

## Experimental and clinical pharmacology

# Leukotrienes - biosynthesis and mechanisms of action

*Stella R. O'Donnell, Professor of Pharmacology, Department of Physiology and Pharmacology, University of Queensland, Brisbane*

## Summary

The leukotrienes are potent inflammatory mediators which may have a role in inflammatory diseases such as allergic rhinitis, inflammatory bowel disease and asthma. The clinically important leukotrienes are LTB<sub>4</sub> and the cysteinyl leukotrienes (CysLTs). To make leukotrienes, cells need 5-lipoxygenase and a protein co-factor, 5-lipoxygenase activating protein (FLAP). Drugs that act on either 5-lipoxygenase or FLAP will inhibit the synthesis, and hence the actions, of both the CysLTs and LTB<sub>4</sub>. There are two distinct receptor types for the CysLTs (CysLT1 and CysLT2 receptors) and one for LTB<sub>4</sub> (BLT receptor). LTB<sub>4</sub> is a potent chemotactic agent and attracts pro-inflammatory cells, e.g. neutrophils and eosinophils, into tissues. The CysLTs contract airway and some vascular smooth muscle, stimulate mucus secretion and increase microvascular permeability.

**Key words:** asthma, pharmacodynamics, inflammatory process, montelukast, zafirlukast.

Aust Prescr 1999;22:55-7

## Introduction

Leukotrienes, together with the prostaglandins and other related compounds, are derived from 20 carbon (eicosa) fatty acids that contain double bonds (enoic). Hence this group of substances is called the eicosanoids. The name leukotriene derives from the original discovery of these substances in white blood cells (polymorphonuclear leucocytes) and the fact that they all have in common 4 double bonds (hence the 4 subscript), 3 of which are in a conjugated triene structure.

Leukotrienes do not exist preformed in cells. They are formed from the breakdown of arachidonic acid, a polyunsaturated 20 carbon fatty acid. In its esterified form, arachidonic acid is bound to the phospholipids of the cell membranes. Both immunological and non-immunological stimuli can release arachidonic acid from membrane phospholipids by activating phospholipase A<sub>2</sub>. The glucocorticosteroid drugs can inhibit phospholipase A<sub>2</sub> and thereby decrease the production of all the leukotrienes and hence leukotriene-mediated responses.

The released arachidonic acid can be metabolised to oxygenated products by several distinct enzyme pathways (Fig. 1). The main ones are:

- the cyclooxygenase pathway that results in the formation of the prostaglandins and thromboxanes (together known as the prostanoids)
- the lipoxygenase pathway that produces several, chemically different leukotrienes (and other intermediate compounds).

## Biosynthesis of leukotrienes from arachidonic acid

The first steps in the generation of leukotrienes are catalysed by the calcium and ATP-dependent enzyme 5-lipoxygenase. This is one of a family of lipoxygenase enzymes that metabolise arachidonic acid to hydroperoxyeicosatetraenoic acids (HPETEs). Each enzyme catalyses the insertion of an oxygen moiety at a specific position in the arachidonic acid backbone. 5-lipoxygenase forms 5-HPETE, the precursor of the leukotrienes.

When cells are activated, cytosolic 5-lipoxygenase is translocated to the nuclear membrane. A nuclear membrane protein, 5-lipoxygenase activating protein (FLAP), is required before 5-lipoxygenase can synthesise 5-HPETE from arachidonic acid. Compounds are now available that block the biosynthesis of the leukotrienes through specific inhibition of 5-lipoxygenase e.g. zileuton (approved for asthma treatment in the U.S.A.). Experimental drugs are also available that inhibit leukotriene synthesis by inhibition of FLAP.

The rearrangement of 5-HPETE to form the unstable LTA<sub>4</sub> is the rate-limiting step in the synthesis of the leukotrienes. This step is catalysed by LTA synthase. LTA<sub>4</sub> is then converted to either LTB<sub>4</sub> or LTC<sub>4</sub>. LTC<sub>4</sub> is actively transported out of cells and rapidly metabolised to LTD<sub>4</sub> and then to LTE<sub>4</sub> (see Fig. 1 for enzymes involved in these steps). LTC<sub>4</sub>, LTD<sub>4</sub> and LTE<sub>4</sub> are referred to as the cysteinyl (Cys) leukotrienes because of their chemical structure. LTE<sub>4</sub> is either excreted in the urine or metabolised to a variety of biologically less active, or inactive, metabolites, including LTF<sub>4</sub>.

In summary, the ability of cells to synthesise leukotrienes depends on

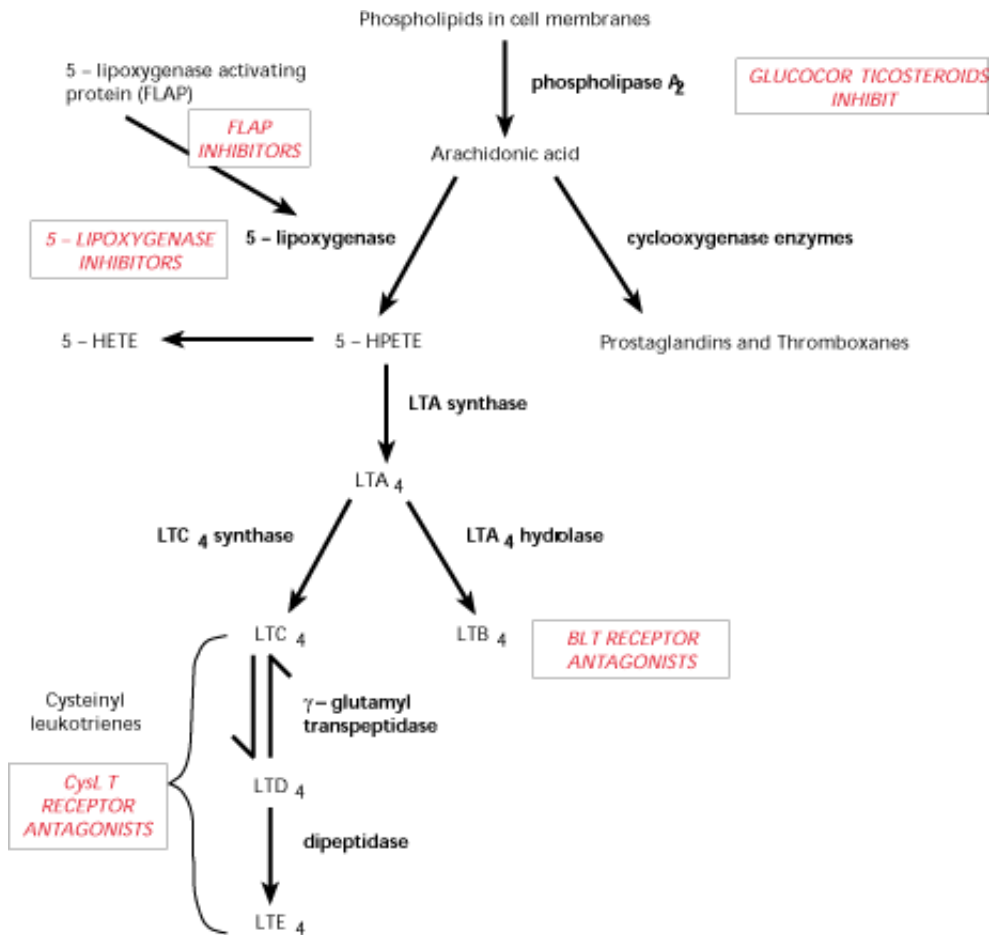
- their enzymic capacity to cleave arachidonic acid from its phosphorylated store
- the 5-lipoxygenase system to synthesise LTA<sub>4</sub>

The lungs contain cells that have the full capacity to synthesise all the leukotrienes *de novo*. Hence, there has been significant interest in their effects on the lung.

**Fig. 1**

The main pathways to the formation of the leukotrienes and the sites of action of the current drug groups (in boxes) that can attenuate

## leukotriene responses.



Normal font - main pathways

Bold - enzymes

Boxed italics - drug groups (in red)

### Leukotriene receptors

Early studies of the leukotrienes focused on their functional responses and their rank order of potency as agonists for various responses. These studies revealed that responses to LTB<sub>4</sub>, and hence possibly its receptor, were distinguishable from those of the CysLTs. There was also an indication that there may be subtypes of receptors for the CysLTs. Since then, attempts have been made to classify and satisfactorily name the leukotriene receptors. An IUPHAR (International Union of Pharmacology) committee on drug classification and nomenclature developed recommendations for the leukotriene receptors. The current (1998) nomenclature is summarised in Table 1, together with the order of potency of the leukotrienes and the names of some selective antagonist drugs.

The classification, based mainly on functional data, recognises a distinct receptor for LTB<sub>4</sub> (now called the BLT receptor) and subtypes of receptors for the CysLTs (now called the CysLT<sub>1</sub> and CysLT<sub>2</sub> receptors). At present, there are no useful selective *agonist* compounds for any of the leukotriene receptor types. Numerous compounds have been shown to be selective *antagonists* of BLT or CysLT<sub>1</sub> receptors in animal studies. Some of the CysLT<sub>1</sub> receptor antagonists are now being used in the treatment of asthma. There is no selective antagonist for the CysLT<sub>2</sub> receptors. The compound, BAY u9773, appears to be a non-selective blocker of both CysLT<sub>1</sub> and CysLT<sub>2</sub> receptors. Hence, at present, those responses that are not blocked by one of the selective CysLT<sub>1</sub> receptor antagonists are assumed to be mediated by CysLT<sub>2</sub> receptors.

The classification of the type(s) of receptor mediating the different responses to the leukotrienes is still evolving because of the lack of a complete range of selective agonists and antagonists and a lack of success in cloning and sequencing the CysLT receptors.

### BLT receptors

LTB<sub>4</sub> is a potent chemotactic agent for neutrophils, eosinophils and monocytes. It promotes the adhesion of neutrophils to the vascular endothelium and enhances their migration across the endothelial wall into the surrounding tissue. LTB<sub>4</sub> also increases the release of toxic oxygen products, lysosomal enzymes and cytokines from pro-inflammatory cells.

### CysLT receptors

It was shown in the 1930s that, if the lungs from sensitised guinea-pigs were perfused with sensitising antigen, a substance was released that could cause a slow contraction of isolated smooth muscle preparations. This substance was called slow reacting substance. It was later renamed slow reacting substance of anaphylaxis and, in the early 1980s, it was identified as a mixture of the CysLTs.

In addition to the contractile responses in the lung, CysLTs have been shown to contract human coronary artery and distal and mesenteric pulmonary artery. They have no effect on most systemic large arteries or on the renal vasculature.

In the early studies, the contractile effects of the leukotrienes in airway, lung, vascular and other tissue preparations were explored. Data from these studies, together with that from ligand binding studies and from experiments with FPL 55712, the first drug shown to block responses to the leukotrienes, indicated

that there might be distinct receptors for LTD<sub>4</sub> and LTC<sub>4</sub>. Subsequently, the effects of the newer, more selective, CysLT<sub>1</sub> receptor antagonists and BAY u9773 on responses to LTD<sub>4</sub>, LTE<sub>4</sub> and/or LTC<sub>4</sub> have allowed us to predict the receptor type(s) likely to be involved in some tissues.

Human bronchi may have a homogeneous population of CysLT<sub>1</sub> receptors, whereas guinea-pig trachea and ileum probably have both CysLT<sub>1</sub> and CysLT<sub>2</sub> receptors. Some tissues, e.g. guinea-pig and human lung, may have an additional receptor, but this is controversial. The current classification may be an oversimplification and it is likely to be modified as more data accumulate, appropriate tools are found and the molecular features of the receptors are unravelled.

*Table 1*

**Current nomenclature for the leukotriene receptors, based on that published by the IUPHAR nomenclature subcommittee.<sup>1</sup> Also shows relative potency of agonists and some key selective antagonists.**

	<i>Leukotriene receptor type</i>		
	<i>BLT receptor</i>	<i>CysLT<sub>1</sub> receptor</i>	<i>CysLT<sub>2</sub> receptor</i>
Previously known as	LTB <sub>4</sub> receptor	LTD <sub>4</sub> receptor	LTC <sub>4</sub> receptor
Order of potency of agonists	LTB <sub>4</sub> >12(R)-HETE (LTC <sub>4</sub> and LTD <sub>4</sub> are mainly inactive)	LTD <sub>4</sub> =LTC <sub>4</sub> >LTE <sub>4</sub> (LTE <sub>4</sub> is a partial agonist in some tissues)	LTC <sub>4</sub> >LTD <sub>4</sub> >LTE <sub>4</sub> (LTE <sub>4</sub> is a partial agonist in some tissues)
Selective antagonists	LY 293111	montelukast	BAY u9773 is a
	SC 53228	iralukast	non-selective
	SB209247	pobilukast	antagonist at
	CP 105696	zafirlukast	CysLT <sub>1</sub> and
	CGS 25019C	pranlukast	CysLT <sub>2</sub> receptors

#### **Action in asthma**

The leukotrienes are primarily endogenous mediators of inflammation. They contribute to the signs and symptoms seen in acute inflammatory responses. This includes responses resulting from the interaction of allergens with IgE antibodies on mast cells. *In vivo*, the CysLTs are bronchoconstrictors. After inhalation of these leukotrienes, bronchospasm occurs in about 30 minutes and lasts up to 2 hours. They also stimulate airway mucus secretion and are very potent at increasing the permeability of post capillary venules, including those in the bronchial circulation - hence they cause plasma protein exudation and oedema. Recent evidence suggests that the CysLTs also promote eosinophil migration into the airways of animals and asthmatic patients. They may also increase bronchial hyperresponsiveness through an action on sensory nerves. Some of these responses can be blocked by the new CysLT<sub>1</sub> receptor antagonists, but other responses are yet to be characterised.

#### **Drug development**

The CysLTs have been implicated as important mediators in the pathogenesis of a number of inflammatory disorders in addition to asthma, including allergic rhinitis, chronic bronchitis and inflammatory bowel disease. To date, most attention has been directed towards their effects in the lungs and to their possible role in asthma. This has led to the development of drugs which prevent the actions of the CysLTs in the airways. Two groups of drugs are currently available for clinical use in the U.S.A. One group prevents the synthesis (and hence effects) of all the leukotrienes by inhibiting the enzyme 5-lipoxygenase (no drug in this group is currently registered in Australia). The other group contains the selective antagonists of CysLT<sub>1</sub> receptors (montelukast and zafirlukast) which block the effects of the CysLTs in human airways.

#### **Reference**

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#### **FURTHER READING**

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#### **Self-test questions**

**The following statements are either true or false (click here for the answers)**

1. Leukotrienes cause bronchoconstriction.
2. Montelukast and zafirlukast act by blocking the synthesis of leukotrienes.

First published online 1 June 1999