

Lipoxins and other arachidonate derived mediators in bronchial asthma

C Chavis, I Vachier, P Godard, J Bousquet, P Chanez

Leukotrienes and lipoxins, arachidonate derived mediators from the lipoxygenase (LO) pathways, are associated with bronchial asthma.¹⁻³ They have been detected in vivo in bronchoalveolar lavage (BAL) fluid⁴⁻⁶ and are biosynthesised in vitro by human alveolar macrophages (AM) and polymorphonuclear cells (PMN) after non-specific stimulation.⁷ Lipoxins were first isolated by Serhan *et al*^{8,9} who incubated granulocytes with 15(S)-hydroxyeicosatetraenoic acid (15(S)-HETE). Lipoxin synthesis was also achieved by the addition of other exogenous substrates to cell cultures.^{8,10,11} In contrast, lipoxin synthesis by co-cultures of two different cell types does not require the addition of any exogenous substrates.¹¹⁻¹³ The biosynthesis of lipoxins was shown to be the result of a cellular cooperation mechanism between two different cell types. The enzymatic mechanism of transcellular metabolism involves the action of at least two different lipoxygenases (fig 1). Thus, lipoxins may be generated following receptor mediated activation of either co-incubated PMN and platelets,¹³ or ionophore stimulation of PMN alone after exposure to exogenous 15(S)-HETE.¹⁴

As shown previously,¹⁵ AM from asthmatic patients display high levels of 5-LO activity. 15(S)-HETE is considered to be specific to human epithelium airways and endothelial cells.¹⁶⁻¹⁸ Human epithelial and endothelial cells are unable to produce lipoxins from endogenous 15(S)-HETE^{16,19} but they are surrounded by several cell types which show 5-LO activity. Moreover, lipoxins have been identified in the BAL fluid of patients with lung disease²⁰ and in stimulated whole blood.²¹ Because of these observations, and assuming that peripheral blood cells were activated

before trafficking to the airways, we have investigated whether AM and peripheral blood cells display sufficient LO activity to transform 15(S)-HETE and have determined whether the generation of lipoxins and their precursors is related to the severity of asthma and can be modulated by treatment. Lipoxin A₄ (LXA₄) has been shown to have a role in immune responses and to possess anti-inflammatory properties,²²⁻²⁴ and the presence of lipoxins in asthma has suggested that they impact on cell regulation. Glucocorticoids, commonly used in the treatment of inflammatory diseases, exert a number of controversial effects on the release of leukotrienes. Thus, lipoxins should be more specific biochemical markers of asthma and treatment efficacy than LTB₄, and may be of interest for exploring glucocorticoid effects on cellular signalling and evaluation of long term treatment.

AM, PMN, and peripheral blood monocytes (PBM) from healthy controls, untreated asthmatics, and patients with steroid dependent asthma were studied for their ability to synthesise lipoxins and their precursors after stimulation with A23187 alone to investigate eicosanoid generation from endogenous arachidonate, and then in the presence of 15(S)-HETE to investigate transcellular metabolism.

Methods

PATIENTS

Asthma was diagnosed according to the criteria of the American Thoracic Society.²⁵ The forced expiratory volume in one second (FEV₁) ranged from 53% to 102% of the predicted values in patients with untreated asthma and from 45% to 117% of the predicted values in those treated with steroids. In the untreated asthmatic patients theophylline and β agonists were stopped one week before analysis. Non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids had been discontinued for at least one month. The steroid dependent patients were included on the basis of their long term treatment (at least one year with oral prednisolone and inhaled beclomethasone) and their continued requirement for oral steroids.

PREPARATION OF CELLS

AM obtained by BAL centrifugation (400g, 10 min) from four control subjects and seven with untreated asthma were processed and cultured as previously described.²⁶ PBM from nine control subjects, 18 with untreated asthma, and 29 steroid dependent asthmatics and PMN from nine controls and 38 untreated asthmatic subjects were isolated and purified from heparinised venous blood by centrifugation

Inserm U454, CHU de Montpellier, Hôpital Arnaud de Villeneuve, 34295 Montpellier, France
C Chavis
I Vachier
P Godard
J Bousquet
P Chanez

Correspondence to:
Dr C Chavis
cchavis@montp.inserm.fr

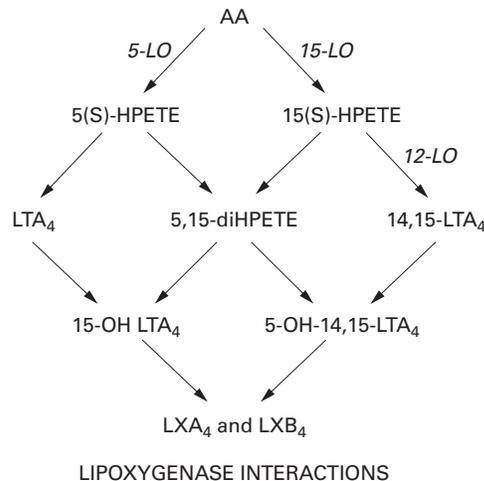


Figure 1 Transcellular metabolism.

(400g, 20 min, 4°C) over a discontinuous Percoll gradient.^{27,28} Cell purity was greater than 95% and viability greater than 90%.

STIMULATION PROCEDURES AND METABOLITE ANALYSIS

After the addition of A23187 (5×10^{-6} M) which optimises eicosanoid production, with or without 15(S)-HETE (3×10^{-6} M), incubations were carried out for five minutes for PMN (10^7 cells/ml) and for 30 minutes for AM (10^6 cells/ml) and PBM (5×10^6 cells/ml). The samples were analysed using reverse phase high performance liquid chromatography (RP-HPLC).²⁷⁻²⁹

ANALYSIS OF DATA

The results are presented as mean (SE) ng product/ 5×10^6 cells. Statistical differences were determined using the Mann-Whitney U test for unpaired samples. A p value of <0.05 was considered significant.

Results

EICOSANOID RELEASE FROM ENDOGENOUS SOURCES

Cell capacity to release eicosanoids from endogenous arachidonic acid was evaluated using ionophore A23187 alone. AM, PMN, and PBM released LTB₄.²⁷⁻²⁹ No detectable amounts of diHETEs and lipoxins were observed under our analysis conditions in AM or PBM. Nevertheless, besides the normally generated leukotrienes, PMN from untreated asthmatic subjects were able to generate considerable amounts of 5(S),15(S)-diHETE as well as lipoxins (135 (14) and 6 (1), respectively). These metabolites were not found in PMN from control subjects.

EICOSANOID RELEASE FROM TRANSCELLULAR METABOLISM

Transcellular metabolism was evaluated by studying 15(S)-HETE metabolism in A23187 stimulated cells. LTB₄, lipoxins, and their precursor 5(S),15(S)-diHETE were detected in all three cell types, whereas 14(R),15(S)-diHETE was released by PBM only (table 1).²⁷⁻²⁹ 5(S),15(S)-diHETE, which was the major product, was at a similar level in AM from patients with untreated asthma and healthy controls. However, it was nearly

twofold higher in PMN from patients with untreated asthma than in those from healthy controls,²⁷ whereas no differences were seen in PBM.²⁸ Total lipoxin levels were significantly higher in the cells from untreated asthmatic subjects than from healthy control subjects for the three cell types. 14(R),15(S)-diHETE levels, generated by PBM only, were almost identical for the two groups of subjects.

EFFECTS OF ORAL CORTICOSTEROIDS ON TRANSCELLULAR METABOLISM

PMN from steroid dependent asthmatics released twofold lower levels of 5(S),15(S)-diHETE than those from untreated asthmatics and a similar level to those from controls (table 1). Cells from patients with steroid dependent asthma also released lower lipoxin levels than those from untreated asthmatics and controls. In contrast, PBM from patients with steroid dependent asthma released higher levels of 5(S),15(S)-diHETE than those from untreated asthmatic patients and controls, whereas lipoxin levels were nearly the same. Simultaneously, PBM from patients with steroid dependent asthma released significantly greater amounts of 14(R),15(S)-diHETE than those from patients with untreated asthma or healthy controls.

Discussion

Human cells were studied *ex vivo* for their ability to synthesise eicosanoids as an indication of cellular activation *in vivo*. They were able to synthesise lipoxins and their precursors 5(S),15(S)-diHETE or 14(R),15(S)-diHETE. In our studies lipoxins were released from endogenous sources in PMN from untreated asthmatic patients only. Nevertheless, Levy *et al* described lipoxin synthesis in the sub-nanogram range in cytokine primed human AM suspensions using an electrochemical detection technique.³⁰ Endogenous generation of 5(S),15(S)-diHETE and lipoxins *in vitro* by human blood PMN, without *in vitro* cytokine priming, has been reported for the first time in rheumatoid disease by Thomas *et al*³¹ and later in asthma,²⁷ although other authors have since claimed that only porcine leucocytes were able to produce lipoxins from endogenous arachidonate.³² It was the first time that lipoxin biosynthesis was described in a cell type with the involvement of 5-LO only. These results can be interpreted by *in vivo* priming induced in bronchial diseases by cytokines or tumour necrosis factor (TNF)- α released by surrounding cells.¹³

In contrast, lipoxins were produced in all cell types when incubated with exogenous mediator 15(S)-HETE.^{27-29,31} AM, PMN, and PBM from untreated asthmatic subjects produced greater quantities of lipoxins and their precursors than those from healthy controls by way of transcellular metabolism. These results suggest that the more active transcellular metabolism of phagocytes from patients with untreated asthma may be associated with previous exposure to specific allergens responsible for the inflammatory process and induced by subsequent lung disease. This may indicate that

Table 1 Eicosanoid generation from transcellular metabolism

Cell type	Subjects	n	5(S),15(S)-diHETE	Total LXs	14(R),15(S)-diHETE
AM	Controls	4	59 (7)	33 (2)	ND
	UTA	7	44 (2)	53 (3)**	ND
PMN	Controls	9	59 (8)	23 (2)	ND
	UTA	13	94 (3)***	57 (4)***	ND
	SDA	14	43 (5)	10 (3)**	ND
PBM	Controls	9	88 (14)	ND	52 (9)
	UTA	18	81 (16)	5 (3)	68 (15)
	SDA	29	102 (21)	3 (1)	133 (18)*

AM = alveolar macrophages; PMN = polymorphonuclear cells; PBM = peripheral blood monocytes; LXs = lipoxins; HETE = hydroxyeicosatetraenoic acid; UTA = untreated asthma; SDA = steroid dependent asthma; ND = non-detectable metabolite amounts.

Cells were stimulated by A23187 after addition of 15(S)-HETE (3×10^{-6} M). Results are presented in ng for 10^6 AM, 10^7 PMN, and 5×10^6 PBM, except for 5(S),15(S)-diHETE which is expressed as a percentage of the amount of 15(S)-HETE initially added. The results are expressed as mean (SE).

Significant differences only are reported: *p<0.02, **p<0.005, ***p<0.0005.

lipoxin can be produced at inflammation sites from a massive amount of 15(S)-HETE generated by airway epithelium and/or human bronchi.^{16,19} This could explain the presence of LXA₄ in BAL fluid from patients with lung diseases involving a cellular cooperation mechanism, thus leading to mediators playing an important part as immunoregulatory molecules in asthma.²⁰

It is commonly accepted that 5-LO activity is reduced in the presence of glucocorticoids. We observed that lipoxin and 5(S),15(S)-diHETE levels released by PMN from steroid dependent asthmatics by way of transcellular metabolism were lower than those from patients with untreated asthma and healthy controls, and were completely inhibited in the endogenous pathway. In contrast, the amounts of diHETEs biosynthesised from exogenous 15(S)-HETE were increased in PBM from patients with steroid dependent asthma compared with those from untreated asthmatics and control subjects, with significant differences for 14(R),15(S)-diHETE. Although lipoxin levels are nearly the same in PBM from both untreated and steroid dependent asthmatic patients, our results have shown that they are not synthesised in control subjects. This result is unexpected since glucocorticoids are thought to reduce lipid mediator production by their anti-inflammatory effects. The role of glucocorticoids in the regulation of 5-LO activity is uncertain. Studies performed in vitro did not show a significant effect on LTB₄ release by human blood neutrophils³³ and monocytes,³⁴ whereas inhibition of specific eicosanoid synthesis and induction of lipocortin-1 by glucocorticoids were reported for macrophages.³⁵ In contrast, a recent study reported increased expression of 5-LO in blood monocytes from healthy volunteers cultured in the presence of dexamethasone,³⁶ and glucocorticoid mediated upregulation of the neuronal 5-LO pathway has been described in rats treated with corticosterone or dexamethasone.³⁷

Our study shows an upregulation of LO activity in PBM following long term treatment with corticosteroids, and lipoxins have been found to regulate leucocyte trafficking in the vascular circulation by inhibition of several inflammatory responses.²⁴ These results suggest that the anti-inflammatory properties of glucocorticoids and the clinical improvement of patients might result in part from the use by target cells of an endogenous anti-inflammatory mediator released by the surrounding cells. Lipoxins and their precursors could be of interest for explaining the effects of glucocorticoids on cellular signalling and for evaluating long term treatments.

- 1 Christie PE, Spur BW, Lee TH. The effects of lipoxin A4 on airway responses in asthmatic subjects. *Am Rev Respir Dis* 1992;145:1281-4.
- 2 Serhan CN. Lipoxins: biosynthesis and evidence for their involvement in respiratory disease. In: Robinson C, ed. *Lipid mediators in allergic diseases of the respiratory tract*. Boca Raton: CRC Press, 1994: 65-78.
- 3 Drazen JM. Effects of leukotrienes on airway function. In: Crystal RG, West JB, Barnes PJ, Weibel ER, eds. *The lung*. 2nd edn. Philadelphia, New York: Lippincott-Raven, 1997: 1287-96.

- 4 Lam S, Chan H, LeRiche JC, et al. Release of leukotrienes in patients with bronchial asthma. *J Allergy Clin Immunol* 1988;81:711-7.
- 5 Wenzel SE, Larsen GL, Johnston K, et al. Elevated levels of leukotriene C4 in bronchoalveolar lavage fluid from atopic asthmatics after endobronchial allergen challenge. *Am Rev Respir Dis* 1990;142:112-9.
- 6 Lee TH, Arm J. Leukotrienes. *Allergy Proc* 1991;12:139-42.
- 7 Borgeat P. Biochemistry of the lipoxygenase pathways in neutrophils. *Can J Physiol Pharmacol* 1989;67:936-42.
- 8 Serhan CN, Hamberg M, Samuelsson B. Lipoxins: novel series of biologically active compounds formed from arachidonic acid in human leukocytes. *Proc Natl Acad Sci USA* 1984;83:1983-7.
- 9 Serhan CN, Hamberg M, Samuelsson B. Trihydroxytetraenes: a novel series of compounds formed from arachidonic acid in human leukocytes. *Biochem Biophys Res Commun* 1984;118:943-9.
- 10 Kim SJ. Formation of lipoxins by alveolar macrophages. *Biochem Biophys Res Commun* 1988;150:870-6.
- 11 Edenius C, Kumlin M, Bjork T, et al. Lipoxin formation in human nasal polyps and bronchial tissue. *FEBS Lett* 1990; 272:25-8.
- 12 Stenke L, Mansour M, Edenius C, et al. Formation and proliferative effects of lipoxins in human bone marrow. *Biochem Biophys Res Commun* 1991;180:255-61.
- 13 Fiore S, Serhan CN. Formation of lipoxins and leukotrienes during receptor-mediated interactions of human platelets and recombinant granulocyte/macrophage colony-stimulating factor-primed neutrophils. *J Exp Med* 1990; 172:1451-7.
- 14 Serhan CN. On the relationship between leukotriene and lipoxin production by human neutrophils: evidence for differential metabolism of 15(S)-HETE and 5-HETE. *Biochim Biophys Acta* 1989;1004:158-68.
- 15 Chavis C, Godard P, Michel FB, et al. Sulfidopeptide leukotrienes contribute to human alveolar macrophage activation in asthma. *Prostaglandins Leukot Essential Fatty Acids* 1991;42:95-100.
- 16 Kumlin M, Hamberg M, Granstrom E, et al. 15(S)-hydroxyicosatetraenoic acid is the major arachidonic acid metabolite in human bronchi: association with airway epithelium. *Arch Biochem Biophys* 1990;282:254-62.
- 17 Ang C, Clark E, Young I, et al. 15 hydroxyicosatetraenoic acid synthesis from exogenous arachidonate by bronchial mucosa. *J Lipid Mediators* 1990;2:263-9.
- 18 Lopez S, Vila L, Breviario F, et al. Interleukin-1 increases 15-hydroxyicosatetraenoic acid formation in cultured human endothelial cells. *Biochim Biophys Acta* 1993;1170: 17-24.
- 19 Hunter JA, Finkbeiner WE, Nadel JA, et al. Predominant generation of 15-lipoxygenase metabolites of arachidonic acid by epithelial cells from human trachea. *Proc Natl Acad Sci USA* 1985;82:4633-7.
- 20 Lee TH, Crea AE, Gant V, et al. Identification of lipoxin A4 and its relationship to the sulfidopeptide leukotrienes C4, D4, and E4 in the bronchoalveolar lavage fluids obtained from patients with selected pulmonary diseases. *Am Rev Respir Dis* 1990;141:1453-8.
- 21 Brezinski DA, Serhan CN. Characterization of lipoxins by combined gas chromatography and electron capture negative ion chemical ionization mass spectrometry: formation of lipoxin A₄ by stimulated human whole blood. *Biol Mass Spectrometry* 1991;20:45-52.
- 22 Lee TH. Lipoxin A4: a novel anti-inflammatory molecule? *Thorax* 1995;50:1112.
- 23 Serhan CN, Takano T, Gronert K, et al. Lipoxin and aspirin-triggered 15-epi-lipoxin cellular interactions anti-inflammatory lipid mediators. *Clin Chem Lab Med* 1999;37: 299-309.
- 24 Diamond P, McGinty A, Sugrue D, et al. Regulation of leucocyte trafficking by lipoxins. *Clin Chem Lab Med* 1999;37: 293-7.
- 25 Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, November 1986. *Am Rev Respir Dis* 1987;136:225-44.
- 26 Godard P, Clot J, Jonquet O, et al. Lymphocytes subpopulations in broncho-alveolar lavages of patients with sarcoidosis and hypersensitivity pneumonitis. *Chest* 1981;80:447-52.
- 27 Chavis C, Vachier I, Chanez P, et al. 5(S),15(S)-Dihydroxyicosatetraenoic acid and lipoxin generation in human polymorphonuclear cells: dual specificity of 5-lipoxygenase towards endogenous and exogenous precursors. *J Exp Med* 1996;183:1633-43.
- 28 Chavis C, Vachier I, Bousquet J, et al. Generation of eicosanoids from 15(S)-hydroxyicosatetraenoic acid in blood monocytes from steroid-dependent asthmatic patients. *Biochem Pharmacol* 1998;56:535-41.
- 29 Chavis C, Godard P, Crastes-de-Paulet A, et al. 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.
- 30 Levy BD, Romano M, Chapman HA, et al. Human alveolar macrophages have 15-lipoxygenase and generate 15(S)-hydroxy-5,8,11-cis-13-trans-icosatetraenoic acid and lipoxins. *J Clin Invest* 1993;92:1572-9.
- 31 Thomas E, Leroux JL, Blotman F, et al. Conversion of endogenous arachidonic acid to 5(S),15(S)-diHETE and lipoxins by polymorphonuclear cells from patients with rheumatoid arthritis. *Inflammation Res* 1995;44:121-4.

- 32 Stahl GL, Morse DS, Martin SL. Eicosanoid production from porcine neutrophils and platelets: differential production with various agonists. *Am J Physiol* 1997;**272**:C1821–8.
- 33 Freeland HS, Pipkorn U, Schleimer RP, *et al.* Leukotriene B₄ as a mediator of early and late reactions to antigens in humans: the effect of systemic glucocorticoid treatment in vivo. *J Allergy Clin Immunol* 1989;**83**:634–42.
- 34 Linden M. The effects of beta-2 adrenoreceptor agonists and a corticosteroid, budesonide, on the secretion of inflammatory mediators from monocytes. *Br J Pharmacol* 1992;**107**:156–60.
- 35 De Caterina R, Sicari R, Giannessi D, *et al.* Macrophage-specific eicosanoid synthesis inhibition and lipocortin-1 induction by glucocorticoids. *J Appl Physiol* 1993;**75**:2368–75.
- 36 Riddick CA, Ring WL, Baker JR, *et al.* Dexamethasone increases expression of 5-lipoxygenase and its activating protein in human monocytes and THP-1 cells. *Eur J Biochem* 1997;**246**:112–8.
- 37 Uz T, Dwivedi Y, Savani PD, *et al.* Glucocorticoids stimulate inflammatory 5-lipoxygenase gene expression and protein translocation in the brain. *J Neurochem* 1999;**73**:693–9.