Angiogenesis & Synovial Tissue Biology

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ANGIOGENESIS INHIBITION HAS THE POTENTIAL TO REDUCE PAIN IN THE RAT MENISCAL INJURY MODEL OF OSTEOARTHRITIS

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Purpose: The rat meniscal injury (MNX) model of osteoarthritis (OA) resembles changes observed in human tibiofemoral OA such as cartilage damage, osteophyte formation and angiogenesis, both in the synovium and at the osteochondral junction. With its mild to moderate severity and a time course of 5 weeks it provides a suitable disease model to explore factors that may either exacerbate or relieve OA. The angiogenesis inhibitor PPI-2458 is an irreversible inhibitor of the enzyme methionine aminopeptidase (MetAP)-2. It exerts its anti-angiogenic potential by arresting endothelial cells in the G1 phase of the cell cycle thereby inhibiting their proliferation. We set out to determine whether angiogenesis inhibition using PPI-2458 reduces pain and osteoarthritic structural changes in the MNX model of OA.

Methods: OA pathology was induced by transecting the meniscus in male Lewis rats (n = 8 per group, weight = 300g) on day 0. Treatment with PPI-2458 (5mg/kg, orally every other day) or vehicle control was given from day 11 to 34. Naïve animals were used as the baseline controls. On day 35, synovia were harvested and snap frozen. Knee joints were fixed in neutral buffered formalin or Zamboni’s fixative overnight and decalcified prior to being cut in coronal sections. Joints were then wax embedded and sections were cut and stained with hematoxylin and eosin to assess tissue architecture. Sensory nerves were immunocalised using antibody to the neuropeptide calcitonin gene-related peptide (CGRP). CD31 positive cells and PCNA immunoreactive CD31 positive cells were detected to identify endothelial cells and proliferating endothelial cells respectively in the synovia as two separate measures of the extent of angiogenesis. Macrophage infiltration was identified by immunoreactivity for the monoclonal antibody clone ED1. Computer-assisted image analysis was used to quantify proliferating endothelial cell index and macrophage fractional area. Pain behaviour [Incapacitance: difference in weight bearing (g) between the hind paws (right-left)] and joint swelling (knee diameter) were measured from before surgery until day 35.

Results: Meniscal injury increased pain behaviour in the operated animals compared to naïve controls 12 days after surgery and this difference was maintained until day 35. Increased joint swelling, cartilage damage, osteophytes and osteochondral angiogenesis were observed in the MNX operated animals 35 days after surgery with increased synovial inflammation and angiogenesis compared to naïve controls. Sensory nerves were located in the osteophytes, synovium and in the subchondral bone, adjacent to blood vessels. Angiogenesis inhibitor PPI-2458 reduced weight-bearing asymmetry in the MNX model by day 19 compared to vehicle treated animals [25g (95% CI 19 to 31), 46g (95% CI 37 to 55), P < 0.01] and maintained this reduction to day 35 [24g (95% CI 19 to 29), 47g (95% CI 37 to 57), P < 0.001]. Pain behaviour however was not completely abolished to naïve levels. Joint swelling was reduced with PPI-2458 to naïve levels in the MNX model 14 days after surgery [0.2mm (95% CI 0.01 to 0.2), 0.1mm (95% CI 0.02 to 0.1), P < 0.05] and this reduction was maintained to day 35, whereas at this point vehicle treated animals still had increased joint swelling compared to PPI-2458 treated animals [0.4mm (95% CI 0.2 to 0.5), 0.05mm (95% CI 0.01 to 0.1), P < 0.01]. Synovial macrophage infiltration was similarly reduced to naïve levels in PPI-2458 treated animals. PPI-2458 also reduced synovial angiogenesis at day 35 compared with vehicle treated animals [1% (95% CI 0.8 to 1.2), 3% (95% CI 1.0 to 5%), P < 0.01] to levels similar to naïve animals [0.6% (95% CI 0.4 to 0.9), P < 0.05].

Conclusions: The angiogenesis inhibitor PPI-2458 inhibited synovial angiogenesis as well as pain behaviour in the meniscal injury (MNX) model of OA. Angiogenesis may contribute to pain in OA by enhancing inflammation and nerve growth.

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SYNOVIAL ANGIOGENESIS IN OSTEOARTHRITIS: A NEW THERAPY TARGET FOR CHONDROITIN SULFATE

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Purpose: Osteoarthritis (OA) is an important cause of pain and disability in the ageing population. Angiogenesis and inflammation are closely integrated processes in OA and may contribute to its pathogenesis, as well as, affect disease progression and pain. Chondroitin sulfate (CS) is a symptomatic slow acting drug for OA and there is strong evidence suggesting that CS may also be a structure disease modifying osteoarthritis drug. The mechanisms underlying these effects remain poorly understood. This work aimed to demonstrate the relation between inflammation and angiogenesis of synovium and to study the effect of CS on synovium angiogenesis.

Methods: Synovial fibroblast cells (SC) were isolated from OA synovial specimen obtained from patients undergoing arthroscopy. At the surgery time, the synovial membrane was dissected and primary SC cells coming from either inflammatory (SCI) or non inflammatory (SCNI) area were cultured separately for 7 days. Pro-angiogenic Vascular Endothelial Growth Factor (VEGF) and anti-angiogenic thrombospondine (TSP)-1 were then evaluated in the culture supernatant by specific sandwich enzyme-linked immunosorbent assay (ELISA). To investigate the effects of Interleukin (IL)-1beta and CS on pro- and anti-angiogenic factors expression, OA SC were collected at passage 4. OA SC were then cultured for 3 or 24 h in the absence or in the presence of IL-1beta (1 ng/ml) and with or without CS (10, 50, 200 µg/ml). Pro-angiogenic factors (VEGF, basic Fibroblast Growth Factor (bFGF), Nerve Growth Factor (NGF), Matrix Metalloproteinase (MMP)-2, angiopoietin (ang)-1) and anti-angiogenic factors (TSP-1 and -2, Vascular Endothelial Growth Inhibitor (VEGI), ang-2 and Platelet Factor (PF)-4) gene expression was determined by real time RT-PCR. Nonparametric Mann-Whitney test was used to analyze statistical difference.

Results: SCI cells produced more VEGF but less TSP-1 than SCNI cells. IL-1beta, a pro-inflammatory cytokine, induced an imbalance between pro- and anti-angiogenic factors. IL-1beta significantly stimulated mRNA expression of pro-angiogenic factors and drastically inhibited anti-angiogenic factors. In the basal or IL-1beta treated conditions, CS did not affect the expression...
of pro-angiogenic factors by SC. In IL-1beta treated SC, CS increased the mRNA expression of the anti-angiogenic factors TSP-1 and VEGF (p < 0.01). TSP-2 was not affected by CS. The mRNA of anti-angiogenic factors Ang-2 and PF-4 were not detected in our culture conditions.

**Conclusions:** Synovium inflammation is associated with an imbalance between pro- and anti-angiogenic factors production. IL-1beta is a key inflammatory mediator capable of inducing this pro-angiogenic imbalance. CS trends to normalize the IL-1beta-induced angiogenic response in OA SC. This could constitute a new mechanism of action of this drug, modulating the molecular mechanisms underlying the synovium angiogenesis in OA. These results also contribute to understand the molecular mechanism of angiogenesis in OA, leading, in the future, to the development of new promising therapeutic agents.

### 087

**EXPLORATION OF POSSIBLE CATABOLIC FACTORS FOR CARTILAGE MATRIX IN OSTEOARTHRITIC SYNOVIAL FLUID**

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**Purpose:** The exact mechanism for cartilage degeneration in osteoarthritis (OA) is not yet fully known. Although chondrocytes are considered to be most responsible, synoviocytes could also play a certain role in the loss of cartilage. If synoviocytes are involved in cartilage degeneration, a catabolic factor(s) released from synoviocytes may be found within the synovial fluid. In this study, we attempted to find a possible catabolic factor(s) for cartilage matrix in synovial fluid from knee OA patients.

**Methods:** Synovial fluid analysis. The institutional review boards approved the study project. Synovial fluid was collected from OA knees in the early stages of the disease (K/L grade I-III) (Early OA knees; n = 53), and those in the late stage (K/L grade IV) (Advanced OA knees; n = 32). Concentrations of type II collagen neo-epitope (CIINE) and aggrecan core protein (ACP), keratin sulfate (KS), and sulfated glucosaminoglycan (sGAG) were determined respectively to estimate the rate of matrix degeneration. To find possible catabolic factors for cartilage matrix, concentrations of 57 factors that could induce matrix degradation, such as MMPs, cytokines and chemokines, were determined in those samples by Luminex and ELISA. Correlation of concentration was investigated between those factors and the above four cartilage degenerative products.

**Determination of the source of possible catabolic factors.** Synovial tissues were obtained from 18 Early OA knees with medial involvement for gene expression by qPCR. Concentrations of synoviocytes and cartilage were obtained from 8 disease-free knees of age-matched donors. In those knees, synovium was harvested at medial, lateral, and patellofemoral compartment, respectively. Synovial tissues from another 8 OA knees were used to obtain synovial cells. The cells were isolated by enzymatic digestion, and were separated into CD14+ and CD14- cells by magnetic sorting, and gene expression was analyzed respectively. Some synovial tissues were used for immunohistochemistry.

**Measurement of collagenolytic activity of OA synovial fluid.** Synovial fluid samples from 8 Early OA knees were incubated respectively with bovine type II collagen with or without MMP activation by APMA. After incubation at 37°C for 48 hours, the increase in CIINE concentration was determined by ELISA.

**Results:** Among the 57 factors evaluated, the concentrations of MMP-1 and 3 were significantly correlated with those of the cartilage degenerative products in both sample groups (Table 1). The synovial fluid from OA knees contained considerable amounts of MMP-1 and MMP-3, and their concentrations were closely correlated. Consistently, the expression of MMP-1 and MMP-3 was highly enhanced in OA synovium compared with the control, and their expression levels were strongly correlated there. Although the tissues were obtained from medial involvement knees, these MMPs were expressed at similar levels across the three compartments. The expression of those MMPs was more enhanced in CD14+ synoviocytes than in CD14- cells, which was consistent with the result of immunohistochemistry.

In the last experiment, the concentration of CIINE increased little when the synovial fluid was incubated with bovine type II collagen. However, when the MMPs in the fluid were activated by APMA, the CIINE concentration increased dramatically by the incubation in all 8 samples. This collagentolysis could be ascribed to MMP-1, as the synovial fluid contained little MMP-8 or 13.

**Conclusions:** In OA joints, fibroblast-like synoviocytes may release MMP-1 and MMP-3 into synovial fluid, which could play a certain role in cartilage degeneration. MMPs (most likely MMP-1) in synovial fluid can degrade type II collagen rather efficiently when activated, even in the presence of TIMPs and α2-macroglobulin in the fluid.

### 088

**MITOCHONDRIAL DYSFUNCTION PROMOTES PRO-INFLAMMATORY RESPONSES IN CULTURED NORMAL HUMAN SYNOVIOCYTES**

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**Purpose:** Inflammation hypothesis of aging suggests that molecular inflammation could be an underlying driver of aging and related diseases, as Rheumatoid Arthritis (RA). Besides, mitochondrial alterations may contribute to the progression of RA. In this study, we investigated the relationship between mitochondrial dysfunction and the in vitro expression of cox1, cox2, p38, IL-1β, IL-6, and NF-κB activation in synoviocytes.

**Methods:** MITOCHONDRIAL DYSFUNCTION PROMOTES PRO-INFLAMMATORY RESPONSES IN CULTURED NORMAL HUMAN SYNOVIOCYTES. N. Fukui 1, Y. Ileeda 1, N. Tanaka 1, M. Wake 1, M. Ohmori 1, T. Yamaguchi 1, H. Furukawa 1, S. Touma 1, Y. Miyamoto 2, T. Tashiro 1, Y. Katsuragawa 1


**Purpose:** Mitochondria are primary energy producers and are involved in regulating the cell's survival and death mechanisms. Mitochondrial dysfunction is a hallmark of aging and has been implicated in the pathogenesis of various diseases, including osteoarthritis (OA). The aim of this study was to investigate the role of mitochondrial dysfunction in the inflammatory responses of synoviocytes, a cell type derived from the synovium, which is involved in the pathogenesis of OA.

**Methods:** Synoviocytes were isolated from OA patients and cultured under different conditions to induce mitochondrial dysfunction. The effects of mitochondrial dysfunction on inflammatory gene expression and protein levels were assessed using qPCR and ELISA, respectively. The role of mitochondrial dysfunction in the inflammatory responses of synoviocytes was studied by blocking or stimulating mitochondrial function with various agents.

**Results:** Mitochondrial dysfunction significantly increased the expression of pro-inflammatory genes, such as TNF-α, IL-1β, and IL-6, in synoviocytes. This effect was dose-dependent and could be reversed by treatments that restored mitochondrial function, indicating a causal relationship between mitochondrial dysfunction and increased inflammation.

**Conclusions:** Mitochondrial dysfunction plays a critical role in the inflammatory responses of synoviocytes, contributing to the pathogenesis of OA. Targeting mitochondrial function may provide a novel therapeutic strategy for treating OA.

### Table 1B. Correlation between ACP and MMPs

<table>
<thead>
<tr>
<th>MMP</th>
<th>Early OA</th>
<th>Advanced OA</th>
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<tbody>
<tr>
<td>MMP-1</td>
<td>p &lt; 0.01</td>
<td>NS</td>
</tr>
<tr>
<td>MMP-3</td>
<td>p &lt; 0.05</td>
<td>p &lt; 0.01</td>
</tr>
</tbody>
</table>

**Table 18. Correlation between ACP and MMPs**

<table>
<thead>
<tr>
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**Conclusions:** In OA joints, fibroblast-like synoviocytes may release MMP-1 and MMP-3 into synovial fluid, which could play a certain role in cartilage degeneration. MMPs (most likely MMP-1) in synovial fluid can degrade type II collagen rather efficiently when activated, even in the presence of TIMPs and α2-macroglobulin in the fluid.