

## Poster Presentations

### 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 or frozen in OCT. Tissue sections were cut and stained with haematoxylin and eosin to assess tissue architecture. Sensory nerves were immunolocalised 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 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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#### SYNOVIUM 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)-1 $\beta$  and CS on pro- and anti-angiogenic factors expression, OA SC were collected at passage 4. OA SC were then cultured for 5 or 24 h in the absence or in the presence of IL-1 $\beta$  (1 ng/ml) and with or without CS (10, 50, 200  $\mu$ g/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-1 $\beta$ , a pro-inflammatory cytokine, induced an imbalance between pro- and anti-angiogenic factors. IL-1 $\beta$  significantly stimulated mRNA expression of pro-angiogenic factors and drastically inhibited anti-angiogenic factors. In the basal or IL-1 $\beta$  treated conditions, CS did not affect the expression