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). No caspase activities were observed in both control and stimulated explants. 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 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

I. Guillén1, J. Megías2, F. Gomar3, M. Alcaraz2, 1Cardenal-Herrera CEU University, Moncada, SPAIN, 2University of Valencia, Department of Pharmacology, Valencia, SPAIN, 3University of Valencia, Department of Surgery, Valencia, SPAIN

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 E2 (PGE2) by RIA. Gene expression was measured by real-time quantitative PCR. Oxidative stress was assayed by laser scanning cytometry analysis. Nuclear factor-xB (NF-xB) and hypoxia inducible factor-1α (HIF-1α) DNA binding was quantified 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-px. 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 PGE2 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-xB-DNA binding. Conclusions: Results from the present study establish the presence of antioxidant and antiinflammatory mechanisms in the beneficial effects elicited by CORM-2 in OA chondrocytes and cartilage. Our data suggest that inhibition of NF-xB 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

F. Scalfo1, S. Davis1, M.A. Karsali2, E. Offord3, L.G. Amye4. 1Nestlé Research Center, Lausanne, SWITZERLAND, 2Nordic Bioscience, Herlev, DENMARK

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-1β 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 dehydrogenase. 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 measuring the amount of CTX-II and fibronectin epitopes released in the culture media over the 21 days of culture. Aggrecanase mediated aggrecan degradation was assessed by measuring the amount of 34K/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 IL-1β 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 with affecting the MMP mediated degradation of aggrecan and type II collagen. The highest tested dose of carnosol (3.31 μg/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

A. Scotto d’Abusco1, C. Cicione1, V. Calamia1, B. Grigolo1, L. Politi1, R. Scandurra1, 1Sapienza, University of Roma, Roma, ITALY, 2Istituto di Ricerca Cavidoli Putsi, Istituti Ortopedici Rizzoli, Bologna, ITALY

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-κB. 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, IBvp55, 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 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: IL-1β stimulation, MPP-1, -3 and -13 productions were strongly increased both at mRNA and protein level. Treatment with GlcN reduced the expression of these metalloproteases. MPP-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