system in vitro was correlated with origin of synoviocytes. Moreover the relation between invasive growth of FLS in the transwell system and growth characteristics and expression of MMP-1, -14, -17, -19, cathepsin K, TIMP-1 and -2 was determined.

Methods FLS were derived from 56 patients (30 rheumatoid arthritis (RA), 17 osteoarthritis (OA), 9 avascular necrosis (AVN) or fractures). Invasive growth of FLS through a collagen matrix was measured in a transwell system coated with matrigel. The number of cells grown through the matrix and the transwell membrane were counted. Growth rate was determined by counting of FLS after seven days of culturing. Expression of MMP's, cathepsin-K and TIMP's was investigated using RT-PCR and related to expression of a household gene, beta-actin.

Results FLS from RA invaded more easily in vitro than FLS from OA and AVN (median: RA: 4788 (cells grown through matrix and transwell membrane), OA: 1875; $p < 0.001$ and AVN: 1530; $p = 0.014$ respectively). The median rate of proliferation of RA FLS was 0.27 per day compared to OA 0.22 per day ($p = 0.012$) and AVN and fractures 0.25 per day (RA versus AVN: $p = 0.242$; OA versus AVN: $p = 0.553$), but there was no correlation between rate of proliferation and invasive growth in vitro. FLS that expressed MMP-1, MMP-3 or MMP-10 were significantly more invasive (median number of invasive cells: 3835, 4248, 4990, respectively) than cells that did not express MMP-1, MMP-3 or MMP-10 (1605, $p = 0.03$; 1970, $p = 0.004$; 2360, $p = 0.012$, respectively). Expression of the other MMP's, cathepsin-K and TIMP's did not show a significant relationship with invasive growth. Expression of MMP-9 showed a trend with higher expression in more invasive cells ($p = 0.066$). There was also a significant relationship between the expression of MMP-1 and MMP-9 and a diagnosis of RA (both $p = 0.013$).

Conclusion FLS of RA invade more easily in a matrigel matrix than OA FLS. This is not due to a greater rate of proliferation of RA FLS than OA FLS, although FLS from RA patients have a 25% higher rate of proliferation than FLS from OA patients. A significant relationship exists between the expression of MMP-1, MMP-3 and MMP-10 and invasive growth in a matrigel transwell system and this relationship was nearly significant for MMP-9. FLS of RA patients expressed significantly more often MMP-1 and MMP-9 than FLS from OA patients.

OP0025 GLUCOSAMINE SULFATE INHIBITS NITRIC OXIDE AND STROMELYSIN PRODUCTION IN CARTILAGE CULTURES AND REVERSES IL-1 INHIBITION OF OSTEOARTHRITIC ARTICULAR CARTILAGE SYNTHESIS

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Background Glucosamine sulfate is used for the management of osteoarthritis of the knee.

The mechanism of its effects needs clarification.

Objectives To evaluate the effects of glucosamine sulfate on the production of nitric oxide (NO) and stromelysin (MMP3) and cartilage synthesis in human osteoarthritic cartilage in the presence of IL-1b or LPS.

Methods Cartilage was obtained in the operation theatre from patients undergoing total knee replacement for osteoarthritis, cut into small pieces and cultured in the presence or absence of IL-1b or LPS.

NO in culture media was measured with the Griess reagent, MMP-3 by ELISA and cartilage synthesis by ^{35}S incorporation.

Results IL-1b (1 ng/ml) and LPS (3 µg/ml) stimulated NO release in human osteoarthritic cartilage cultures about four fold. Glucosamine sulfate (1 µg/ml, 10 µg/ml, 100 µg/ml) inhibited stimulated NO production in a dose dependent manner, reaching statistical significance ($p < 0.05$) at 100 µg/ml glucosamine sulfate (60% and 38% inhibition respectively).

Glucosamine sulfate (100 µg/ml) significantly ($p < 0.05$) inhibited MMP-3 release in IL-1b and LPS stimulated cartilage cultures. IL-1b and LPS inhibited cartilage synthesis by 70% and 50% respectively ($p < 0.001$). Glucosamine sulfate reversed this inhibition in a dose dependent manner reaching statistical significance ($p < 0.05$) at 10 µg/ml glucosamine sulfate.

Conclusion Glucosamine sulfate may protect human osteoarthritic articular cartilage by inhibiting local NO and MMP-3 production and by reversing IL-1b inhibition of cartilage synthesis.

OP0026 IN VIVO INHIBITION OF ENDOGENOUS TGF-BETA ACTIVITY REDUCES OSTEOPHYTE SIZE AND CARTILAGE PROTEOGLYCAN CONTENT

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Background Transforming Growth Factor-beta (TGF-beta) is considered to be a beneficial factor during cartilage repair. However, TGF-beta can also induce fibrosis and osteophytes. TGF-beta signalling starts with the binding of TGF-beta to the type II receptor, a constitutively active serine/threonine kinase. This complex then binds the type I receptor, which is subsequently phosphorylated by the type II receptor. This results in phosphorylation of downstream mediators by the type I receptor. Since the binding of TGF-beta to the extracellular domain of the type II receptor is one of high affinity, the soluble TGF-beta type II receptor, lacking cytoplasmic and membrane domains, was used as a TGF-beta antagonist.

Objectives The objective of this study was to determine the effect of inhibition of endogenous TGF-beta during an experimental model of cartilage damage.

Methods For the production of recombinant solRII, the methylotrophic yeast *Pichia pastoris* was used. Intra-articular injection of papaine was used as a model for experimental cartilage damage and osteophyte formation. The effect of systemic delivery of solRII on endogenous TGF-beta was investigated in this model. Therefore, Alzet osmotic pumps containing either 10 mg of solRII or pumps containing solvent were implanted i.p. in C57Bl/6 mice. One day later, a 1.2% papaine solution was injected in the right knee joint. Knees were dissected on day 7 and processed for routine histology.

Results Injection of papaine into murine knee joints resulted in the formation of osteophytes and a decrease in proteoglycan (PG) content of non-calcified articular cartilage. Blocking of endogenous TGF-beta via systemic administration of solRII resulted in a reduction of 75% in osteophyte size compared to controls. Moreover, cartilage PG content was significantly decreased in solRII treated animals compared to control animals.

Conclusion This study shows for the first time that inhibition of endogenous TGF-beta leads to a reduction in osteophyte size in the papaine model of cartilage damage and osteophyte formation. This finding implicates a direct role for TGF-beta in the