Inflamm Res 44: 121–124 (1995)

1023-3830/95/030121-04 $ 1.50+0.20/0

Conversion of endogenous arachidonic acid to 5,15-diHETE and lipoxins by polymorphonuclear cells from patients with rheumatoid arthritis

E. Thomas[2], J. L. Leroux[2], F. Blotman[2], and C. Chavis[1]

[1]Centre de Recherches INSERM, 70 rue de Navacelles, F-34090 Montpellier, France
[2]Service de Réadaptation Fonctionnelle, CHU Lapeyronie, F-34295 Montpellier, France

Received 14 June 1994; returned for revision 16 August 1994; accepted by G. Carter 8 December 1994

Abstract. The conversion of endogenous arachidonic acid (AA) by polymorphonuclear cells (PMN) from patients with rheumatoid arthritis (RA) was studied before (D0) and one day (D1) after antiinflammatory drug therapy. The biosynthesis of 5,15-diHETE and lipoxins (LXS), were investigated *ex vivo*, after PMN stimulation by ionophore A23187 without exogenous addition of 15-HETE. The eicosanoids were resolved by RP-HPLC and simultaneously detected at 246 and 302 nm respectively. Large amounts of 5,15-diHETE (50 to 400 ng/10^7 PMN) and significant levels of LXS (from 2 to 20 ng/10^7 PMN) were produced with individual differences between donors. Metabolite levels varied between patients but this work showed for the first time a linear relationship between the amounts of 5,15-diHETE and LXS. Moreover LXS production after treatment may be related to long-term clinical improvement of patients.

Key words: Rheumatoid arthritis – Polymorphonuclear cells – Lipoxins – 5,15-dihydroxyeicosatetraenoic acid

Introduction

Low dose methotrexate (MTX) therapy and glucocorticoid pulse were found to be effective in patients with active rheumatoid arthritis (RA) [1, 2, 3, 4]. Their mechanism of action in RA remains controversial. Arachidonic acid *ex vivo* metabolism by polymorphonuclear cells (PMN) from patients with RA is dependent on the type of the treatment. However after 24 h MTX injection [5, 6] or long-term weekly oral administration [7] a significant decrease of leukotriene B_4 (LTB_4) production was described. The results after methylprednisolone pulse therapy were quite different: some works reported eicosanoid biosynthesis inhibition by glucocorticoids [8] but several studies showed that glucocorticoids failed to inhibit neutrophil LTB_4 production [9, 10]. Nevertheless, the effect of antiinflammatory drug treatments are usually associated with inhibition of neutrophil chemotactic activity [11, 12] which was found to be related to a decreased capacity of PMNs to generate LTB_4. We thus investigated the 5-lipoxygenase pathway (5-LO) before (D0) and 24 h after (D1) antiinflammatory therapy through biosynthesis, from endogenous sources, of other arachidonic acid metabolites with a hydroxyl function at carbon 5 of the molecule: lipoxins (LXS) and 5,15-dihydroxyeicosatetraenoic acid (5,15-diHETE). The physiological effects of the conjugated diene 5,15-diHETE have yet to be determined [13], although its precursor 15-hydroxyeicosatetraenoic acid (15-HETE) was described to have minor antiinflammatory properties in colitis [14]. However, conjugated tetraene LXS, particularly LXA_4, were reported to act as immunoregulators through their action on $O2^{\varrho}$ production, chemotaxis and cellular adhesion [15]. The aim of the present study was thus to determine whether LXS and 5,15-diHETE, produced *ex vivo* by PMN, are inflammatory state biomarkers that are more specific than LTB_4 and whether they are related to clinical improvement of patients after treatment.

Materials and methods

Reagents

5,15-diHETE, purchased from Euromedex (Strasbourg, France) were from Cascade (Reading, UK). Ionophore A23187 was from Sigma Chemical Co. (St Louis, MO, USA). The solvents were HPLC grade (Carlo Erba). Culture material, phosphate buffer saline (PBS) with $CaCl_2$ and $MgCl_2$ came from Flow Laboratories (France). Lipoxins were a generous gift from Dr. C. Picket (Merck Frosst Research Laboratory, Pointe-Claire Dorval, Canada).

Subject selection

9 patients (mean ages 50.6 ± 11.2) with active rheumatoid arthritis (RA) according to American College of Rheumathology criteria,

Correspondence to: C. Chavis

without peripheral blood eosinophilia, were studied. They received neither glucocorticoid and methotrexate injection for 3 months before the study, nor non steroidal antiinflammatory drug (NSAID) treatment. Arachidonic acid (AA) metabolites released by PMN were analyzed before (D0) and 24 h after (D1) intravenously injected methyl prednisolone (250 mg for 4 patients) or after intramuscularly injected MTX (10 mg for the other 5 patients). The patients were recalled 3 months later (D90) to evaluate clinical improvement (reduction of nocturnal awakenings, of morning stiffness duration, of painful joint number and of Ritche score). Simultaneously 7 healthy subjects (HS, mean ages 37.2 ± 6.5), who have not received aspirin for at least one week, were studied to test whether eventual daily variation was observed when the eicosanoid biosynthesis by PMN was evaluated from one day to another.

Human PMN isolation

Blood cell analysis routinely carried out showed the same PMN/platelet ratio before or after treatment. Cells were isolated and purified from heparinized blood by centrifugation of samples over discontinuous Percoll gradient as previously described [6]. Briefly a 5 ml volume of 63% percoll solution in 0.15 M NaCl was layered over 5 ml of 72% percoll solution. Whole blood was then layered over percoll gradient and the tubes were spun at 400 g for 20 min at 20 °C. The upper layer constituted mononuclear cells and platelets. The PMN were recovered in the intermediary layer between the mononuclear cells and the 72% percoll solution. Purity of the PMN suspension was evaluated by microscope observation of the cells after cytocentrifugation and May Grünwald staining. The preparations, at least 95% purity, were greater than 85% viability, before and after glucocorticoid or methotrexate injection, as measured by trypan blue exclusion.

Stimulation procedures

Isolated PMN were resuspended in phosphate buffer saline (PBS) (pH 7.4) with Ca^{++}/Mg^{++} (final concentrations 2.10^{-3} M and $0.5.10^{-3}$ M respectively). The cell concentration was adjusted to 10^7 cells/ml. Eicosanoids were released into the incubation medium after ionophore A23187 stimulation (final concentration 5.10^{-6} M) for 5 min at 37 °C, as previously described [16]. After cell suspension cooling at 0 °C, the reaction was stopped by addition of the same volume of cold methanol and the samples were stored at −20 °C until reverse-phase HPLC analysis (RP-HPLC).

Eicosanoid identification and quantification

Before analysis the samples were centrifuged to pellet the proteins denatured by methanol and aliquots of the supernatants were directly injected onto RP-HPLC (Waters apparatus, model 510 pump, U6K injector) without any purification. The eicosanoids were resolved on a 100 RP-18 column (Merck) by isocratic elution with the methanol/water/acetic acid 65/35/0.1 (v/v/v) solvent mixture, at a flow rate of 0.5 ml/min for 15 min and then at 1 ml/min. 5,15-diHETE and LXS were simultaneously detected at 246 and 302 nm respectively (Waters programmable multiwavelength detector model 490). They were identified by comparing their retention times and UV spectra recorded in the stop-flow mode with those of synthetic reference products and quantitated by measuring the areas under the respective peaks as described elsewhere [17].

Results

The 5-LO activity of human PMN was investigated via eicosanoid biosynthesis under stimulation by Ca^{++} ionophore A23187 for 5 min, the day before (D0) and the day after therapy (D1). Besides LTB4, 20-hydroxy-LTB4, 6-trans-LTB4 and 12-epi-6-trans-LTB4 as previously reported [6], PMN from patients with RA were able to release 5,15-diHETE and LXS into the culture medium (Table 1), without any addition of exogenous 15-HETE and without intermediate formation of 15-HETE (data not shown). High amounts of 5,15-diHETE and substantial amounts of LXS were thus biosynthesized from the endogenous arachidonic acid pool at D0, except in one subject (S8). However, the therapies suppressed the capacity of PMN to biosynthesize these eicosanoids, except in cells from two patients (S8 and S9) (Table 1). Individual variations were observed between patients, but there was still a linear relationship between the LXS and 5,15-diHETE production with a correlation coefficient value of 0.908 (Fig. 1). The clinical state of the patients three months later was reported in the Table 1 and no long term clinical improvement was observed except in the case of the patients S8 and S9. As reported in the Table 1 PMN from HS did not release detectable amounts of LXS and 5,15-diHETE.

Discussion

LXS biosynthesis requires either the addition of exogenous substrate to incubated human cells or tissues [18, 19, 20] or transcellular metabolism between two different cell types [21, 22, 23]. Production of LXS and 5,15-diHETE by isolated human PMN was first reported by Serhan *et al.* [24] in cells from healthy subjects incubated in the presence of 15-HETE. 5,15-diHETE is obtained by 5-LO dioxygenation of 15-HETE whereas LXS are the result of opening of the intermediate epoxytetraene 15-hydroxy LTA_4 produced from LTA_4 synthase activity of 5-LO. The present study demonstrates for the first time simultaneous production of LXS and 5,15-diHETE *ex vivo* by PMN from patients with inflammatory disease only, and in the absence of exogenous 15-HETE. The cells did not generate

Table 1. LXS and 5,15-diHETE biosynthesized *in vitro* by PMN from patients with RA, at D0 and D1 and clinical state of patients 3 months later (D90). The results are expressed as ng/10^7 PMN (ND: non-detectable levels). Clinical improvement, expressed as +++, was assessed by less than 50% reduction in number of nocturnal awakenings, morning stiffness duration, painful joint number and Ritchie score at D90.

	5,15-diHETE		LXS		Clinical improvement
Subjects	D0	D1	D0	D1	D90
S1	60	ND	1.0	ND	–
S2	48	ND	5.0	ND	–
S3	152	ND	6.0	ND	–
S4	263	ND	14.3	ND	–
S5	180	ND	7.2	ND	–
S6	90	ND	5.0	ND	–
S7	130	ND	7.1	ND	–
S8	ND	112	ND	5.0	+++
S9	263	404	14.3	20.9	+++
HS ($n = 7$)	ND	ND	ND	ND	

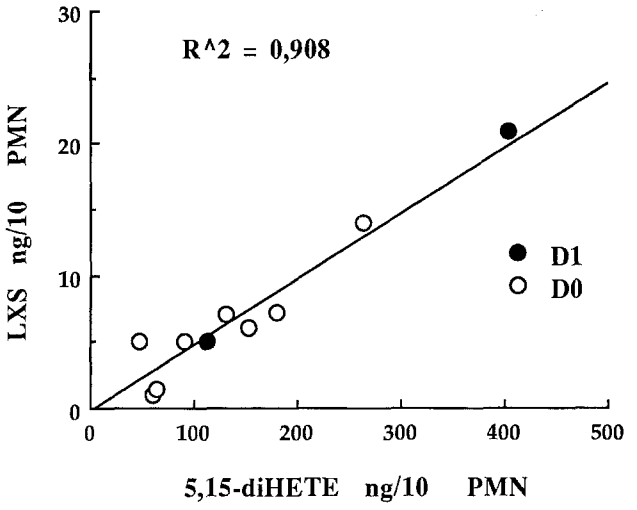

Fig. 1. Linear relationship between the LXS and 5,15-diHETE generation by PMN from patients with RA. Data are expressed as ng/10^7 PMN.

detectable amounts of 12-HETE, 15-HETE nor LTC_4 (data not shown). So the nature and the levels of the products cannot be interpreted as the result of a cellular cooperation between contaminating platelets and neutrophils [25]. The eicosanoid biosynthesis was studied under the same experimental conditions in a group of 7 healthy subjects to demonstrate that the effects observed in PMN from RA after treatment were not due to a simple daily variation in cellular responsiveness or preparation. These results revealed that PMN 5-LO from RA subjects had a special activation state. This indicated *in vivo* priming by the surrounding cells, such as monocytes or endothelial cells, thus allowing PMN to directly biosynthesize conjugated diene and tetraene containing eicosanoids from endogenous arachidonic acid, without any exogenous precursor supply. Although LTB_4 production by PMN at D1 [6, 7] was decreased by antiinflammatory treatments, LXS and 5,15-diHETE biosynthesis was completely inhibited, except in two patients. PMN were therefore unable to produce LXS and 5,15-diHETE as expected considering the described inhibitory effect of antiinflammatory drugs on 5-LO activity. Moreover, this result was not dependent on the nature of the administrated drugs. However, for two patients, this production was enhanced after therapy at D1 but a simultaneous long term clinical improvement may be observed at D90, in contrast to the other patients. In addition to the character of early biomarkers, LXS and 5,15-diHETE could be of interest for choosing and evaluating long-term therapy. Further studies with a higher number of patients are required to determine the pathophysiological significance of 5,15-diHETE and LXS biosynthesis.

Acknowledgement. We would like to thank Dr. Cecil B. Picket and Suzanne Longpré (Merck Frosst Inc., Dorval, Quebec, Canada) for generous gift of LXS and LXS isomers.

References

[1] Weinblatt ME, Coblyn JS, Fox DA. Efficacity of low-dose methotrexate in rheumatoid arthritis. N Engl J Med 1985;312:818–22.

[2] Cronstein BN. Molecular mechanism of methotrexate action in inflammation. Inflammation 1992;16:411–23.

[3] Songsiridej N, Furst DE. Methotrexate: the rapidly acting drug. Clin Rheumatol 1990;4:575–93.

[4] Sebaldt RJ, Yang XH, Adachi JD, Bensen W. Pulse glucocorticosteroid (GCS) therapy in R.A. patients inhibits whole blood leukotriene B_4 biosynthesis ex vivo. Arthritis Rheum 1993;36 (suppl.):F269.

[5] Sperling RI, Benincaso AI, Anderson RJ, Coblyn, JS, Austen KF, Weinblatt ME. Acute and chronic suppression of leukotriene B_4 synthesis ex vivo in neutrophils from patients with rheumatoid arthritis beginning treatment with methotrexate. Arthritis Rheum 1992;35:376–84.

[6] Leroux JL, Damon M, Chavis C, Crastes de Paulet A, Blotman F. Effects of a single dose of methotrexate on 5- and 12-lipoxygenase products in patients with rheumatoid arthritis. J Rheum 1992;19:863–6.

[7] Sperling RI, Coblyn JS, Larkin JK, Benincaso AI, Austen KF, Weinblatt ME. Inhibition of leukotriene B_4 synthesis in neutrophils from patients with rheumatoid arthritis by a single oral dose of methotrexate. Arthritis Rheum 1990;33:1149–55.

[8] Sebaldt RJ, Sheller JS, Oates JR, Roberts LJ, Fitzgerald GA. Inhibition of eicosanoid biosynthesis by glucucorticoids in humans. Proc Nat Acad Sci 1990;87:6974–8.

[9] Freeland HS, Pipkorn U, Schleimer RP, Bascom R, Lichtenstein LN, Naclerio RM, Peter SP. Leukotriene B_4 as a mediator of early and late reactions to antigens in humans: the effect of systemic glucocorticoid treatment *in vivo*. J Allergy Clin Immunol 1987;83:634–42.

[10] Schleimer RP, Freeland HS, Peter SP, Brown KE, Derse CP. An assessment of the effects of glucocorticoids on degranulation, chemotaxis, binding to vascular endothelium and formation of leukotriene B_4 by purified human neutrophils. J Pharmacol Exp Therapeutics 1989;250:598–605.

[11] O'Callahan P, Forrest MJ, Brooks PM. Inhibition of neutrophil chemotaxis in methotrexate treated rheumatoid arthritis patients. Rheumatol Int 1988;8:41–5.

[12] Goldstein RA, Bowen DL, Fauci AS. Adrenal corticosteroids. In: Gallin JI, Goldstein IM, Snyderman R, eds. Inflammation: Basic principles and clinical correlates, 2nd ed. New York: New York Raven Press, 1992:1061–81.

[13] Maas RL, Turk J, Oates JA, Brash AR. Formation of a novel dihydroxy acid from arachidonic acid by lipoxygenase-catalyzed double oxygenation in rat mononuclear cells and human leukocytes. J Biol Chem 1992;257:7056–67.

[14] Vand Dijk APM, McCafferty DM, Wilson JHP, Zijlstra FJ. 15-Hydroxy-eicosatetraenoic acid has minor anti-inflammatory properties in colitis. Agents Actions 1993;38:120–1.

[15] Serhan CN. Lipoxin biosynthesis and its impact in inflammatory and vascular events. Biochem Biophys Acta 1994;1212:1–25.

[16] Radeau T, Chavis C, Damon M, Michel FB, Crastes de Paulet A, Godard P. Enhanced arachidonic acid metabolism and human neutrophil migration in asthma. Prostagland Leuk Essent Fatty Acids 1990;41:131–8.

[17] Chavis C, Godard P, Crastes de Paulet A, Damon M. Formation of lipoxins and leukotrienes by human alveolar macrophages incubated with 15(S)-HETE: a model for cellular cooperation between macrophages and airway epithelial cells. Eicosanoids 1992;5:203–11.

[18] Sehan CN. On the relationship between leukotriene and lipoxin production by human neutrophils: evidence for differential metabolism of 15-HETE and 5-HETE. Biochem Biophys Acta 1989;1004:158–68.

[19] Chavis C, Godard P, Michel FB, Crastes de Paulet A, Damon M. Cell to cell interaction between human alveolar macrophages and epithelial cells: lipoxins A and lipoxins B synthesis. In Baily JM eds. Prostaglandins, Leukotrienes and PAF, XIth Washington International Spring Symposium, Abstract book. New York: Raven Press, 1991:196.

[20] Edenius C, Kumlin M, Bjork T, Anggard A, Lindgren JA. Lipoxin formation in human nasal polyps and bronchial tissue. FEBS Lett 1990;272:25–8.

[21] Edenius C, Haeggström J, Lindgren JA. Transcellular conversion of endogenous arachidonic acid to lipoxins in mixed human platelet-granulocyte suspensions. Biochem Biophys Res Commun 1988;157:801–7.

[22] Fiore S, Serhan CN. Formation of lipoxins and leukotrienes during receptor-mediated interactions of human platelets and recombinant human granulocyte/macrophage colony stimulating factor-primed neutrophils. J Exp Med 1990;172: 1451–7.

[23] Levy BD, Bertram S, Tai HH, Israel E, Fisher A, Drazen JM, Serhan CN. Agonist-induced lipoxin A_4 generation: detection by a novel lipoxin A_4-ELISA. Lipids 1993;28: 1047–53.

[24] Serhan CN, Hamberg M, Samuelsson B. Lipoxins. Novel series of biologically active compounds formed from arachidonic acid in human leukocytes. Proc Nat Acad Sci USA 1984;81:5335–9.

[25] Maclouf JA, Murphy R. Transcellular metabolism of neutrophil-derived leukotriene A_4 by human platelets. J Biol Chem 1988;263:174–81.