol since the anti-catabolic effect of carnosol and rosemary extract on articular cartilage only partly overlap. These findings provide a rationale basis for the in vivo testing of rosemary extract in osteoarthritis.

174 GLUCOSAMINE INTERFERES WITH MITOGEN-ACTIVATED PROTEIN KINASE PATHWAY BY INHIBITING JNK AND p38 PHOSPHORYLATION, IN HUMAN CHONDROCYTES

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Purpose: Previous studies demonstrated the ability of Glucosamine (GlcN) to inhibit mRNA transcription level of Interleukin-1β (Il-1β)-stimulated genes. These genes are under the control of two transcription factors Activator Protein (AP)-1 and NF-kB. The aim of this study was to determine the effects of GlcN on mitogen-activated protein (MAP) kinase phosphorylation and on activation of AP-1, in human chondrocytes.

Methods: Human immortalized cell line, lbpva55, and human chondrocytes, obtained from healthy donors, were challenged with 10 ng/ml Il-1β cytokine after pre-treatment with 2.5 or 10 mM GlcN. mRNA expression levels of some matrix metalloprotease (MMP) genes were evaluated by Quantitative-Real Time PCR (Q-RT-PCR), protein production levels were evaluated in the culture supernatant by Enzyme Linked ImmunoSorbent Assay (ELISA). MAP kinase phosphorylation was evaluated by Western Blotting. AP-1 transcription factor activation was evaluated by measuring AP-1 component DNA binding activity (TransAM AP-1 family kit).

Results: After Il-1β stimulation, MMP-1, -3 and -13 productions were strongly increased both at mRNA and protein level. Treatment with GlcN reduced the expression of these metalloproteases. MMP-1, -3 and -13 expression is regulated by transcription factors such as AP-1, which is activated by phosphorylated MAP kinases. Il-1β stimulated phosphorylation

of c-jun N terminal kinase (JNK), p38 MAP kinase and extracellular-signal regulated kinase (ERK)-1/2. GlcN inhibited JNK and p38 phosphorylation and consequently c-jun and to a lower degree junD DNA binding activity. Moreover, we found also down-regulation of c-jun mRNA transcription level.
Conclusions: These results demonstrated for the first time, in human chondrocytes, that GlcN inhibits cytokine-stimulated MMP production, by affecting MAP kinase phosphorylation and consequently AP-1 transcription factor.

175 THE UBIQUITIN-PROTEASOME PATHWAY AND VIRAL INFECTIONS IN ARTICULAR CARTILAGE OF PATIENTS WITH OSTEOARTHRITIS

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Purpose: It has long been suspected that viruses have a role in the aetiology and pathogenesis of various forms of rheumatic diseases. Many viruses can evolve different strategies to exploit the ubiquitin-proteasome pathway (UPP) for their own benefit. Some data have recently established connections between UPP and osteoarthritis (OA). The objective of this study was to determine the possible involvement of viral infections linked with the UPP in the physiopathology of OA.
Methods: Samples of human cartilage were obtained from 12 patients with clinical and radiological features of OA and from 12 normal controls. DNA was extracted from cultured chondrocytes from these patients, and quantitative real time PCR was performed to analyse the DNA/RNA prevalence and viral loads of herpes simplex virus (HSV), Epstein-Barr virus (EBV), human cytomegalovirus (CMV), enterovirus, and human T-cell leukaemia virus 1 (HTLV-1).
Results: The prevalence of total viral DNA/RNA among patients with OA was 16.7% (2/12), with a mean viral load of 7.86 copies/μg DNA. The positive samples were positive for EBV. We did not find any positive samples among control subjects for the viruses analysed. No statistically significant differences in DNA/RNA prevalence and viral loads were found in OA patients compared to controls.
Conclusions: Our study of viruses related to UPP in articular cartilage of OA patients does not support their possible involvement in the aetiology of this disease.

176 CHICORY EXTRACTS AND THEIR 3 MAJOR SESQUITERPENE LACTONES INHIBIT GLYCOSAMINOGLYCAN DEGRADATION AND NITRIC OXIDE PRODUCTION IN BOVINE ARTICULAR CARTILAGE EXPLANTS

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Purpose: The objective of the study was to investigate the effects of chicory, an important agricultural crop, and of lactucopicrin, 8-deoxylactucin and lactucin, its 3 major sesquiterpene lactones (SQLs), on cartilage metabolism after catabolic stimulation.
Methods: Ethyl acetate extracts from dried and roasted chicory roots were prepared. To increase the efficiency of the extraction, part of these extracts was digested with cellulase whereas another part was treated by acid hydrolysis or enzymatic digestion with glucosidase and esterase to partly mimic human digestion. The 3 major SQLs (lactucopicrin, 8-deoxylactucin and lactucin) present in these extracts were purified and an SQL metabolite, α-methylene γ-butyrolactone, was purchased. The effect of these extracts (at the doses of 100, 50, 25 and 10 μg/ml) and SQLs (at doses ranging from 60 to 3 μg/ml) on IL1β-induced NO production and glycosaminoglycan turnover was tested in bovine articular cartilage explants in 3 independent experiments. After 2 days of culture, NO production was assessed by quantifying the amount of nitrite in the supernatant using the Griess spectrophotometric method while glycosaminoglycan turnover was assessed by quantifying the amount of glycosaminoglycans present in the supernatant with the dimethyl methylene blue assay. Cell viability was assessed by the release of lactate deshydrogenase. To specifically assess the effects of chicory on glycosaminoglycan degradation, bovine articular cartilage explants prelabelled with S35 were cultured for 3 days with IL1-β in the presence or absence of various doses of the dried root extract (50, 25, 10, 5, 1 μg/ml) in 2 independent experiments. Glycosaminoglycan degradation was measured by quantifying the amount of S35 released in the culture media.
Results: Extracts from dried and roasted roots dose-dependently decreased the IL1β-induced NO production and glycosaminoglycan release. Extracts from dried roots were more active than extracts from roasted roots. The untreated extract from dried root had an IC50 of 15 μg/ml for NO production and 34 μg/ml for glycosaminoglycan turnover. Digestion of the extracts with cellulase, or with digestive enzymes like glucosidase and esterase, did not affect their activity much but acid hydrolysis destroyed the activity of the dried root extract. Regarding NO production, a statistical significant correlation was observed between the efficacy and the SQL content of the extracts. In addition, the 3 SQLs and α-methylene-γ-butyrolactone dose-dependently decreased the IL1β-induced NO production and glycosaminoglycan release with IC50 ranging from 10 to 40 μg/ml. LDH release was increased by 25 and 50 μg/ml of lactucopicrin in one out of 3 experiments and by 100 μg/ml of the untreated dried root extract in all 3 experiments. The other extracts and compounds had no effect on LDH release. The extract from dried roots, at the doses of 50, 25 and 10 (even down to 1 μg/ml in one of the 2 experiments) inhibited IL1-β induced S35 release indicating that chicory extracts decreased the glycosaminoglycan turnover of articular cartilage through an anti-catabolic effect.
Conclusions: Taken together, these results indicate that chicory extracts counteract some of the deleterious effects of IL1β on articular cartilage metabolism and indicate that these properties are partly due to the sesquiterpene lactones and their metabolites. These findings provide a rationale basis for the in vivo testing of chicory extract in osteoarthritis.

177 AUTOCRINE MOTILITY FACTOR INDUCES THE RESISTANCE AGAINST APOPTOSIS IN HUMAN CHONDROCYTES

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Purpose: Autocrine motility factor (AMF) is a cytokine that regulates locomotion and metastasis of tumor cells, and accumulates the angiogenesis and ascites fluid. It was reported that AMF-high secretion tumor cells have a resistance against the apoptosis. It is well known that chondrocyte apoptosis is related to the onset and progression of osteoarthritis (OA). In this study, we investigated AMF expression in human cartilage, and investigated the function of apoptosis mechanisms of AMF in human chondrocytes with the transfection of AMF plasmid.
Methods: Cartilage tissues were obtained during total joint replacement surgery with OA. Normal cartilage tissues were obtained during surgery of femoral neck fracture with no history of joint disease and with macroscopically normal cartilage. Chondrocytes were isolated and cultured from cartilage tissues. Expressions of AMF, caspase-9, and Apaf-1 in chondrocytes were analyzed by RT-PCR and realtime PCR. We transfected AMF plasmid into NHAC-kn (normal human chondrocyte) with lipofection method. Survival of AMF transfected NHAC-kn were manually counted with a hemocytometer up to 7 days after seeding in serum-free condition. Expressions of AMF, caspase-9, and Apaf-1 in chondrocytes were analyzed by RT-PCR. To explore the anti-apoptotic ability of AMF, shear stress was introduced to NHAC-kn for 6 h after transfection of AMF. Chondrocyte apoptosis was detected by DNA fragmentation and western blotting with the anti-body of caspase-9. After incubation with PD98059, MAPK inhibitor, or LY294002, PI3K inhibitor, expression of caspase-9 and Apaf-1 were analyzed by RT-PCR and chondrocyte apoptosis with shear stress was detected by DNA fragmentation to investigate the intracellular signaling of AMF.
Results: AMF, caspase-9, and Apaf-1 were expressed in OA and normal chondrocytes (Fig. 1a). However expression levels among AMF, caspase-9 and Apaf-1 were not significantly coefficient (Fig.1b). The expression level of AMF increased significantly in AMF transfected NHAC-kn, compared with the control (Fig. 2). AMF transfected cells survived compared with control NHAC-kn and empty vector transfected cells (Fig. 3a). And the expression levels of caspase-9 and Apaf-1 decreased significantly in AMF transfected cells (Fig. 3b,c). DNA fragmentation was not observed in AMF transfected cells after shear stress induction (Fig. 4a), and cleaved caspase-9 was not expressed in AMF tansfected cells (Fig. 4b). When AMF transfected cells were treated with PD98059 and LY290042, expression of caspase-9 and Apaf-1 was fully recovered by RT-PCR (Fig. 5a), and DNA fragmentation was could be detected (Fig. 5b).