Biological Actions of Leukotrienes
State of the Art Lecture
ANTHONY FORD-HUTCHINSON AND GORDON LETTS

SUMMARY  Leukotrienes are novel mediators derived from arachidonic acid through the 5-lipoxygenase enzyme system. Leukotriene B4 has potent effects on leukocyte function and in vivo induces leukocyte accumulation and changes in vascular permeability and modulates pain responses. Peptidolipid leukotrienes are potent smooth muscle-contracting agents. They may have important cardiovascular actions through mechanisms involving either vasoconstriction or indirect vasodilatation. Evidence for leukotriene production has been found in subjects with allergic conditions and psoriasis, indicating a putative role for these substances in human disease.
(Hypertension 8 [Suppl II]: II-44-II-49, 1986)

KEY WORDS • leukotriene C4 • leukotriene D4 • leukotriene B4 • arachidonic acid metabolism

THE leukotrienes are a group of mediators derived from arachidonic acid through the action of the 5-lipoxygenase enzyme system (Figure 1).1-2 The initial product of this reaction is 5-hydroperoxy-6,8,11,14-eicosatetraenoic acid, which may then be converted either to the corresponding monohydroxyeicosatetraenoic acid, by the glutathione peroxidase system, or to leukotriene A4, by a dehydrase step.3-4 Leukotriene A4 is an unstable epoxide intermediate that can be converted by two specific enzymes, leukotriene A4 hydrolase and leukotriene C4 synthetase, to leukotrienes B4 and C4, respectively. Leukotriene A4 hydrolase is a highly specific enzyme that has been isolated to homogeneity from rat and human polymorphonuclear leukocytes.3-4 This enzymatic step leads to the production of a 5,12-dihydroxy-6,8,10,14-eicosatetraenoic acid (leukotriene B4) with precise stereochemical characteristics. The enzyme is eventually inactivated through covalent coupling of the substrate to the active site.5 The inactivation rate of the enzyme is increased in the presence of the alternative substrates leukotriene A5 and leukotriene A5, which are derived from the alternative fatty acids eicosatrienoic acid and eicosapentaenoic acid, respectively.3-6 Subsequent metabolism of leukotriene B4 occurring through omega-oxidation produces 20 OH-leukotriene B4 and 20 COOH-leukotriene B4. This metabolism results in loss of biological activity.7

Leukotriene C4 synthetase also appears to be a specific enzyme, distinct from the generalized glutathione transferase system, which catalyzes the insertion of glutathione, producing the peptidolipid conjugate leukotriene C4. Subsequent metabolism involves loss of the glutamic acid residue and loss of the glycine residue, producing leukotrienes D4 and E4. These peptidolipid conjugates collectively account for the biological activity known as the slow-reacting substance of anaphylaxis.1-28 Leukotriene F4 may be produced in biochemically driven systems through the addition of glutamic acid to leukotriene E4.

Leukotriene B4

Leukotriene B4 was first described chemically as one of a group of substances produced by polymorphonuclear leukocytes.9-10 Subsequently, it was demonstrated that leukotriene B4, accounted for a potent chemokinetic and aggregating activity released from polymorphonuclear leukocytes after exposure to ionophore A23187.11,12 Leukotriene B4 has high-affinity receptor sites on the polymorphonuclear leukocyte.13 These receptor sites are associated with activation of a number of cellular functions, including chemotaxis, chemokinesis, aggregation, and enzyme release.12,14 Evidence has also been obtained to indicate that subsets of both helper and suppressor T lymphocytes possess receptor sites for leukotriene B4.15 A number of

From the Department of Pharmacology, Merck Frosst Canada Inc., Quebec, Canada.
Address for reprints: Dr. A. W. Ford-Hutchinson, Merck Frosst Canada Inc., P.O. Box 1005, Pointe Claire-Dorval, Quebec, Canada H9R 4P8.
ACTIONS OF LEUKOTRIENES/Ford-Hutchinson and Letts

FIGURE 1. Arachidonic acid conversion by lipooxygenase pathways. HPETE = hydroperoxyeicosatetraenoic acid; HETE = hydroxyeicosatetraenoic acid; LT = leukotriene.

Peptidolipid Leukotrienes

Collectively, leukotrienes C₄, D₄, and E₄ are thought to account for the slow-reacting substance of anaphylaxis.¹ ¹ ² ³ Separate receptor sites have been identified for both leukotrienes C₄ and D₄, and these sites are readily distinguished by either radioligand receptor binding or differing responses to antagonists, such as FPL-55712.³¹ Interaction with the receptor sites can lead to smooth muscle contraction, which is expressed, for example, as either bronchoconstriction or vasoconstriction.³ ³ Thus, peptidolipid leukotrienes are potent inducers of the contraction of human airway smooth muscle preparation. When given by aerosol to either humans or conscious squirrel monkeys, leukotriene D₄ induced a prolonged bronchoconstriction similar to that observed after antigen challenge.³₂ ³³ In squirrel monkeys known to develop a late-phase response after exposure to ascaris antigen, a late-phase response after exposure to an aerosol of leukotriene D₄ has been observed.³² Peptidolipid leukotrienes may also mediate a number of other processes important in the lung, including impaired mucociliary clearance, mucous secretion, and changes in vascular permeability.³ Thus, leukotrienes may be important mediators of bronchoconstriction in diseases such as human bronchial asthma.

Cardiovascular Actions of Leukotrienes

The cardiovascular actions of leukotrienes are summarized in Table 1. In the majority of animal studies the peptidolipid leukotrienes have been shown to have potent vasoconstrictor actions. Vasoconstriction was first observed in the microvascular system of the skin, where intradermal injections of leukotriene C₄ or D₄
induced an initial blanching (vasoconstriction) followed by an enhanced extravasation of fluid (edema). Subsequent studies have shown that the peptidolipid leukotrienes also exhibit vasoconstrictor actions in most other vascular systems, including the renal, mesenteric, cerebral, pulmonary, and gastric vascular beds. These pharmacological actions are specific, since they are not blocked by a wide variety of selective receptor antagonists either in vitro or in vivo. Recent ligand binding studies have supported these findings by showing that there are specific and selective leukotriene C₄ binding sites on vascular smooth muscle. It has also been reported that there are no comparable leukotriene D₄ binding sites with high specificity on vascular smooth muscle. However, this is not consistent with the observation that leukotriene D₄ can exert potent vasoconstrictor actions in vitro and in vivo.

One important observation is that leukotrienes are potent constrictors of the coronary vasculature. Reductions in coronary flow are seen in vitro and in vivo and are usually concurrent with a negative inotropic action. The myocardial depressant actions of leukotrienes in vivo cause marked reductions in cardiac output. The reduced cardiac output most likely contributes to the secondary and more prolonged hypotension often seen after intravenous administration of leukotrienes. It has also been demonstrated that blood vessels stimulated by the calcium ionophore A23187 generate the peptidolipid leukotrienes in physiologically relevant amounts.

Although animal studies have clearly shown that bolus doses of peptidolipid leukotrienes reduce blood flow, recent data indicate that a sustained reduction in flow may not occur with a continuous infusion. During a continuous intracoronary infusion of peptidolipid leukotrienes, an initial reduction in myocardial blood flow and contractility was followed by recovery of myocardial function, even though circulating blood levels of the leukotrienes were high. The importance of this observation is unclear, since the studies were performed in healthy animals. Compensatory homeostatic mechanisms are intact in healthy animals and thus able to counteract any cardiac insult.

Leukotriene B₄ has no direct hemodynamic activity, yet its inhibition may cause important modulatory actions in diseased cardiac muscle. Animal models of myocardial infarction have recently shown that an inflammatory response accompanies the infarction. This action appears to correlate with a recruitment of polymorphonuclear leukocytes into the ischemic muscle. Animals that are either depleted of leukocytes or treated with 5-lipoxygenase inhibitors have significantly less myocardial damage, as compared with the respective control animals. It is therefore anticipated that leukotriene inhibitors will show beneficial actions in myocardial infarction therapy.

If a 5-lipoxygenase inhibitor can offer a beneficial reduction in the size of an infarct in an animal model, it is important to understand how this class of drug exerts its actions. Studies in dogs have shown that after the instigation of coronary reperfusion, there is a slow infiltration of white blood cells into the ischemic tissues. These leukocytes migrate into the area at risk over several hours, and mononuclear phagocytes in particular may provide a source of further peptidolipid leukotriene generation. Since the kinetics of leukocytes is influenced by leukotriene B₄, it may be postulated that during ischemia itself or during the reperfusion of ischemic muscle, there is a burst of arachidonate lipoxigenase activity. The release of cyclooxygenase products of arachidonic acid metabolism has been reported to coincide with the onset of ischemia. Letts et al. (unpublished data) have confirmed this observation and extended it to show that in guinea pig heart either during ischemia alone or during reperfusion, there is no concurrent release of leukotriene B₄ of the peptidolipid leukotrienes. These results indicate that the ischemic heart itself is not a source of leukotriene generation. Since no leukotrienes could be detected during ischemia, it may be postulated that their production in vivo is associated primarily with circulating cells. Clearly, further research is required to determine whether the 5-lipoxygenase inhibitors tested have other actions, such as radical scavenger actions.

Other important modulatory actions of leukotrienes may be associated with the platelet. It is well established that in many diseases of the cardiovascular system there is an abnormal platelet function. The peptidolipid leukotrienes have recently been reported to be the most potent aggregatory agent of porcine platelets identified (10-fold more potent than platelet activating factor). The release of a potent, platelet-derived vasoconstrictor was also associated with the interaction between leukotriene C₄ and the platelet. This platelet-derived mediator, which has a very short half-life in vivo, may represent an important part of the homeostatic process. A deficiency of the mediator may allow full expression of the vasoconstrictor actions of various stimuli (including the peptidolipid leukotrienes) to be expressed.

There are indications that the leukotrienes may be

**Table 1. Effects of the Leukotrienes in the Cardiovascular System**

<table>
<thead>
<tr>
<th>Effect</th>
<th>LTB₄</th>
<th>LTC₄</th>
<th>LTD₄</th>
<th>LTE₄</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vasoconstriction</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Coronary</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mesenteric</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cerebral</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Renal</td>
<td>( + )</td>
<td>( + )</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Myocardial depression</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Edema</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Symbols indicate level of cardiovascular action: highly active (+ +), active (+), variable (in parentheses), no effect (-).
involved in various cerebrovascular disorders, such as migraine and cerebral edema. Evidence of their involvement includes their potent constrictor actions on cerebral vessels in vitro and in vivo, the generation of leukotrienes during cerebral ischemia, and the identification of an active transport system for leukotrienes in the rabbit choroid plexus and their ability to alter the permeability of the blood–brain barrier in rats.

Although there is considerable information on the cardiovascular actions of synthetic leukotrienes in different animal species (see review), few data are available from studies in humans. Of particular interest is the limited evidence that peptidolipid leukotrienes reduce blood pressure and increase forearm blood flow after systemic administration. Intradermal injection has also been shown to increase skin blood flow. These actions contrast with those seen in the majority of animal species and may call into question the relevance of some animal models. Of greater potential importance will be the actions of leukotriene antagonists and inhibitors in disease-related animal models that can show the generation of leukotrienes in vivo. The actions of leukotrienes as modulators of the function of other mediators point to another important role.

Leukotriene Production in Human Disease

Leukotrienes are rapidly broken down within the circulation, and metabolism involves extensive β-oxidation. For this reason and because of problems associated with background reactivity with antiserum, it has been difficult to measure leukotrienes in plasma. Other approaches may be possible, such as measurement of leukotriene E2 or 20-OH leukotriene B4 in urine or measurement of N-acetyl leukotriene E4 in bile. Leukotrienes have been measured in fluids other than blood, including nasal washings, tear fluids, samples from the skin, and bronchoalveolar lavage fluids. Thus, for example, peptidolipid leukotrienes have been detected in tear fluid, nasal washings, and skin chamber fluid after antigen challenge in the eye, the nose, and the skin, respectively. Similarly, both leukotriene B4 and peptidolipid leukotrienes have been identified in skin chamber fluids obtained from patients with psoriasis. Such evidence has been used in support of a role for leukotrienes in various pathological processes. More definitive evidence for the role of such mediators in human disease will require the use of selective leukotriene D4 receptor antagonists or 5-lipoxygenase inhibitors in humans.

References

47. Voelkel NF. Species variations in the pulmonary responses to arachidonic acid metabolites. Prostaglandins 1985;25:867–889
54. Clark MA, Cook M, Song K, Crooke ST. The binding of leukotriene C4 and leukotriene D4 to membranes of a smooth muscle cell line (BCH3) and evidence that leukotriene induced contraction in these cells is mediated by thromboxane, protein and RNA synthesis. Eur J Pharmacol 1985;116:207–220
60. Fiedler VB, Mardin M, Abram TS. Leukotriene D4-induced vasocostriction of coronary arteries in anesthetized dogs. Eur Heart J 1984;5:235–236
64. Lefer AM, Roth DM, Lefer DJ, Smith JB. Potentiation of leukotriene formation in pulmonary and vascular tissue. Naunyn Schmiedebergs Arch Pharmacol 1984;326:186–189
ACTIONS OF LEUKOTRIENES/Ford-Hutchinson and Letts

of compounds with properties of leukotrienes C₄ and D₄ in gerbil brains after ischemia and reperfusion. Science 1984; 224:886-889


Biological actions of leukotrienes. State of the art lecture.
A Ford-Hutchinson and G Letts

Hypertension. 1986;8:II44
doi: 10.1161/01.HYP.8.6_Pt_2.II44

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/8/6_Pt_2/II44