Biological Actions of Leukotrienes
State of the Art Lecture
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SUMMARY  Leukotrienes are novel mediators derived from arachidonic acid through the 5-lipoxygenase enzyme system. Leukotriene B$_4$ has potent effects on leukocyte function and in vivo induces leukocyte accumulation and changes in vascular permeability and modulates pain responses. Peptido-lipid 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 C$_4$ •  leukotriene D$_4$ •  leukotriene B$_4$ •  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). 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 A$_4$, by a dehydrase step. Leukotriene A$_4$ is an unstable epoxide intermediate that can be converted by two specific enzymes, leukotriene A$_4$ hydrolase and leukotriene C$_4$ synthetase, to leukotrienes B$_4$ and C$_4$, respectively. Leukotriene A$_4$ hydrolase is a highly specific enzyme that has been isolated to homogeneity from rat and human polymorphonuclear leukocytes. This enzymatic step leads to the production of a 5,12-dihydroxy-6,8,10,14-eicosatetraenoic acid (leukotriene B$_4$) with precise stereochemical characteristics. The enzyme is eventually inactivated through covalent coupling of the substrate to the active site. The inactivation rate of the enzyme is increased in the presence of the alternative substrates leukotriene A$_4$ and leukotriene A$_5$, which are derived from the alternative fatty acids eicosatrienoic acid and eicosapentaenoic acid, respectively. Subsequent metabolism of leukotriene B$_4$ occurring through omega-oxidation produces 20 OH-leukotriene B$_4$ and 20 COOH-leukotriene B$_4$. This metabolism results in loss of biological activity. Leukotriene C$_4$ 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 C$_4$. Subsequent metabolism involves loss of the glutamic acid residue and loss of the glycine residue, producing leukotrienes D$_4$ and E$_4$. These peptidolipid conjugates collectively account for the biological activity known as the slow-reacting substance of anaphylaxis. Leukotriene F$_4$ may be produced in biochemically driven systems through the addition of glutamic acid to leukotriene E$_4$.

Leukotriene B$_4$
Leukotriene B$_4$ was first described chemically as one of a group of substances produced by polymorphonuclear leukocytes. Subsequently, it was demonstrated that leukotriene B$_4$ accounted for a potent chemokinetic and aggregating activity released from polymorphonuclear leukocytes after exposure to ionophore A23187. Leukotriene B$_4$ has high-affinity receptor sites on the polymorphonuclear leukocyte. These receptor sites are associated with activation of a number of cellular functions, including chemotaxis, chemokinesis, aggregation, and enzyme release. Evidence has also been obtained to indicate that subsets of both helper and suppressor T lymphocytes possess receptor sites for leukotriene B$_4$. A number of
functional studies point to a role for leukotriene B4 in immune regulation. Thus, in vitro leukotriene B4 may induce a T-lymphocyte suppressor cell population, which inhibits the proliferation of mitogen-stimulated helper inducer T lymphocytes. In addition, leukotriene B4 has been reported to replace the helper cell or interferon-2 requirement for γ-interferon production by murine Lyt-1−,2+ cells and may augment the activity of human natural cytotoxic cells. When added to cultured human keratinocytes, leukotriene B4, but not biologically inactive structural analogues of leukotriene B4, has been shown to stimulate DNA synthesis as assessed by the incorporation of [3H]thymidine.

The in vivo properties of leukotriene B4 can be related to its in vitro properties. Thus, leukotriene B4 causes leukocyte accumulation after injection into or application to rabbit skin or eye, human skin, or guinea pig peritoneal cavity. Two other properties of leukotriene B4 have also been related to neutrophil accumulation: induction of changes in vascular permeability, which has been shown to occur in the rabbit, rat, guinea pig, and pig, and induction of pain responses, which occurs after injection into the rat paw either alone or in the presence of other agents, such as yeast. Both the induction of pain and the induction of vascular permeability changes are thought to be due to the secondary release of mediators from polymorphonuclear leukocytes after stimulation with leukotriene B4 and adherence to the vascular endothelium. The effects of leukotriene B4 on keratinocyte proliferation in vitro have also been observed in vivo after topical application to the guinea pig ear. In these studies increased epidermal proliferation was observed, as assessed either histologically or through the incorporation of [3H]thymidine into DNA.

Peptidolipid Leukotrienes

Collectively, leukotrienes C4, D4, and E4 are thought to account for the slow-reacting substance of anaphylaxis. Separate receptor sites have been identified for both leukotriene C4 and D4, and these sites are readily distinguished by either radioligand receptor binding or differing responses to antagonists, such as FPL-55712. Interaction with the receptor sites can lead to smooth muscle contraction, which is expressed, for example, as either bronchoconstriction or vasoconstriction. 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. 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. 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. 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. Together, these observations may indicate an involvement of leukotrienes in some vasospastic disorders of cardiac function, such as unstable or variant angina.

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 kinesis 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 or 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 vasodilator 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

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

<table>
<thead>
<tr>
<th>Action</th>
<th>LTB₄</th>
<th>LTC₄</th>
<th>LTD₄</th>
<th>LTE₄</th>
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<tbody>
<tr>
<td>Vasoconstriction</td>
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<tr>
<td>Coronary</td>
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<td>+</td>
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<td>+</td>
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<td>Mesenteric</td>
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<td>Cerebral</td>
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<td>Renal</td>
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<td>Myocardial depression</td>
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<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 [-]. LT = leukotriene.
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 \( \beta \)-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 \( E_2 \) or 20-OH leukotriene \( B_3 \) in urine or measurement of N-acetyl leukotriene \( E_2 \) 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 \( B_4 \) 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 \( D_4 \) receptor antagonists or \( \beta \)-lipoxygenase inhibitors in humans.

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II-47
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