Arachidonic acid and leukotriene synthesis in relation to lung disease

JOHANNES F. G. VLIEGENTHART,
JAN VERHAGEN and GERRIT A. VELDINK
Department of Bio-Organic Chemistry, University of Utrecht, Padualaan 8, NL-3584 CH Utrecht, The Netherlands

Lipoxygenase (EC 1.13.11.12) catalyses the incorporation of molecular oxygen into unsaturated fatty acids containing one or more 1*Z*,4*Z*-pentadiene systems. The initial products are *EZ*-conjugated hydroperoxides:

$$R\text{–}CH{=}CH\text{–}CH_2\text{–}CH{=}CH\text{–}R' + O_2/\text{lipoxygenase} \rightarrow$$

$$R\text{–}\underset{\displaystyle OOH}{\underset{|}{CH}}\text{–}CH{=}CH\text{–}CH{=}CH\text{–}R'$$

Scheme 1.

The introduction of oxygen at one of the terminal carbon atoms of the pentadiene system and the abstraction of one of the hydrogen atoms from the central methylene group of the pentadiene system occur stereospecifically. The process of hydrogen abstraction, which is generally looked upon as being the initial step in this reaction sequence, and the insertion of a molecule of dioxygen take place at opposite sides of the planar pentadiene system. The enzymes studied so far have been found to contain 1 mol of iron which is essential for catalytic activity. The involvement of mammalian lipoxygenases in the biosynthesis of leukotrienes has evoked new research to establish the biological role of this type of compound. For recent reviews covering physiological, structural and biochemical aspects of the leukotrienes the reader is referred to Samuelsson (1983), Hammarström (1983) and Verhagen & Bruynzeel (1985). Leukotrienes have been shown to exert important biological effects, for example leukotriene B_4 is strongly chemotactic towards human granulocytes and stimulates the adherence of granulocytes to the endothelial cell wall (Ford-Hutchinson *et al.*, 1980; Palmblad *et al.*, 1983). Sulphidopeptide leukotrienes cause the contraction of smooth muscle tissue (Dahlen *et al.*, 1980) and an increase of the permeability of capillary vessel walls leading to oedema (Drazen *et al.*, 1980). Furthermore, they have been shown to stimulate the secrection of bronchial mucus (Marom *et al.*, 1982) and to slow down the rate of mucus removal in asthmatic patients (Ahmed *et al.*, 1981) after allergen exposure.

Abbreviations used: SRS-A, slow-reacting substance of analphylaxis; PAF, platelet-aggregating factor; fMLP, *N*-formyl-methionyl-leucyl-phenylalanine.

Sulphidopeptides and asthma

Originally named 'slow-reacting substance of anaphylaxis' (SRS-A), it was later established that SRS-A was in fact a mixture of sulphidopeptide leukotrienes C_4, D_4 and E_4 (Samuelsson, 1983). The biological effects of sulphidopeptide leukotrienes, and in particular those of leukotriene C_4, suggest that they have a role in the asthmatic process. Compared with histamine, the sulphidopeptide leukotrienes show major differences in contracting smooth muscle tissue. Leukotriene D_4 has been shown to be 20 000 times more powerful than histamine in contracting a guinea-pig lung parenchymal strip (Drazen *et al.*, 1980), while leukotriene C_4 turned out to be 5900 times more active than histamine in causing bronchoconstriction of human airways

Table 1 *Leukotriene C_4 formation by human eosinophils after stimulation with Ca-ionophore A23187, opsonized zymosan, fMLP or PAF*

From Bruynzeel *et al.* (1985*b*).

Stimulant	*n*	10^6 × Leukotriene C_4 (molecules/cell)
A23187 (10 μM)	14	56 ± 8
Opsonized zymosan (5 mg/ml)	45	17 ± 2
fMLP (100 nM)	14	0.9 ± 0.1
PAF (0.1 μM)	11	0.7 ± 0.1
PAF (10 μM)	23	4 ± 1
Opsonized zymosan (5 mg/ml) + fMLP (100 nM)	14	24 ± 4
Opsonized zymosan (5 mg/ml) + PAF (0.1 μM)	11	24 ± 5

in vivo (Weiss *et al.*, 1983). It has recently been demonstrated that eosinophilic granulocytes infiltrate the bronchioli at the beginning of the late-phase asthmatic reaction (De Monchy *et al.*, 1985). We have determined leukotriene profiles of purified eosinophilic and neutrophilic granulocytes from peripheral human blood after stimulating the cells with various agents.

Purification of eosinophilic and neutrophilic granulocytes

Platelet-rich plasma was removed from citrated human blood by centrifugation. The buffy coat was then centrifuged on Ficoll-Paque to separate mononuclear cells and granulocytes. A separation of neutrophils and eosinophils and a final purification of the eosinophil preparation was accomplished by centrifugation over Percoll-layers as described before (Verhagen *et al.*, 1984*a*; Bruynzeel *et al.*, 1985*a*,*b*). Eosinophils and neutrophils (5×10^6 cells/ml) were then incubated at 37°C with arachidonic acid, Ca-ionophore A23187, Ca^{2+} and reduced glutathione (Verhagen *et al.*, 1984*a*). In a subsequent series of experiments with eosinophils the artificial stimulus A23187 was replaced by compounds that are more likely to occur in living systems, e.g. platelet-aggregating factor (PAF).

Leukotriene production by human eosinophils and neutrophils

The product patterns of eosinophilic and neutrophilic granulocytes were determined by reverse-phase h.p.l.c. on Nucleosil 5C18 or CPspher 8C18 columns with the solvent system tetrahydrofuran/methanol/water/acetic acid (25:30:45:0.1, by vol.; pH5.5) (Verhagen *et al.*, 1984*b*). It was found that leukotriene C_4 is almost exclusively produced by the eosinophilic cell, whereas leukotriene B_4, 20-hydroxyleukotriene B_4 and two non-enzymically formed leukotriene B_4 isomers result from neutrophil action. This remarkable specificity further corroborates the view that eosinophils, after having invaded the bronchioli in an allergen-induced asthmatic reaction, have an important role in the process of leukotriene formation.

In subsequent experiments this specific potency of human eosinophilic granulocytes was further investigated. Purified eosinophils were then subjected to challenge with a variety of compounds, namely *N*-formyl-methionyl-leucyl-phenylalanine (fMLP), leukotriene B_4, Val-Gly-Ser-Glu, phorbol myristate acetate, opsonized zymosan, zymosan and Ca-ionophore A23187. The product patterns after incubation with arachidonic acid, Ca^{2+} and glutathione were determined by radioimmunoassay and reverse-phase h.p.l.c. (Bruynzeel *et al.*, 1985*b*). Opsonized zymosan proved to be a very effective stimulant in the formation of leukotriene C_4 by eosinophils. By contrast, neither zymosan as such, nor zymosan treated with heat-inactivated serum, was capable of mediating leukotriene C_4 synthesis by eosinophils. Although PAF at concentrations below 1 μM did not induce a measurable leukotriene C_4 synthesis, significant amounts of leukotriene C_4 were formed at a relatively high concentration (10 μM) of PAF. None of the other agents mentioned above was able to induce leukotriene C_4 synthesis. However, it is interesting to note that both fMLP and PAF significantly enhanced the capacity to synthesize leukotriene C_4 induced by opsonized zymosan (Table 1).

The observation that opsonized zymosan specifically induces leukotriene C_4-formation by human eosinophils suggests that the underlying mechanism is C3b- and/or IgG-mediated. In this respect it interesting to note that Shaw *et al.* (1985) have observed a stimulatory effect of IgG-coated Sepharose beads on leukotriene C_4 formation. Therefore, bronchoconstriction *in vivo* might occur as a consequence of the formation of leukotriene C_4 during phagocytosis of immunocomplexes or mast cell granules by eosinophils.

Ahmed, T., Greenblatt, D. W., Birch, S., Marchette, B. & Wanner, A. (1981) *Am. Rev. Respir. Dis.* **124**, 110–114

Bruynzeel, P. L. B., Kok, P. T. M., Viëtor, R. & Verhagen, J. (1985*a*) *Prostaglandins Leukotrienes Med.* **20**, 11–22

Bruynzeel, P. L. B., Kok, P. T. M., Hamelink, M. L., Kijne, A. M. & Verhagen, J. (1985*b*) *FEBS Lett.* **189**, 350–354

Dahlen, S. -E., Hedqvist, P., Hammarström, S. & Samuelsson, B. (1980) *Nature (London)* **288**, 484–486

De Monchy, J. G. R., Kauffman, H. F., Venge, P., Koëter, G. H., Jansen, H. M., Sluiter, H. J. & De Vries, K. (1985) *Am. Rev. Resp. Dis.* **131**, 373–377

Drazen, J. M., Austen, K. F., Lewis, R. A., Clark, D. A., Goto, G., Marfat, A. & Corey, E. J. (1980) *Proc. Natl. Acad. Sci U.S.A.* **77**, 4354–4358

Ford-Hutchinson, A. W., Bray, M. A., Doig, M. V., Shipley, M. E. & Smith, M. J. H. (1980) *Nature (London)* **286**, 264–265

Hammarström, S. (1983) *Annu. Rev. Biochem.* **52**, 355–377

Marom, Z., Shelhamer, J. H., Bach, M. R., Morton, D. R. & Kaliner, M. (1982) *Am. Rev. Resp. Dis.* **126**, 449–451

Palmblad, J., Malmsten, C. L., Uden, A. -M., Radmark, O. & Samuelsson, B. (1983) *Science* **220**, 568–575

Samuelsson, B. (1983) *Angew. Chem.* **95**, 854–864

Shaw, R. J., Walsh, G. M., Cromwell, O., Moqbel, R., Spry, C. J. F. & Kay, A. B. (1985) *Nature (London)* **316**, 150–152

Verhagen, J., Bruynzeel, P. L. B., Koedam, J. A., Wassink, G. A, De Boer, M., Terpstra, G. K., Kreukniet, J., Veldink, G. A. & Vliegenthart, J. F. G. (1984*a*) *FEBS Lett.* **186**, 23–28

Verhagen, J., Walstra, P., Veldink, G. A., Vliegenthart, J. F. G. & Bruynzeel, P. L. B. (1984*b*) *Prostaglandins Leukotrienes Med.* **13**, 15–20

Verhagen, J. & Bruynzeel, P. L. B. (1985) *Allergol. Immunopathol.* **13**, 531–537

Weiss, J. W., Drazen, J. M., McFadden, Jr., E. R., Weller, P., Corey, E. J., Lewis, R. A. & Austen, K. F., (1983) *J. Am. Med. Assoc.* **249**, 2814–2817

Received 1 December 1986