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Targeted lipidomics reveals activation of resolution pathways in knee osteoarthritis in humans

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SUMMARY

Objective: To investigate the presence of inflammation and resolution pathways in osteoarthritis (OA).
Design: Tissues were obtained from knee OA patients and control rheumatoid arthritis (RA) patients. Cells in synovial fluid (SF) were visualized by flow cytometry. Cytokines and chemokines were measured by multiplex assay. Lipid mediators (LMs) were determined by targeted lipidomics using liquid-chromatography mass spectrometry.
Results: SF of OA patients contained less cells, especially neutrophils, less cytokines and comparable levels of chemokines compared to RA controls.
Thirty-seven lipids were detected in the soluble fraction of SF, including polyunsaturated fatty acids (PUFAs) and their pro-inflammatory and pro-resolving lipoxygenase (LOX) and cyclooxygenase (COX) pathway markers in both OA and RA patients. Among these, pro-inflammatory LM such as prostaglandin E_2 (PGE_2) and thromboxane B_2, as well as precursors and pathway markers of resolution such as 17-HDHA and 18-HEPE were detected. Interestingly, the pro-resolving lipid RvD2 could also be detected, but only in the insoluble fraction (cells and undigested matrix). Ratios of metabolites to their precursors indicated a lower activity of 5-LOX and 15-LOX in OA compared to RA, with no apparent differences in COX-derived products. Interestingly, synovial tissue and SF cells could produce 5-LOX and 15-LOX metabolites, indicating these cells as possible source of LM.
Conclusions: By using a state-of-the-art technique, we show for the first time that resolution pathways are present in OA patients. A better understanding of these pathways could guide us to more effective therapeutic approaches to inhibit inflammation and further structural damage in OA and RA.

Introduction

Osteoarthritis (OA) is the most common form of arthritis, with a prevalence of more than 70% in the elderly population. Characteristic radiographic features of OA are cartilage degradation and the presence of osteophytes (bone spurs). It has recently become evident that synovitis is an accompanying feature of OA in a significant number of patients and that inflammation is an important player in OA (reviewed in 1 and 2) as it is associated with pain, as well as radiographic progression. The association with radiographic progression seems to be even stronger in patients with persistent inflammation[3]. The reason for persistent inflammation in some patients is unclear, but one intriguing possibility is that the essential resolution pathways are incompletely/not activated.

Inflammation is usually a self-resolving process initiated as a response to danger signals. This response is tightly regulated and involves the concerted and timely action of several molecular and

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cellular players. Extensive studies of acute inflammation in a model of self-resolving inflammation in mice indicated that the initial phases of inflammation are characterized by neutrophil recruitment, followed by macrophage accumulation during the resolution process[4,5].

At the molecular level, cytokines and lipids are involved in regulating inflammation. Pro-inflammatory mediators, such as cytokines, chemokines and eicosanoids (e.g., prostaglandins and leukotrienes), a class of lipid mediators (LMs) derived from arachidonic acid (AA), are released during the initial phases of inflammation, driving recruitment and activation of immune cells[4]. Resolution of inflammation has been shown to be an active process originating early in inflammation, being driven by anti-inflammatory and pro-resolving mediators[6–8]. Among these, several families of specialized pro-resolving mediators (SPMs) have been identified (lipoxins, resolvins, protectins and maresins) that can induce resolution of inflammation in murine acute inflammatory models[9–11]. Moreover, they appear to be regulated during the disease course in asthma, Alzheimer's disease[12–14], multiple sclerosis[15], cystic fibrosis[16], as well as ulcerative colitis patients[17], indicating a possible role for SPMs in regulating inflammation in human disease.

The SPMs known to date are synthesized enzymatically through lipoxygenase (LOX), cyclooxygenase (COX), or cytochrome P450 (CYP) pathways from polyunsaturated fatty acids (PUFAs) such as AA, docosahexaenoic acid (DHA), or eicosapentaenoic acid (EPA), mostly through transcellular processes involving different types of cells[18,19]. More recently, other mechanisms such as microparticle uptake, phagocytosis and sequential stimulation with different stimuli have been implicated in the generation of SPMs[20,21].

In contrast to cytokines and chemokines, the presence of bioactive (oxy)lipids has only scarcely been investigated in OA. The available studies indicated the presence of the 15-LOX product 15-HETE and the COX product prostaglandin E_2 (PGE_2) in plasma and these appeared to be higher in OA patients than healthy controls[22]. Similarly, the 5-LOX product leukotriene B_4 (LTB_4) was described in synovial fluid (SF) of OA patients[23], as well as $PGF_{2\alpha}$, the non-enzymatically made 8-iso-$PGF_{2\alpha}$, and the deactivation product 15-keto-13,14-dihydro-$PGF_{2\alpha}$ in both SF and plasma[24]. Both LTB_4 and PGE_2 have been shown to be secreted by OA synovial explants[25]. Interestingly, 5-LOX and 15-LOX have been shown to be present in the OA synovium, however most of their LM products were not yet studied in detail[26]. Specifically, the presence of LMs associated with resolution of inflammation, SPMs or their precursors, has not yet been investigated in OA, despite the important role inflammation plays in the progression of structural damage.

The aim of this study was to investigate the activation of resolution in OA by studying the presence of bioactive lipids associated with resolution pathways in SF of OA patients. To this end, we employed a state-of-the-art targeted lipidomics approach to detect SPMs and their precursors in end-stage knee OA patients. Moreover, we extensively characterized inflammatory cells, cytokines and chemokines in SF and compared the results to rheumatoid arthritis (RA), as a control chronic inflammatory disease.

Materials and methods

Chemicals and materials

Listing of chemicals and other materials can be found in Supplementary Materials and Methods.

Patients and tissue sample collection

SF and synovial tissues from knee OA and RA patients were obtained as anonymized leftover material from patients undergoing knee arthroscopy at the department of Rheumatology or undergoing knee-replacement surgery at the Departments of Orthopaedic Surgery in the LUMC or Alrijne Hospital in Leiden, performed for standard clinical care. Diagnosis in all patients was established by the treating physician. Age, gender, and BMI are reported in Supplementary Table 1. This procedure was approved by the local ethical committee. SF samples were treated as described below and in Supplementary Fig. 1 and were stored at −80 °C until analysis. The average time to analysis was 7 months (range: 1 day−19 months).

Isolation of soluble and insoluble fraction of SF

One mL SF was treated with hyaluronidase, followed by centrifugation at 931 ×*g* for 10 min as described in Supplementary Fig. 1. The supernatant (soluble fraction) was removed and the pellet (insoluble fraction) was resuspended in 1 mL water. Proteins were precipitated from both soluble and insoluble fractions with 3 mL methanol (MeOH) (3184 ×*g* for 15 min at 4 °C). The MeOH supernatant was removed, the protein pellet washed again with 1 mL MeOH and internal standard (IS) was added (LTB_4-d4, 15(*S*)-HETE-d8 and PGE_2-d4, 150 pg each and DHA-d5 1500 pg). Next, the sample was spun again before combining the MeOH supernatants. After drying down the MeOH, diluting it with water and acidifying, the samples were loaded on 3 mL 500 mg Bond Elut C-18 solid-phase extraction (SPE) columns (Agilent Technologies Santa Clara, CA, USA) as described in the legend of Supplementary Fig. 1 and lipids were analyzed as described below.

Lipid analysis

Targeted lipidomics analysis of the SF was carried out after SPE as previously described[27] with some modifications (Supplementary Fig. 1). Liquid-chromatography combined with mass spectrometry (LC-MS/MS) analysis was carried out as previously published[28] with some modifications (Supplementary Materials and Methods).

Synovial tissue cells

Synovial tissue cells (synoviocytes) were isolated from fresh synovial tissue digested for 1.5 h with 1 mg/mL collagenase type 2 (Worthington Biochemical Corporation, Lakewood, NJ, USA) in serum free IMDM medium (Lonza, Basel, Switzerland). Digested tissue was filtered over a 70 μm cell strainer (Falcon, Corning Incorporated, Life Sciences, Durham, NC, USA) to obtain the cells present in synovium. The cells were washed 3 times with serum free IMDM medium before use.

Stimulation of SF cells and synoviocytes

Both SF cells (SFCs) and synoviocytes were first filtered over a 70 μm cell strainer (Falcon, Corning Incorporated, Life Sciences, Durham, NC, USA), then isolated cells suspended in PBS with calcium and magnesium (PBS (+/+), Sigma Aldrich, Steinheim, Germany) were stimulated with 4 μM calcium ionophore A23187 (Sigma, Saint Louis, MO, USA) or vehicle control for 10 min. For LPS stimulation experiments, SFC and synoviocytes were suspended in PBS (+/+) containing 0.1% fatty acid free BSA (Sigma, Saint Louis, MO, USA) and stimulated with 10 ng/mL LPS (Sigma, Saint Louis, MO, USA) for 72 h. Next, proteins were precipitated by adding 3 volumes of MeOH and IS (0.75 ng/mL final concentration). Samples were stored under argon at −80 °C until analysis. Before LC/MS−MS analysis, samples were centrifuged at 16,100 ×*g* for 10 min at 4 °C and supernatants diluted 1:1 with water. Precipitated protein was