Role of arachidonic acid metabolites in inflammatory and thrombotic responses

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Arachidonic acid is converted by mammalian enzymes to a variety of oxygenated metabolites which include prostaglandins, thromboxanes and leukotrienes (collectively called eicosanoids). These products may contribute to the pathogenesis of several conditions including inflammation, asthma, thrombotic disease, Bartter's syndrome, dysmenorrhoea, threatened abortion, premature labour and cancer. In this paper the evidence that eicosanoids contribute to inflammatory and thrombotic responses will be considered. As such a discussion requires an understanding of the biosynthesis of the eicosanoids this aspect will be considered first.

Biosynthesis of prostaglandins, thromboxanes and leukotrienes

Eicosanoids are not stored in tissues but must be biosynthesized immediately before release. The initial and rate-limiting step in the formation of eicosanoids upon cell stimulation is the enzymic liberation of free fatty acid precursor, arachidonic acid, mainly from cell-membrane phospholipids (Fig. 1; see Irvine, 1982). Free arachidonic acid can be metabolized by the fatty acid cyclo-oxygenase (CO) enzyme complex to prostaglandin endoperoxides (PGG₂ and PGH₂; see Fig. 1). The endoperoxides, which are unstable at physiological pH and temperature, are pivotal in the formation of several other products: they are converted enzymically or non-enzymically to prostacyclin (PGI₂), TXA₂, the 'primary' prostaglandins (PGE₂, PGF₂α, and PGD₂), a C₁₅ hydroxy fatty acids (HHT) and malondialdehyde (MDA) (see Fig. 1). The metabolite formed varies from cell to cell; for example, blood platelets convert arachidonic acid to TXA₂, whereas vascular endothelium produces mainly prostacyclin. For a more detailed account of the biosynthesis of CO products readers are referred to Samuelsson et al. (1978).

A second, more recently discovered, pathway of arachidonic acid metabolism is an oxidation controlled by lipoygenase (LO) enzymes. The fatty acid is converted into hydroperoxy derivatives (hydroperoxy-eicosatetraenoic acids; HPETEs) which are readily reduced to the corresponding hydroxy-acids (HETEs) by glutathione peroxidase. The first lipoxigenation of arachidonic acid in mammalian tissues to be described was that occurring in blood platelets which resulted in the formation of 12-HETE (see Fig. 1). However, another hydroperoxy derivative, 5-HPETE (Fig. 1), is of more interest since it can be converted to a novel series of biologically active compounds known as leukotrienes. The first report of 5-LO activity was in polymorphonuclear leucocytes. The initial enzymic reaction in the conversion of 5-HPETE to leukotrienes is the loss of water to form the unstable 5,6-epoxide, LTA₄ (Fig. 1). As can be metabolized by the fatty acid cyclo-oxygenase (CO) enzyme complex to prostaglandin endoperoxides (PGG₂ and PGH₂; see Fig. 1). The endoperoxides, which are unstable at physiological pH and temperature, are pivotal in the formation of several other products: they are converted enzymically or non-enzymically to prostacyclin (PGI₂), TXA₂, the 'primary' prostaglandins (PGE₂, PGF₂α, and PGD₂), a C₁₅ hydroxy fatty acids (HHT) and malondialdehyde (MDA) (see Fig. 1). The metabolite formed varies from cell to cell; for example, blood platelets convert arachidonic acid to TXA₂, whereas vascular endothelium produces mainly prostacyclin. For a more detailed account of the biosynthesis of CO products readers are referred to Samuelsson et al. (1978).

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Fig. 1. Pathways of arachidonic acid metabolism

The enzymes controlling the reactions are shown in italics and broken lines indicate non-enzymic reactions.
with the endoperoxides in the synthesis of prostaglandins, LTA₄ is pivotal in the formation of other leukotrienes. It is hydrolysed to 5(S), 12(R)-dihydroxy-6,14-cis,8,10-trans-eicosatetraenoic acid (LTB₄) under the influence of LTA₄ hydrolase. Also, LTA₄ can be non-enzymically hydrolysed to other 5,12- and 5,6-dihydroxy acids. Additionally, glutathione can react with LTA₄ under the influence of a specific glutathione S-transferase to form 5-hydroxy-6-glutathionyl derivative (LTC₄), which can be metabolized successively by γ-glutamyl-transpeptidase and cysteinyl-glycine dipeptidase to LTD₄ and LTE₄ respectively. As with prostaglandins, different leukotrienes are formed in a specific cell type; for example, human eosinophils and neutrophils synthesize predominantly LTC₄ and LTB₄ respectively.

Role of arachidonic acid metabolites in inflammation

Inflammation is a complicated response involving several cell types (e.g. polymorphonuclear leucocytes, macrophages, lymphocytes and mast cells) and many putting mediators and modulators have been implicated (e.g. histamine, 5-hydroxytryptamine, kinins, chemotactic peptides, interleukins, interferons, prostaglandins, thromboxanes and leukotrienes). These mediators act in concert to amplify the inflammatory response which is characterized by erythema, oedema, hyperthermia, hyperalgesia, cell influx and loss of function. Since there is probably 'mediator redundancy' it may seem unlikely that either inhibition of synthesis or antagonism of the response of one group of mediators could provide significant reduction of the inflammatory response. However, there is persuasive evidence that inhibition of arachidonic acid metabolism can give important anti-inflammatory effects. The evidence that CO products are important mediators of some cardinal signs of inflammation (e.g. erythema, oedema, hyperalgesia and hyperthermia) is conclusive; prostaglandins have been detected in inflammatory exudate at biologically active concentrations (reviewed by Higgs et al., 1983) and the CO is selectively inhibited by non-steroidal anti-inflammatory drugs (e.g. aspirin, indomethacin). There is also much data which indicates that metabolites of arachidonic acid formed under the control of CO enzymes (particularly by the 5-LO) are involved in inflammatory reactions and this is discussed below.

LTB₄ has powerful effects on polymorphonuclear leucocyte function; it is a potent chemokinetic, chemotactic and degranulating agent for polymorphonuclear leucocytes of several species in vitro and causes polymorphonuclear leucocytes accumulation in vivo (for review, see Bray, 1983). In the presence of a vasodilator prostaglandin (e.g. PGE₂; LTB₄ also increases plasma exudation which is probably mediated by its effects on polymorphonuclear leucocytes. LTB₄ is formed during inflammatory reactions; for example, we have detected it in inflammatory exudates produce by the subcutaneous implantation in rats of 0.5% carrageenan-soaked polyester sponges (Simmons et al., 1983). The peak of LTB₄ concentration correlated with the maximum rate of polymorphonuclear leucocytes influx into the exudate. In experiments in which the animals were pre-dosed with colchicine, which limits leucocyte movement, the concentration of LTB₄ in the exudate, as well as the polymorphonuclear leucocyte count, was decreased. These findings suggest that polymorphonuclear leucocytes were the source of LTB₄ and probably, therefore, LTB₄ is not the initial signal for polymorphonuclear leucocyte infiltration, but may contribute to an amplification of the response. Synovial fluids from patients with rheumatoid arthritis and gout and fluids from involved skin of psoriatics contain elevated levels of LTB₄ (see Salmon, 1986).

The HPETE and HETE compounds formed from arachidonic acid via different LO pathways are biologically less potent than LTB₄ (Palmer et al., 1980). However, they could be influential in promoting cell influx into areas of inflammation since the concentrations of these metabolites may be much higher than those of LTB₄ in some diseases (e.g. psoriasis; Hammarstrom et al., 1975).

The peptido-lipid leukotrienes (LTC₄, LTD₄ and LTE₄) also have inflammatory activities in the skin, causing wheal and flare responses and increased vascular permeability. Thus these leukotrienes could modulate the vascular responses occurring in inflammation but, since they do not have chemotactic activity, they are unlikely to affect the cellular phase. These leukotrienes are biosynthesized in inflammatory cells (e.g. mastophages; eosinophils) and there is some evidence that they are formed in increased amounts at inflammatory sites.

Although peptido-lipid leukotrienes may be involved in inflammation, most interest is directed to their role as putative mediators of the bronchoconstriction occurring in asthma and similar hypersensitivity reactions: this aspect is considered by Vliegenthart et al. (1987).

Role of arachidonic acid metabolites in thrombosis

Addition of arachidonic acid or PG endoperoxides to suspensions of blood platelets causes aggregation and induces the release of platelet constituents. During this process the endoperoxides are converted almost exclusively to TXA₂ and HHT by the thromboxane synthase in the platelets. Hamberg et al. (1975) proposed that TXA₂ is the arachidonic acid metabolite that mediates platelet aggregation and release, although it remains to be established whether it is an important mediator of thrombotic events in vivo. Products of arachidonic acid metabolism represent only one pathway of platelet aggregation and it seems that pathophysiological platelet aggregation is a multifactorial phenomenon in which there may be participation of several pro-aggregatory substances including TXA₂, ADP, thrombin and collagen.

TXA₂ induces aggregation of platelets but another arachidonic acid metabolite, prostacyclin, is the most potent naturally occurring inhibitor of aggregation known (Moncada et al., 1976). Prostacyclin is formed from PG endoperoxides in blood vessel walls, particularly by the endothelium. These two arachidonate metabolites mediate their effects on platelets by opposite effects on the adenylate cyclase (prostacyclin increases levels of cyclic AMP, but TXA₂ prevents elevation). The compounds also have opposing effects on the vasculature; TXA₂ is a potent vasoconstrictor whereas prostacyclin causes vasodilatation. These biological properties suggest that normal haemostasis could be controlled by the generation of TXA₂ in platelets and prostacyclin in the vascular endothelium (see Moncada & Vane, 1978). An imbalance in the formation of TXA₂ and prostacyclin will affect thrombosis and haemostatic plug formation. Indeed several diseases have now been related to a disturbance of the TXA₂/prostacyclin ratio (see Bunting et al., 1983). In some instances, administration to patients of prostacyclin or a stable analogue has normalized the ratio and this has resulted in decreased platelet consumption.

The above hypothesis has also provided an explanation for previously observed phenomena as well as stimulating new anti-thrombotic initiatives. For example, aspirin has a unique mode of action which results in a more marked inhibition of platelet CO compared with inhibition of the enzyme in vascular endothelium and therefore the TXA₂/prostacyclin ratio is altered in favour of prostacyclin. Thus this may account for the reported reduction of myocardial re-infarction in patients given low doses of aspirin. However, there are theoretical advantages in employing more specific inhibitors
of TXA₂ synthesis and several such compounds have been described but, as yet, they have not proved useful in the clinic. Several explanations for their lack of therapeutic success can be offered; for example, one reason may be that these compounds do not block the formation of the endoperoxides which, although less active than TXA₂, do have pro-thrombotic activities.

Dietary manipulation can also modify the TXA₂/prostacyclin balance. An increased intake of eicosapentaenoic acid reduces the formation of TXA₂ and, as yet, it has not proved useful in the clinic. Thus the high intake of eicosapentaenoic acid in the diet of Greenland Eskimos could explain their low tendency to bleed (Dyerberg et al., 1979). Supplementation of the diet with eicosapentaenoic acid may offer a novel anti-thrombotic strategy.

Summary

In conclusion, there is strong evidence that arachidonic acid metabolites mediate some inflammatory and thrombotic responses. The non-steroidal anti-inflammatory drugs (the non-steroidal anti-inflammatory drugs such as aspirin and indomethacin) act by inhibiting the synthesis of prostaglandins. There are now data which indicate that leukotrienes, which are not reduced by non-steroidal anti-inflammatory drugs, also contribute to inflammatory reactions. Consequently, agents which reduce leukotriene formation may provide an important improvement in the treatment of inflammatory disorders. Modification of arachidonate metabolism by drugs or by dietary manipulation also offers the possibility of anti-thrombotic therapy. Administration of prostacyclin (or stable analogues) and aspirin can reduce the incidence and severity of thrombotic episodes. Also, supplementation of the diet with eicosapentaenoic acid may improve the thrombotic status of patients.

References


Received 1 December 1986

Archidonic acid and leukotriene synthesis in relation to lung disease

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

\[ \text{R}^1\text{CH}=\text{CH}-\text{CH}_2\text{CH}==\text{CH}-\text{R}' + \text{O}_2/\text{lipoxygenase} \rightarrow \]

\[ \text{R}^1\text{CH}==\text{CH}-\text{CH}==\text{CH}-\text{CH}==\text{CH}-\text{R}' \]

\[ \text{OOH} \]

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 evolved 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 B₄ 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 (Dracen et al., 1980). Furthermore, they have been shown to stimulate the secretion of bronchial mucus (Marom et al., 1982) and, in particular, those of leukotriene C₄, 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 D₂ has been shown to be 20000 times more powerful than histamine in contracting guinea-pig lung parenchymal strip (Dracen et al., 1980), while leukotriene C₄ turned out to be 5900 times more active than histamine in causing bronchoconstriction of human airways...