cyte mitochondria as well as the effect on signalling pathways leading to altered collagenase expression.
Methods: RNA intereference was used to characterise whether the reduction of SOD2 levels affects matrix metalloproteinase (MMP)-1 and MMP-13 gene expression along with superoxide levels produced by the mitochondria in human articular chondrocytes (HAC). For the MMP-1 and MMP-13 expression, cells were then serum starved for 24 hours and stimulated with IL-1. MMP-1 and MMP-13 mRNA levels were determined by real-time RT-PCR. To assess the levels of superoxide and SOD2 protein, HAC were stained with MitoSOX™ -Red, a specific indicator for superoxide in the mitochondria, or an anti-SOD2 antibody. To compare the mitochondrial respiratory activity between HAC from healthy patients (neck of femur fractures, NOF) and from osteoarthritic patients (OA), cells were cultured for 3 days after extraction from the cartilage and analysed for respiratory activity using the Oroboros Oxygraph-2k respirometry system.
Results: SOD2 depletion by RNA interference led to a significant decrease in basal MMP-1 mRNA expression and a significant reduction in the level of MMP-1 and MMP-induction following IL-1 stimulation. SOD2 depletion by RNA interference also led to a significant increase in mitochondrial superoxide levels.
In terms of respiratory capacity, OA chondrocytes have on average 65% higher respiratory chain capacity than NOF chondrocytes. However, although OA chondrocytes have a more active respiratory chain, it appears less efficient (25%) to that of HAC from NOF patients.
Conclusions: Depletion of SOD2 in OA chondrocytes leads to a significant decrease in the expression levels of the collagenases MMP-1 and MMP13, indicating that the decrease of SOD2 expression in OA cartilage may represent a chondroprotective mechanism. However, this depletion leads to a significant increase of mitochondrial superoxide levels. Combined with the increased levels of unutilised protons and electrons in the respiratory chain, these effects can potentially alter the mitochondrial membrane potential of the cells causing cellular dysfunction.

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GLATIRAMER ACETATE (GA), A PEPTIDE IMMUNOMODULATORY DRUG, INHIBITS INFLAMMATORY MEDIATORS, MMP-13 ACTIVITY AND COLLAGEN DEGRADATION IN OA CARTILAGE

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Purpose: Glatiramer acetate (GA), the generic name for Copaxone, is an immunomodulatory agent used in the treatment of multiple sclerosis, which has been to shown to induce interleukin-1 receptor antagonist (IL-1Ra) production in macrophages and microglial cells. In osteoarthritis, the production of inflammatory mediators, particularly IL-1 by chondrocytes, may be important in the pathogenesis and progression of OA. We therefore tested the effects of GA on the catabolic activities of chondrocytes in OA cartilage explants cultures.
Methods: Cartilage slices were obtained from the knees of patients with advanced OA and undergoing knee replacement surgery. Non-arthritic knee cartilages were obtained from autopsy patients within 24h (NDRI, Philadelphia). Matrix metalloproteinases proMMP-13 ELISA kits were from R&D Systems, (Minneapolis, MN). Predesigned TaqMan PCR primers were purchased from Applied Biosystems (CA).
Results: We have previously shown that OA cartilage explant cultures spontaneously release inflammatory mediators such as nitric oxide, Prostaglandin E2 (PGE2), interleukins including IL-1betaand matrix metalloproteinases (MMPs). In this study we evaluated the chondroprotective property of GA in monolayer cultures of primary OA chondrocytes and cartilage explant cultures. GA (5-100ug/ml) treatment dose dependently increased transcription (QPCR) and production (ELISA) of sIL-1Ra ($p>0.001$) in normal and OA Chondrocytes. GA also intrinsically increased ($p<0.001$) sIL-1Ra production by OA cartilage explant cultures. Furthermore, addition of GA: 1) inhibited both spontaneous and IL-1 induced inflammatory mediators such as nitric oxide (NO) (9.0+1.5 to 1.5+0.5 uM; $p<0.01$) and PGE2 (100.6+18.5 to 15.1+6.1 ng/ml; $p<0.01$) production; 2) inhibited spontaneous and IL-1 induced proMMP-13 secretion (200.6+90.5 to 78.5+15.1ng/ml; $p<0.001$); 3) inhibited APMA activated total MMP-13 activity by more than 30-50% ($p<0.01$); 4) inhibited collagen degradation as assayed by CTXII ELISA (17.4+1.7 to 4.5+1.9 ng/ml; $p<0.01$). All these chondroprotective effects of GA were dose dependent and significance was reached between 10-100 ug/ml.
Conclusion: Glatiramer acetate is a complex heterogeneous mixture of polypeptides that exhibits "chondroprotective" properties in OA cartilage, inhibiting the production of inflammatory mediators as well as MMP-13 expression/activation. The data suggest that these effects may be due to upregulation of IL-1Ra. Based on these studies, we propose that GA may have potential for disease modifying properties in OA and should be evaluated in vivo animal studies.

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ADIPONECTIN AND LEPTIN EXHIBIT DIFFERENT PATTERN OF PRODUCTION IN CARTILAGE FROM PATIENTS WITH OSTEOARTHRITIS

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Purpose. Based on the association between obesity and osteoarthritis (OA), increasing studies aimed to determine the contribution of adipokines in OA. As adiponectin and leptin may exhibit opposite inflammatory effects and display different patterns of distribution between the joint and the circulating compartment, we compared the production of both adipokines in cartilage from human OA-affected joints in relation with the grade of cartilage destruction and with the Body Mass Index (BMI) of the patients.
Methods. The production of leptin and adiponectin was determined by ELISA in conditioned media from cultured full-depth cartilage biopsies obtained from patients with OA. The severity of OA cartilage lesion was then evaluated after histological analysis of each specimen and was graded using the Mankin score.
Results. The results indicated that the production of adiponectin in OA cartilage are quite different from that of leptin. A positive association has been found between the BMI of the patients and the production level of leptin while the synthesis of adiponectin did not change with the BMI. In addition, a grade-dependent increase in the production level of leptin was shown for non obese patients ($BMI<30kg/m^2$). The synthesis of leptin strongly increased in obese patients ($BMI>30kg/m^2$) between the low and the moderate OA grades, but did not change anymore for the most severe OA grade. The adiponectin production was slightly elevated in cartilage samples with moderate or advanced OA compared to specimens with low histological OA score, but the difference did reach statistical significance.
Conclusion. These findings indicated that leptin and adiponectin exhibit different pattern of production in OA cartilage. The leptin level is strongly associated with both the grade of cartilage destruction and the BMI of the patients. Conversely, the production of adiponectin is slightly up-regulated in damaged cartilage independently of the OA score and the BMI of the patients.

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WNT3A MODULATES CHONDROCYTE PHENOTYPE THROUGH ACTIVATION OF BOTH CANONICAL AND NON-CANONICAL PATHWAYS

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Purpose: Injury to the articular cartilage results in the activation of Wnt signalling which may represent a homeostatic mechanism. Indeed disruption of Wnt signalling results in osteoarthritis both in humans and experimental models. Wnt ligands are traditionally categorized based on their capacity to activate either the β-catenin-dependent canonical pathway or any of the non canonical pathways including the Ca^{2+}/Calmodulin-dependent kinase II (CaMKII)-dependent or the PKA-dependent pathways, the planar cell polarity pathway and the Wnt/ROR-mediated pathway. The clear-cut separation between canonical and non-canonical Wnts has been recently challenged and therefore the purpose of the study was to investigate through which pathway Wnt3a modulates the cartilage phenotype and regulates cartilage homeostasis in human articular chondrocytes.
Methods: Primary adult human articular chondrocytes were isolated from preserved areas of the cartilage of patients undergoing knee arthroplasty for osteoarthritis. Detection of protein and protein phosphorylation upon Wnt3a stimulation was evaluated by western blotting. Intracellular calcium mobilization was detected by cellular accumulation of FURA-4 dye. The activation of the canonical pathway upon Wnt3a stimulation was evaluated