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Lipid and lipid mediator profiling of human synovial fluid in rheumatoid arthritis patients by means of LC–MS/MS

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ABSTRACT

Human synovial fluid (SF) provides nutrition and lubrication to the articular cartilage. Particularly in arthritic diseases, SF is extensively accumulating in the synovial junction. During the last decade lipids have attracted considerable attention as their role in the development and resolution of diseases became increasingly recognized. Here, we describe a capillary LC–MS/MS screening platform that was used for the untargeted screening of lipids present in human SF of rheumatoid arthritis (RA) patients. Using this platform we give a detailed overview of the lipids and lipid-derived mediators present in the SF of RA patients. Almost 70 different lipid components from distinct lipid classes were identified and quantification was achieved for the lysophosphatidylcholine and phosphatidylcholine species. In addition, we describe a targeted LC–MS/MS lipid mediator metabolomics strategy for the detection, identification and quantification of maresin 1, lipoxin A_4 and resolvin D5 in SF from RA patients. Additionally, we present the identification of 5S,12S-diHETE as a major marker of lipoxygenase pathway interactions in the investigated SF samples. These results are the first to provide a comprehensive approach to the identification and profiling of lipids and lipid mediators present in SF and to describe the presence of key anti-inflammatory and pro-resolving lipid mediators identified in SF from RA patients.

1. Introduction

SF is a rarely studied body fluid that is present in only minute amounts in healthy joints. Its physiological function is to provide nutrition and lubrication to the articular cartilage. SF is composed primarily of hyaluronic acid, secreted by fibroblasts in the synovial membrane, interstitial fluid filtered from plasma and a low number of cells. In pathologic circumstances, such as inflammatory conditions of the joints (e.g. arthritic diseases–rheumatoid arthritis (RA)), infections or trauma, SF can accumulate in the joint reflecting synovial pathology [1]. In RA, SF is enriched in inflammatory cytokines and immune cells which could further enhance synovial inflammation and subsequent cartilage and bone pathology [2]. Consistent with a pathological role for SF in arthritis, removal of SF during arthroscopy

Abbreviations: 5S-HETE, 5S-6E,8Z,11Z,14Z-hydroxyeicosatetraenoic acid; 5S,12S-diHETE, 5S,12S-6E,8Z,10E,14Z-dihydroxyeicosatetraenoic acid; 17(R)HDoHE, 17(R)-hydroxy docosahexaenoic acid; AAd_8, arachidonic acid d8; AT-RvD1, 7S,8R,17R-trihydroxy-4Z,9E,11E,13Z,15E19Z-docosahexaenoic acid; CE, cholesterol ester; DHA, docosahexaenoic acid; ESI (±), electrospray ionization (positive/negative); FA, fatty acid; FTICR-MS, Fourier transform ion cyclotron mass spectrometry; GC–MS, gas chromatography–mass spectrometry; hFA, hydroxylated fatty acid; HRMS, high resolution mass spectrometry; IT-MS, ion trap mass spectrometer; LC–MS/MS, liquid chromatography–tandem mass spectrometry; LOX, lipoxygenase; LPC, lysophosphatidylcholine; LPC(19:0), 1-nonadecanoyl-2-hydroxy-sn-glycero-3-phosphocholine; LPE, lysophosphatidylethanolamine; LPI, lysophosphatidylinositol; LTB_4, 5S,12R-dihydroxy-6Z-8E,10E,14-Z-eicosatetraenoic acid; LTB_4 d_4, 5S,12R-dihydroxy-6Z,8E,10E,14Z-eicosatetraenoic-6,7,14,15-d4 acid; LXA_4, 5S,6R,15S-trihydroxy-7E,9E,11Z,13E-eicosatetraenoic acid; MALDI, matrix assisted laser desorption ionization; MaR, maresin, macrophage mediator in resolving inflammation; MaR1, 7R,14S-dihydroxydocosa-4Z,8E,10E,12Z,16Z,19Z-hexaenoic acid; MeOH, methanol; PBS, phosphate buffered saline; PC, phosphatidylcholine; PC (14:0/14:0 d_{54}), 1,2-dimyristoyl(d54)-sn-glycero-3-phosphocholine; PC (19:0/19:0), 1,2-dinonadecanoyl-sn-glycero-3-phosphocholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PG (14:0/14:0), 1,2-dimyristoyl-sn-glycero-3-phospho-(1'-rac-glycerol)(sodium salt); PGE_2 d_4, prostaglandin E2 d4; PI, phosphatidylinositol; PIP, phosphatidylinositolphosphate; PS, phosphatidylserine; PS (16:0/18:1 d_{31}), 1-palmitoyl(d31)-2-oleoyl-sn-glycero-3-[phospho-L-serine] (sodium salt); PUFA, poly-unsaturated-fatty acid; RA, rheumatoid arthritis; RP18, reversed phase octadecyl silica; RT, retention time; RRT, Relative retention time; RvD5, 7S,17S-dihydroxy-docosa-5Z,8E,10Z,13Z,15E,19Z-hexaenoic acid; SF, synovial fluid; SM, sphingomyelin; TG, triglyceride

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is an efficient intervention for immediate symptom relief [3]. During the last decade it has become clear that on-set and termination of inflammation are tightly controlled processes [4]. Particularly lipid species such as prostaglandins (PG) and lipid mediators play crucial roles in the tight regulation of inflammation. Prostaglandins, including PGE_2, and leukotrienes, such as LTB_4, are considered to play important roles in the onset and development of arthritic diseases [5–7]. Along these lines the major treatment strategies for RA include COX targeting substances such as NSAID, inhibiting the key enzyme(s) in the production of prostaglandins from AA. On the other hand, anti-inflammatory and pro-resolving lipid mediators, such as lipoxins and other specialized pro-resolving mediators are crucial for the active resolution of inflammatory processes [8]. The underlying mechanisms and their implications for the rheumatic diseases were summarized by Yacoubian and Serhan [9]. In this context, Lima-Garcia et al. recently provided evidence for the anti-hyperalgesic actions of both 17(R) HDoHE and AT-RvD1 in an arthritis rat model [10]. Of interest, a clinical trial showed that ω-3 PUFA had positive effects on reducing disease activity in RA patients [11]. Taken together, many clues from the literature point to an important role of lipids and particularly lipid mediators in arthritic diseases such as RA. Hence, we are interested in the lipid composition of human SF in RA patients in order to gain a better insight into the ongoing inflammatory processes occurring in the arthritic joint.

To date, the vast majority of published studies investigating SF were dedicated to proteome analysis [12,13]. Only a few recent studies investigated non-peptide metabolites or mediators present in SF. Goto et al. determined the PGE_2 levels in SF [14] after sodium hyaluronate injections, while Huffman et al. focused on glucose and lactate levels [15]. Referring to lipid species present in SF, earlier studies based on MALDI-MS mainly presented the detection of a limited number of different LPC and PC species in SF from RA patients, including altered LPC/PC ratios in RA patients [16,17]. Other studies report differences between normal SF and SF from RA patients, in particular higher amounts of cholesterol, cholesterol esters and changes in the phospholipid composition [18]. A more recent study presented a more detailed investigation of LXA_4 levels in SF [19]. In addition to this de Grauw et al. recently published an investigation of the lipid mediators present in the SF of horses with acute synovitis [20]. Thus all these studies investigated either total cholesterol- free FA- or TG-levels [21], and were mainly limited to LPC and PC species, focusing on a single substance, or have not investigated human materials. As emphasized above lipid species and lipid mediators are increasingly recognized as important key factors in the development and regulation of inflammatory processes [4]. Hence detailed profiling of the lipid species found in the SF of RA patients is of considerable interest and will enable further investigations and understanding of the underlying mechanisms.

Several analytical approaches are used for the profiling of lipids in human body fluids, including MALDI [16], LC–MS/MS, direct infusion experiments in combination with either FTICR-MS [22], or Orbitrap MS [23] and in some cases also GC–MS [24,25]. The most versatile and frequently used technique certainly is LC–MS/MS in combination with ESI [26]. Besides commonly employed 2 mm i.d. columns, also nano-LC–MS/MS and capillary LC–MS/MS platforms have been described [27,28].

In this manuscript, we report on the use of a capillary LC-ESI (±)–MS/MS platform, employing a fast scanning IT-MS and a core-shell based capillary RP18 phase for an in-depth characterization of the lipids present in SF of RA patients. The decoded lipid profiling data indicated the presence of PUFA, hFA and lipoxygenase products in human SF. Hence, we followed up on this finding with a targeted investigation of the biochemically expected downstream products by a dedicated LC–MS/MS platform for lipid mediator screening.

2. Experimental

2.1. Chemicals and materials

Isopropanol LC–MS grade, methyl-*tert.*-butylether LC grade, MeOH gradient grade, glacial acetic acid p.a., formic acid p.a., ammonium formate p.a. grade, acetonitrile gradient grade, cholesterol linoleate and LC–MS grade water were from Sigma Aldrich (Schnelldorf, Germany). LPC (19:0/0:0), PG (14:0/14:0), PC (19:0/19:0), PC (14:0/14:0 d_{54}), and PS (16:0/18:1 d_{31}) were from Avanti Polar Lipids (Alabaster, AL, USA). 5S-HETE, AAd_8, LTB_4, LTB_4 d_4, PGE_2 d_4 and LXA_4 were from Cayman chemicals (Ann Arbor, MI, USA). C18 solid phase extraction cartridges (Sep-Pak C_{18} 500 mg, 6 mL) were from Waters (Boston, MA, USA). MaR1 and RvD5 were prepared in house. PBS was from Life technologies (Paisley, UK).

2.2. Collection of synovial fluid

Synovial fluid was obtained as discarded waste material from knee arthroscopy of RA patients visiting the outpatient clinic at the department of Rheumatology in the LUMC. This procedure is part of the standard clinical care and the use of waste material for research was approved by the local ethical committee. Upon informed consent, SF samples were stored at −70 °C until use.

2.3. Lipid extraction

The methyl-*tert.*-butylether extraction as described by Matyash et al. [29] was applied with some modifications. The obtained SF samples were centrifuged at 13,200 ×*g* for 3 min to remove tissue contaminations. To 50 μL SF were added 160 μL MeOH, 20 μL internal standard solution consisting of PC (14:0/14:0 d_{54}), PS (16:0/18:1 d_{31}) and AAd_8 (33.3 μg/mL each) and 600 μL methyl-*tert.*-butylether in a 2 mL Eppendorf plastic centrifuge tube. The suspension was shaken for 30 min on a benchtop shaker at room temperature. For phase separation 200-μL water was added and the sample tube was centrifuged for 3 min at 13,200 ×*g*. The upper layer was transferred into a 1.5 mL Eppendorf plastic tube and the extraction was repeated for an additional 5 min by adding 100 μL MeOH, 100 μL water and 300 μL methyl-*tert.*-butylether. The combined organic extracts were concentrated under a gentle stream of nitrogen and fully dried in an Eppendorf concentrator 5310 at 45 °C (approximately 30 min). To the dried residue 250 μL reconstitution solution (65% acetonitrile, 30% isopropanol, 5% water) was added and the tube was vortexed for 10 s and ultrasonicated for 20 s. The reconstituted sample was diluted 1:1 with water and transferred to the autosampler vial. A water sample treated in the same way was used as blank.

2.4. LC–MS/MS for untargeted lipidomics analysis

The HPLC system was a Dionex Ultimate 3000, consisting of a binary pump, connected to an autosampler equipped with a 1.0 μL injection loop and a column oven which was maintained at 50 °C. The column was an Ascentis express C-18, 5 cm × 300 μm, 2.7 μm from Sigma Aldrich (Schnelldorf, Germany). The flow rate was 9.0 μL/min. The gradient program started at 65% eluent A (water: acetonitrile 80:20, containing 5 mM ammonium formate and 0.05% formic acid) and 35% eluent B (isopropanol:acetonitrile:water 90:9:1, containing 5 mM ammonium formate and 0.05% formic acid) kept constant for 2 min, then linearly increasing to reach 95% B at 30 min, held for 5 min.

The IT-MS was a Bruker amaZon speed, which was operated in the ultrascan mode (Bruker Daltonics, Bremen, Germany). The dry temperature was set to 180 °C. Nitrogen 99.9990% was used as dry gas (8 psi) and nebulizer gas (4 L/min). The capillary voltage was set to ±3.5 kV. The MS was operated in the ESI ± mode and auto MS^n