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Specialised pro-resolving mediators of inflammation in inflammatory arthritis

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ABSTRACT

Introduction: Specialised pro-resolving mediators (SPM) are derived from n-3 long chain polyunsaturated fatty acids (n-3FA). They promote resolution of inflammation and may contribute to the beneficial effects of n-3FA in patients with arthritis. This study compared SPM in knee effusions and plasma of patients with arthritis taking n-3FA, and plasma of healthy volunteers taking n-3FA.
Methods: Thirty six patients taking n-3FA undergoing arthrocentesis for an inflammatory knee effusion and 36 healthy volunteers who had taken n-3FA (2.4 g/day) for 4 weeks were studied. SPM in synovial fluid and plasma were measured by liquid chromatography-tandem mass spectrometry included 18-hydroxyeicosapentaenoic acid (18-HEPE), the precursor of the E-series SPM (RvE1, RvE2, RvE3, 18R-RvE3), and 17-hydroxydocosahexaenoic acid (17-HDHA), the precursor of the D-series SPM (RvD1, 17R-RvD1, RvD2). Other SPM included protectin D1 (PD1), 10S,17S-dihydroxydocosahexaenoic acid (10,17S-DHDHA), maresin-1 (MaR-1) and 14-hydroxydocosahexaenoic acid (14-HDHA) derived from docosahexaenoic acid (DHA).
Results: E- and D-series SPM and the precursors 18-HEPE and 17-HDHA were present in synovial fluid and plasma of the patients with inflammatory arthritis. Plasma SPM were negatively related to erythrocyte sedimentation rate in arthritis patients ($P < 0.01$) and synovial fluid RvE2 was negatively associated with pain score ($P = 0.02$). Conversion from 18-HEPE and 17-HDHA to E- and D-series SPM was greater in synovial fluid ($P < 0.01$). Most plasma SPM in arthritis patients were elevated ($P < 0.05$) compared with healthy volunteers, and conversion to E- and D-series SPM was greater ($P < 0.01$).
Conclusions: SPM are present in chronic knee effusions and although the levels are lower than in plasma, the association between synovial fluid RvE2 and reduced pain scores suggests that synthesis of SPM at the site of inflammation is a relevant mechanism by which n-3FA alleviate the symptoms of arthritis.

1. Introduction

Specialised pro-resolving mediators (SPM) are a family of oxylipids that include resolvins, protectins, maresins and lipoxins. SPM contribute actively to the resolution of inflammation through engagement, at nanomolar concentrations, of cognate G-protein coupled receptors [1]. SPM arise from n-3 long chain polyunsaturated fatty acids (n-3FA) through the action of lipoxygenase enzymes and other remodelling steps [2]. The lipoxins are formed from arachidonic acid (AA, 20:4 n-6) [1,3] but there is particular interest in SPM derived from eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA; 22:6 n-3). The E-series resolvins, (RvE1-E3) are formed from EPA via a hydroxyl intermediate 18-hydroxyeicosapentaenoic acid (18-HEPE) and initially require acetylated COX-2 or cytochrome P450. 18-HEPE is then converted by 5-lipoxygenase to RvE1, RvE2 or by 15-lipoxygenase to RvE3 [4,5] (Fig. 1). DHA can be metabolised by acetylated COX-2 or 15-lipoxygenase, to the unstable intermediate 17-hydroperoxydocosahexaenoic acid (17-HpDHA) that

Abbreviations: SPM, specialised pro-resolving mediators; n-3FA, n-3 long chain polyunsaturated fatty acids; 18-HEPE, 18-hydroxyeicosapentaenoic acid; 17-HDHA, 17-hydroxydocosahexaenoic acid; 10S, 17S-DHDHA, 10S, 17S-dihydroxydocosahexaenoic acid; MaR-1, maresin-1; 14-HDHA, 14-hydroxydocosahexaenoic acid; AA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; RvE1, resolvin E1; RvE2, resolvin E2; RvE3, resolvin E3; 18R-RvE3, 18R-resolvin E3; RvD1, resolvin D1; 17R-RvD1, 17R resolvin D1; RvD2, resolvin D2; LC–MS/MS, liquid chromatography–tandem mass spectrometry; CCC, concordance correlation co-efficient

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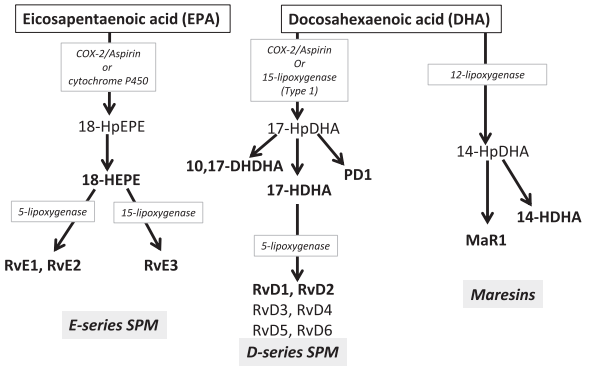

Fig. 1. Synthesis of E-series SPM from EPA and D-series SPM and maresins from DHA. The SPM measured in this study are identified in bold letters.

can form protectin D1 (PD1), 10S,17S-dihydroxydocosahexaenoic acid (10S,17S-DHDHA) and 17-hydroxydocosahexaenoic acid (17-HDHA). The D-series resolvins (RvD1–RvD6) form as a result of metabolism of 17-HDHA by 5-lipoxygenase [6] (Fig. 1). In humans, the maresins are formed by metabolism of DHA by macrophage 12-lipoxygenase giving rise to 14-hydroxydocosahexaenoic acid (14-HDHA) and maresin-1 (MaR-1) [7] [8] (Fig. 1).

Fish oils are a rich source of EPA and DHA, that have been shown to enhance management of rheumatoid arthritis (RA) [9] and systemic lupus [10,11] and to reduce reoccurrence rates in Crohn's disease in patients who are at high risk for relapse [12]. While a number of potential anti-inflammatory actions of n-3FA have been identified [13], it is conceivable that conversion of EPA and DHA to SPM could be a significant contributor to the disease mitigating effects of fish oil in inflammatory diseases. However, in spite of animal studies, which have demonstrated the presence of SPM in experimentally-induced inflammation [14,15] and the demonstration of SPM in healthy subjects taking fish oil [3], reports regarding SPM in human arthropathies are confined to a limited exploratory analysis of a small number of synovial fluid samples [16].

Based on favourable results of randomised controlled trials [17], at the Royal Adelaide Hospital patients with inflammatory arthropathies are routinely given advice to take fish oil supplements in addition to disease-modifying anti-inflammatory drugs. This study was undertaken to determine whether SPM are present in chronic inflammatory knee effusions of patients with arthritis of various aetiologies, to examine how well plasma SPM concentrations reflected those of synovial fluid from the inflamed joint, and to compare plasma SPM concentrations in patients with arthritis with those of healthy controls taking n-3FA.

2. Patients and methods

2.1. Recruitment of patients and healthy volunteers

2.1.1. Arthritic patients

All patients gave informed written consent and the study protocol was approved by the Human Research Ethics Committee, Royal Adelaide Hospital. All procedures were performed in accordance with the Declaration of Helsinki. Synovial fluid and peripheral blood samples were obtained contemporaneously from 36 patients undergoing arthrocentesis of an inflammatory knee effusion. Most patients had been told previously to take n-3FA as fish oil 10–15 mL daily as a complement to therapy with anti-rheumatic drugs. Subjects completed a Vital Activities and Lifestyle Index (VALI) form [18] and erythrocyte sedimentation rate (ESR) and CRP were measured as a routine component of clinical assessment. Our modification of the VALI form includes questions on type, dose and mode of ingestion of fish oil taken (fish oil on juice or as capsules). Answers to these questions allowed calculation of the dose of n-3FA (18% EPA + 12% DHA w/w, 1 mL fish oil weighs 0.92 g) as g/d. A small minority of patients took a concentrate of fish oil n-3FA as natural triglycerides, in which case the daily dose was computed as the equivalent dose, with regard to EPA and DHA content, of standard fish oil in g/day. Pain score was estimated from the VALI form as previously described [19].

2.1.2. Healthy volunteers

Thirty six healthy volunteers were selected as a comparison group on the basis of age and gender. They were recruited from the general population in Perth, Western Australia and were non-smokers; with no history of chronic disease, not taking anti-hypertensive or lipid lowering agents; aspirin or non-steroidal anti-inflammatory drugs; and not consuming fish meals or n-3FA supplements prior to the study. The volunteers gave informed written consent and the study protocol was approved by the Human Research Ethics Committee, at the University of Western Australia. All procedures were performed in accordance with the Declaration of Helsinki. Plasma SPM were measured after 4 weeks of supplementation with daily dose of 4 capsules of Omega Daily (Blackmores Australia), each 1 g capsule provides 360 mg of EPA and 240 mg of DHA as natural marine triglycerides In terms of EPA + DHA content, the dose given this equates to ~8 g/d of standard fish oil given to the arthritis patients.

2.2. Measurement of plasma and synovial fluid SPM

SPM in plasma and synovial fluid were measured by liquid chromatography-tandem mass spectrometry (LC–MS/MS) as previously described [20]. Briefly, internal standard (leukotriene B_4-d_4, 0.5 ng) was added to 1 mL of plasma or synovial fluid that was acidified to pH 3, and applied to a Bond Elut C18 cartridge (500 mg, Agilent Technologies, Lake Forrest, CA, USA). After washing with water and hexane, the SPMs were eluted with ethyl acetate (2 mL), dried under nitrogen and reconstituted in 5 mM ammonium acetate (pH = 8.9) and methanol (1/1; v/v) for analysis by LC–MS/MS, using a Thermo Scientific TSQ Quantum Ultra triple quadrupole LC–MS system equipped with an electrospray ionisation source operated in the negative ion mode. LC was performed on a Zorbax Eclipse XDB C18 column under conditions previously described [20]. Plasma and synovial fluid SPM were identified on the basis of the retention time, mass spectrum, and the parent and product ions of authentic standards as previously reported [20]. The standards 18-HEPE, 17-HDHA, resolvin D1 (RvD1) 17R-resolvin D1 (17R-RvD1), resolvin D2 (RvD2), 10S,17S-DHDHA, MaR-1 and leukotriene B_4-d_4 (LTB_4-d_4) were purchased from Cayman Chemicals (Ann Arbor, MI, USA). PD1 standard was provided by Professor Charles N Serhan (Harvard Medical School, Boston, Massachusetts, USA). Resolvin E1 (RvE1) and 14-HDHA were made available by Cayman Chemicals. Resolvin E2 (RvE2), resolvin E3 (RvE3) and 18R-resolvin E3 (18R-RvE3) standards were provided by Professor Makoto Arita (Department of Health Chemistry, Graduate School of Pharmaceutical Sciences, University of Tokyo, Japan).

2.3. Assessment of E series and D-series SPM in relation to their precursors

The ability to form E-series SPM and D-series SPM from their respective precursor substrates 18-HEPE and 17-HDHA was inferred from the ratios (RvE1 + RvE2 + RvE3 + 18R-RvE3)/18-HEPE and (RvD1 + 17R-RvD1 + RvD2)/17-HDHA, respectively. MaR-1, 14-HDHA, PD1 and 10S,17S-DHDHA that derive from DHA were excluded from this equation as the pathway for their synthesis does not involve 17-HDHA.