returned to control levels when synovial fibroblasts were co-incubated with PGF2α inhibitor AL8810 whereas inhibition of TGFβ signaling by SB505124 had no effect. sMA gene expression and collagen production were unaffected by inhibition of TGFβ or PGF2α signaling. The increased synovial fibroblast migration in response to FCM could not be counteracted by SB505124, but was partially counteracted by AL8810. Synovial fibroblast proliferation in response to FCM was unaffected either by SB505142 or AL8810.

Conclusions: These results indicate that infrapatellar fat can contribute for the development of synovial fibrosis by increasing collagen production, PLOD2 gene expression, cell proliferation and cell migration; all characteristics of a fibrotic process. Based on our results, not TGFβ but the more recently discovered pro-fibrotic factor PGF2α seems partly responsible for the observed effects.

96 EFFECT OF CHONDROTIN SULFATE ON THE FACTORS INVOLVED IN SYNOVIAL INFLAMMATION

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Purpose: Chondroitin sulfate (CS), an interesting candidate for the therapeutic treatment of osteoarthritis (OA), has been shown in clinical trials to reduce swelling and effusion in knee OA. We thus aimed to further investigate the effect of CS alone or in combination with glucosamine sulfate (GS), an anti-COX-2 (cecloxib), or acetylaminophen on some synovial membrane anti-angiogenic and inflammatory factors.

Methods: Treatment with CS (200 μg/ml; CsBio-Active®, Bioibérica, Spain), GS (5 mM), cecloxxib or acetylaminophen alone, and CS in combination with the other mentioned products was investigated on human OA synovial fibroblasts in the presence or absence of IL-1β at 10 and 100 ng/ml. To determine whether CS has an additional effect with cecloxxib or acetylaminophen, preliminary experiments were performed to find concentrations of these products that induced about a 50% reduction in prostaglandin E2 production under IL-1β at 100 ng/ml. A concentration of 10 nM was found for cecloxxib and 25 μM for acetylaminophen. The expression levels (real time PCR) and/or protein production of vascular endothelial growth inhibitor (VEGI), thrombospandin-1 (TSP-1), hyaluronic acid (HA), secreted phospholipase 2 (sPLA2), and cytosolic phospholipase A2 (cPLA2) were determined using specific primers (expression) and ELISAs (protein).

Results: On the anti-angiogenic factors VEGI and TSP-1, IL-1β dose-dependently decreased their levels. On cells under basal conditions or treated with IL-1β, CS significantly induced the levels of VEGI expression and TSP-1 production. All the other products tested (CS, cecloxxib, and acetylaminophen) alone had no effect or decreased these anti-angiogenic factors, but in conjunction with CS their levels were significantly increased. HA production was slightly but significantly increased by IL-1β at 10 ng/ml and a heightened induction was found at 100 ng/ml. CS significantly increased HA production under basal conditions and in the presence of IL-1β at 10 ng/ml. GS had no effect and cecloxxib and acetylaminophen significantly decreased it. Concomitant incubation of CS with GS, cecloxxib, or acetylaminophen significantly increased HA production. Interestingly, sPLA2, which is considered an anti-inflammatory (resolving) factor, demonstrated a significant decrease under IL-1β. Incubation with CS alone and in conjunction with cecloxxib or acetylaminophen significantly increased its level. The inflammatory factor cPLA2 showed significantly decreased expression levels by CS, GS, and the combination of CS and GS. IL-1β markedly and significantly increased it at both concentrations and under IL-1β, CS alone had no effect, but in combination with GS, its level was significantly reduced.

Conclusions: The anti-inflammatory effect of CS appears to occur through a number of mechanisms including the inhibition of the anti-angiogenic factors TSP-1 and VEGI as well as sPLA2, a factor associated with an anti-inflammatory effect, and through increasing HA production and decreasing cPLA2. Importantly, these effects of CS occurred in the presence of other products, even though those products alone had no or a reverse effect.

97 EXPRESSION OF SPECIFIC PATHWAYS IN THE INFLAMED SYNOVIAL MEMBRANE OF OSTEOPATHITIS PATIENT: IDENTIFICATION OF NEW POTENTIAL KEY INTERMEDIATES

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Purpose: Synovitis is a key factor in osteoarthritis (OA) pathophysiology, contributing to both patient symptoms and disease progression. In this study, using an original methodology comparing normal/reactive (N/R) and inflammatory (I) synovial membranes zones, we investigated the gene expression profiles of synovial cells from these areas and identified differentially regulated pathways.

Methods: Synovial cells (SC) were isolated from OA synovial specimens obtained from 12 patients undergoing knee replacement. The inflammatory status of the synovial membrane was characterized by the surgeon according to macroscopic criteria including the synovial vascularization, the vili formation and the hypertrophic aspect of the tissue. At the surgery time, the synovial membrane was dissected and biopsies from N/R and I areas cultured separately for a period of 7 days. Total RNA was extracted using the RNeasy Mini Kit. RNA purity and quality were evaluated using the Experion RNA StdSens Analysis kit (Bio-rad Laboratories). Gene expression profiling between N/R and I areas was performed using Illumina’s multi-sample format Human HT-12 BeadChip (Illumina Inc.). Differential analysis was performed with the BRB array tools software. Class Comparison test between N/R and I areas was based on paired t-test where N/R and I were paired for each patient. The biological relevance of up- and down-regulated genes was analysed with Ingenuity Pathways Analysis (Ingenuity® Systems). Western blot was performed to confirm certain intermediate expression.

Results: From among 47000 probes, 17500 were filtered out. Probes with a p-value below than 0.005 were chosen and classified as up- or down-regulated ones. By this way, 896 differentially expressed genes between N/R and I zones were identified. Among these, 576 genes were upregulated (I/NR > 1.5) and 320 downregulated (I/NR < 0.75). With Ingenuity Pathways Analysis, a significant number of the top ranking differentially expressed genes were identified as inflammatory, Wnt and angiogenic pathways. Interleukin (IL)-6 and –8, chemokines (CXCL1, CXCL2, CXCL5, CXCL6, CXCL16) and arachidonate 5-lipoxygenase (ALOX5) were identified as the most upregulated in I zones in the inflammatory pathway. Interestingly, the alamin S100A9 was found strongly upregulated in this pathway. Wnt5A and LRP (Low density lipoprotein receptor-related protein) 5 were upregulated whereas FZD (Frizzled homolog) 2 and DKK (dickkopf homolog) 3 were downregulated in the Wnt signaling pathway. Finally, stanniocalcin (STC)-1, an intermediate in angiogenesis was identified as the most upregulated gene in I zones compared to N/R zones. This difference of expression was confirmed at the protein level.

Conclusions: Using a unique culture system, this study is the first to identify different expression pattern between two areas of synovial membrane from the same OA patient. These differences concern several key pathways involved in OA pathogenesis, i.e. inflammation, Wnt and angiogenesis. This analysis also provided interesting information regarding new potential intermediates as S100A9 and STC-1. They could be potential targets for chondroitin sulfate, one of the most used molecules in the management of OA. New experiments are being performed at the moment to elucidate the potential effect of this molecule on these specific differentially expressed genes in the same culture system.

98 SYNOVIAL INFLAMMATION CORRELATES WITH MENISCAL PATHOLOGY IN A COHORT OF PATIENTS UNDERGOING ACL RECONSTRUCTION FOR TRAUMATIC ACL RUPTURE

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