increase in IL1ra. This was especially seen in the M2 macrophages, indicating that OA SF enhances this phenotype. Surprisingly, RA SF also seemed to decrease pro-inflammatory processes as shown by a decrease of IL10 and an increase of IL1ra, but also by the decrease of IL6. In conclusion, SF from diseased joints does not induce pro-inflammatory processes, even not when from RA joints. Based on our results, SF from diseased joints might even induce anti-inflammatory processes.

506 SYNOVOCYTE INFLAMMATORY RESPONSES TO CALCIUM PYROPHOSPHATE DIHYDRATE CRYSTALS INVOLVES CD14 AND TLR-4.

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Purpose: Low-grade synovial inflammation is seen in the setting of osteoarthritis (OA), even in early-stages, although the molecular triggers of inflammation in OA are as yet unclear. Stimulation of Toll-like receptors (TLRs) by endogenous molecules released during cartilage matrix damage and chondrocyte stress is hypothesized to be one mechanism leading to synovial inflammation in OA. We previously reported that a TLR co-receptor, soluble CD14 (scCD14), is found in synovial fluid (SF) from patients with early and advanced stage OA, and can augment responses of fibroblast-like synoviocytes (FLS) to typical TLR-2 and TLR-4 ligands. The purpose of the current study was to determine if scCD14 can impact FLS responses to crystalline ligands (specifically MSU and CPPD) that have relevance to OA pathogenesis.

Methods: Synovial tissue specimens for establishment of FLS lines were obtained from asymptomatic organ donors with no documented history of joint disease via IRB approved protocols. Recombinant human CD14 (0.5 ug/ml, based on concentrations found in patient SF) in combination with MSU crystals or CPPD crystals (both from Invivogen) used in concentrations from 0.05 to 50 ug/ml were used to stimulate primary FLS at passage 4. Stimulation was measured by IL-8 release into the culture media measured by ELISA (Invitrogen). Responses of HEK-293 cell lines transfected with TLR-2 or TLR-4 were measured to determine the dependence of observed responses on these receptors. A peptide inhibitor of TLR-4 signaling (CLI-095, Invivogen) was used to test whether FLS responses were related to TLR-4 mediated signaling.

Results: CPPD, but not MSU, was able to stimulate IL-8 production from FLS cells in the presence of CD14. Experiments in three separate cell lines demonstrated that addition of CD14 augmented the effect of CPPD down to a concentration of 0.5 ug/ml (repeated measures ANOVA, p = 0.02 at 0.5 ug/ml, with CD14 vs. without CD14). CPPD, in combination with rhCD14, was able to stimulate HEK-293 cells transfected with TLR-4, but not TLR-2. In FLS cells, addition of the TLR-4 inhibitor CLI-095 prevented the response to CD14+CPPD.

Conclusions: CPPD crystals stimulate FLS production of IL-8 in the presence of CD14. Our experiments indicate that TLR-4 is involved in this response. As CPPD crystals are known to be common in the joint tissues and fluid of patients affected by OA, it is possible that the synovial response to these crystals may contribute to inflammation in OA.

507 ANTI-INFLAMMATORY EFFECTS OF LANSOPRAZOLE BY INHIBITING NITRIC OXIDE PRODUCTION VIA REACTIVE OXYGEN SPECIES IN MURINE MACROPHAGE RAW 264.7 CELLS


Purpose: Lansoprazole (LPZ), proton pump inhibitor, is widely used clinically to treat gastrointestinal mucosal disorders. The mucosal-protective effects of PPIs are due primarily to their inhibition of P-ATPase. More recently, however, PPIs were shown to have local pleiotropic effects in the gastric mucosa, including having anti-inflammatory effects, inhibiting the generation of reactive oxygen species (ROS), and inhibiting the expression of macrophage adhesion molecules. aberrantly activated macrophages overproduce inflammatory mediators such as nitric oxide (NO) and prostaglandin E2 (PGE2), both of which are involved in the pathogenesis of many inflammatory diseases including rheumatoid arthritis. Agents that target macrophages, inhibiting the secretion of multiple inflammatory mediators, may provide a breakthrough as novel therapy for patients with inflammatory disorders. The purpose of this study is to investigate the effects of LPZ on activated macrophages and the mechanisms by which LPZ inhibits the production of inflammatory mediators such as NO and PGE2.

Methods: RAW264.7 murine macrophages were used in this study. The cells were incubated with various concentrations of LPZ for 24 h and the cell viability was evaluated by MTS assay. The cells were treated with various concentrations of LPZ in the presence or absence of lipopolysaccharide (LPS), and the concentration of NO and PGE2 in supernatant was measured. iNOS and COX-2 expression were analysed by Western blotting. We used RT-PCR to determine whether P-ATPase mRNA is expressed in RAW264.7 cells. We measured intracellular ROS concentrations of LPS-stimulated cells by flow cytometry in the presence or absence of 100 μM LPZ. To determine whether LPS-induced ROS affects NO and PGE2 production, we measured NO and PGE2 levels after controlling ROS levels using antioxidants such as N-acetyl-L-cysteine (NAC), an ROS scavenger, and Diphenylene iodonium (DPI), an inhibitor of NADPH oxidase.

Results: LPZ, at concentrations of 0–100 μM, had no effects on the viability of RAW264.7 cells. LPZ inhibited the LPS-induced production of NO and PGE2 in a concentration-dependent manner. The expression of iNOS and COX-2 was also suppressed by LPZ at a concentration of 100 μM. P-ATPase expressed in mouse gastric mucosal tissue but not in RAW264.7 cells. In addition, LPZ alone did not alter ROS levels in the absence of LPS, whereas preincubation with LPZ prior to LPS stimulation significantly reduced the levels of ROS. NAC had no effect on NO levels and, DPI, an inhibitor of NADPH oxidase, decreased NO levels, but neither NAC nor DPI altered PGE2 levels.

Conclusions: We found that LPZ not only inhibited NO and PGE2 production but also suppressed iNOS and COX-2 expression. This result suggests that LPZ exerts anti-inflammatory effects by suppressing the expression of iNOS and COX-2. We found that LPS stimulation increased the production of both ROS and NO and that DPI suppresses LPS-induced NO production. These findings suggest that, LPS stimulation increases ROS production and is associated with inflammatory responses by inducing NO production. NAC did not inhibit LPS-induced NO production, indicating that once ROS has been produced, it is difficult to control NO production. Our findings, showing that LPZ suppressed the inflammatory activity of activated macrophages in vitro, suggest that LPZ may be promising in the treatment of inflammatory diseases involving activated macrophages including rheumatoid arthritis. Studies in animal models may show these therapeutic effects.
MARKERS OF ATHEROSCLEROSIS IN RELATION TO PRESENCE AND PROGRESSION OF KNEE OSTEOARTHRITIS: THE ROTTERDAM STUDY


Purpose: Several observational studies have found an association between subclinical measures of atherosclerosis and osteoarthritis (OA) of the hands and knees, predominantly among women. Different mechanisms have been suggested to explain the potential relation between atherosclerosis and OA; systemic low-grade inflammation caused by visceral adipose tissue is particularly mentioned and may consequently highlight a route to improve prevention and treatment of atherosclerosis and OA. However, the reported associations between subclinical measures of atherosclerosis and OA were modest in effect size, derived mainly from cross-sectional studies and generally attenuated after adjustment for cardiovascular risk factors. Furthermore, previous results were inconsistent for the different imaging markers of atherosclerosis. In previous work, we found no relation between peripheral measurements of atherosclerosis (including carotid intima-media-thickness or carotid plaque) and progression of knee, hand, or hip OA in a large sample of a prospective cohort study. Hence, it remains unclear whether atherosclerosis and OA are related or whether they are simply independent disorders with shared risk factors. Coronary artery calcification (CAC) is considered a late stage pathognomonic feature of coronary atherosclerosis. CD40L, VCAM-1, and VEG-f are serum biomarkers that reflect initial inflammatory stages of vascular wall damage in the ischemic cascade. In the vascular endothelium, VEG-f is a protein that stimulates angiogenesis, CD-40L and VCAM-1 are molecules that initiate coagulation and immune responses. We therefore investigated the association between these markers of early and late atherosclerosis and presence and progression of knee OA, a possible cardiometabolic phenotype of OA, in a large population-based cohort study.

Methods: The analyses on prevalence of knee OA were performed in 3,465 participants from the prospective population-based Rotterdam Study (mean age 73.1 years, 61% women). Data on coronary artery calcification (CAC) were available for 1,699 participants and plasma levels of CD40L, VCAM-1, and VEG-f in 975. For the analyses on progression of knee OA, data on CAC was available for 979 participants (17% progressors), and 246 participants (20% progressors) had plasma levels of CD40L, VCAM-1, and VEG-f. We scored radiographs of the knee with the Kellgren-Lawrence (K&L) score for osteoarthritis (knee OA present with a K&L graded score equal or greater to 2) at baseline and follow-up (average follow-up time 4.5 years ±0.5). Overall progression of knee OA was defined as the combination of the incidence and the progression of existing OA at baseline and was considered present if the K&L score increased 1 grade between baseline and follow-up visit. After stratification by gender, multivariate logistic regression models with generalized estimated equations on knee level were used to calculate odds ratios (95% confidence intervals) for prevalence and progression of knee OA per each SD increase in marker levels.

Results: Within the study population, 18% had radiographic knee OA, 11% of the men, 23% of the women. CAC and VEG-f were not associated with prevalent knee OA. Among women, CD40L (adjusted odds ratio (aOR) 1.31 (1.12 to 1.56)) and VCAM-1 (aOR 1.31 (1.08 to 1.59)) were associated with prevalent knee OA (table). No associations with progression were found in women. In men, too few progressors were available to assess associations.

Table. Markers of atherosclerosis in relation to prevalent knee OA, stratified by gender

<table>
<thead>
<tr>
<th></th>
<th>Knee OA (Women) OR (95% CI)*</th>
<th>Knee OA (Men) OR (95% CI)*</th>
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<tbody>
<tr>
<td>Coronary artery</td>
<td></td>
<td></td>
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<tr>
<td>calcification</td>
<td>1.11 (0.95–1.30)</td>
<td>1.11 (0.86–1.43)</td>
</tr>
<tr>
<td>CD40L</td>
<td>1.32 (1.12–1.56)**</td>
<td>1.05 (0.80–1.37)</td>
</tr>
<tr>
<td>VCAM-1</td>
<td>1.31 (1.08–1.59)**</td>
<td>1.08 (0.82–1.42)</td>
</tr>
<tr>
<td>VEG-f</td>
<td>1.08 (0.93–1.24)</td>
<td>1.07 (0.90–1.28)</td>
</tr>
</tbody>
</table>

Conclusions: In this population-based study, coronary artery calcification and VEG-f were not associated with presence or progression of knee OA. However, plasma levels of CD40L and VCAM-1 were higher in women with knee OA and not in men. This might reflect an association between early atherosclerosis and knee OA through low-grade systemic inflammation in women.

MECHANISMS INVOLVED IN INHIBITION OF INFLAMMATION IN THP-1 CELLS BY THE HEXADECYLAMIDE DERIVATIVE OF HYALURONIC ACID.

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Purpose: Intra-articular injections of hyaluronic acid are widely used in the treatment of inflammatory and degenerative joint diseases. The immune regulation exerted by hyaluronic acid is modulated by its interaction with different receptors including CD44 and the toll-like receptors 2 and 4. A novel hexadecylamide derivative of hyaluronic acid (HA), HYADD®4, has recently been tested in animal models of osteoarthritis, showing both anti-inflammatory and anabolic effects. The purpose of this study is to investigate the possible mechanisms involved on the effect of unmodified and hexadecylamide derivative