

Role of Leukotriene in Inflammation and Antileukotriene Therapy

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ABSTRACT

The leukotrienes arise from oxidative metabolism of arachidonic acid through the action of the 5-lipoxygenase enzyme, leading to the unstable allylic epoxide leukotriene A₄. This intermediate represents the substrate for two different specific enzymes, namely leukotriene A₄-hydrolase and leukotriene C₄-synthase, generating LTB₄ and cysteinyl leukotrienes, respectively. LTC₄ and LTD₄ are potent contracting agents of smooth muscle in airways and blood vessels. LTB₄ is known as a potent chemokinetic and chemotactic agent. A number of evidences reported in the literature underline the potential role of leukotrienes in the inflammatory responses that characterizes asthma and other pathological conditions. These potent lipid bioeffectors are synthesized during the course of inflammatory reactions and their pharmacological modulation is able to significantly attenuate the clinical manifestations associated with different inflammatory pathologies. Selective leukotriene inhibitors and receptor antagonists are currently under evaluation in the treatment of various inflammatory diseases.

Key words: Leukotriene, Inflammation, LTRAs, FLAP, Cys LTs, LTB₄

INTRODUCTION

Introduction

All organisms respond to local injury. Inflammation is the response of living tissue to damage. The inflammatory process is the reaction of blood vessel, which brings about an accumulation of fluid and white blood cell in the extravascular tissue.¹ The acute inflammatory response has functions of destroying and eliminating the components of exudate. The damaged tissue can be broken down and partially liquefied, and the debris removed from the site of damage.¹

Inflammation is caused by release of chemicals from tissues and migrating cells after injury. Most strongly implicated are the prostaglandins (PGs), leukotrienes (LTs), histamine, bradykinin, more recently, platelet-

activating factor (PAF) and interleukin-1 and other various mediators.²

A number of evidences reported in the literature underline the potential role of leukotrienes in the inflammatory responses. The name "leukotriene" is referring to the cellular source (leukocytes are one of the major sources) as well as the conjugated triene that characterizes their structure. The cysteinyl leukotrienes C₄, D₄, and E₄ account for the biologic activity that was previously termed "slow-reacting substance of anaphylaxis".

LTC₄ and LTD₄ are potent contracting agents of smooth muscle in airways and blood vessels; in addition, they induce mucus secretion and promote plasmatic exudation with direct action on endothelial cells. On the other side, LTB₄ is known as a potent chemokinetic and chemotactic agent. These potent lipid bioeffectors are synthesized during the course of inflammatory reactions and their pharmacological modulation

is able to significantly attenuate the clinical manifestations associated with different inflammatory pathologies. Given the differences between the two classes of LTs (LTB_4 versus LTC_4 , LTD_4 , and LTE_4 , i.e., cysteine-containing leukotrienes or cysteinyl-LTs) in terms of both structure and profile of biological activity, it is not unexpected that the receptors for LTB_4 are totally different from those for cysteinyl-LTs and no cross reactivity has been shown among these two classes. Leukotrienes (LTs) exert their actions through high-affinity specific receptors, LTB (LTB_1 and LTB_2) receptor and CysLT ($CysLT_1$ and $CysLT_2$) receptor.⁴

The leukotriene activity is inhibited by using leukotriene inhibitors, leukotriene receptor antagonists (LTRAs) or 5-lipoxygenase activating protein (FLAP) inhibitors. The LTRAs selectively antagonize the $CysLT_1$ receptor, unlike 5-LO and FLAP inhibitors, the current LTRAs do not affect LTB_4 .

Leukotriene

Leukotrienes, together with prostaglandins, thromboxanes, and lipoxins, are the major constituents of a group of biologically active oxygenated fatty acids known as eicosanoids¹⁸ and constituent family of lipid mediators with potent biological activities.¹⁹

Because myeloid cells contain substantial amounts of esterified arachidonic acid (AA)²⁰ and constitutively express all of the enzymes necessary to hydrolyze it and metabolize it via the 5-lipoxygenase (5-LO) pathway, they are capable of generating large quantities of products termed leukotrienes (LTs) within seconds to minutes after encountering an activating stimulus. The systemic name of arachidonic acid is 5,8,11,14-eicosatetraenoic acid, symbolized as $C_{20:4}$, indicating a total of 20 carbons (twenty in greek is eicosi) and presence of four double bonds at the indicated positions.²¹ Unlike many other inflammatory mediators, LT are not stored but synthesized de novo in response to inflammatory stimuli.²²

The leukotrienes can be divided into two different classes, based upon their chemical structures and biological activity: 1) the cysteinyl leukotrienes (Cys-LTs), namely leukotriene C_4 (LTC_4), leukotriene D_4 (LTD_4), and leukotriene E_4 (LTE_4), containing different amino acid residues, and 2) the dihydroxy derivative leukotriene B_4 (LTB_4). Both classes arise from the oxidative metabolism of arachidonic acid (AA) through the action of the 5-lipoxygenase enzyme (5-LO). Leukocytes are one of the major sources of leukotrienes. The name "leukotriene" is indeed referring to the cellular source as well as the conjugated triene that characterizes their structure.^{23, 24} Cys-LTs were originally described as the slow reacting substance of anaphylaxis (SRS-A).¹⁹

SRS-A was identified in the lungs of guinea pigs and described as a smooth muscle contracting compound as early as in 1938.¹⁸ Since the structure of leukotrienes was described in 1979,²⁴ Leukotriene B_4 (LTB_4) was the first of the leukotrienes to be isolated.²⁵

Biosynthesis of Leukotrienes

a. Mobilization of Arachidonic Acid

Free AA concentration is tightly controlled in inflammatory cells. AA is present mainly in ester form within endogenous stores located in membrane phospholipids, and the first step toward leukotriene generation is represented by the activation of specific phospholipases.²⁶ Together with AA mobilization, 5-LO activity requires cell activation and influx of extracellular calcium levels to produce leukotrienes. The initial step in biosynthesis of eicosanoids is a receptor-mediated influx of Ca^{2+} ions that causes translocation of a phospholipase enzyme, cytosolic phospholipase A_2 , to the cell membrane.²⁷ This enzyme then catalyzes the hydrolysis of the esterified form of arachidonic acid. Several phospholipase A_2 enzymes of varying molecular weight have been identified in cells such as macrophages, neutrophils, platelets, and mast cells, all of which are involved in eicosanoid biosynthesis.²⁸ The activity of phospholipase A_2 is increased by a phospholipase A_2 -activating protein that, when activated by cytokines such as tumor necrosis factor and interleukin-1, can lead to arachidonic acid release and leukotriene formation in leukocytes.²⁹

b. Role of 5-Lipoxygenase in the Formation of Leukotriene A_4

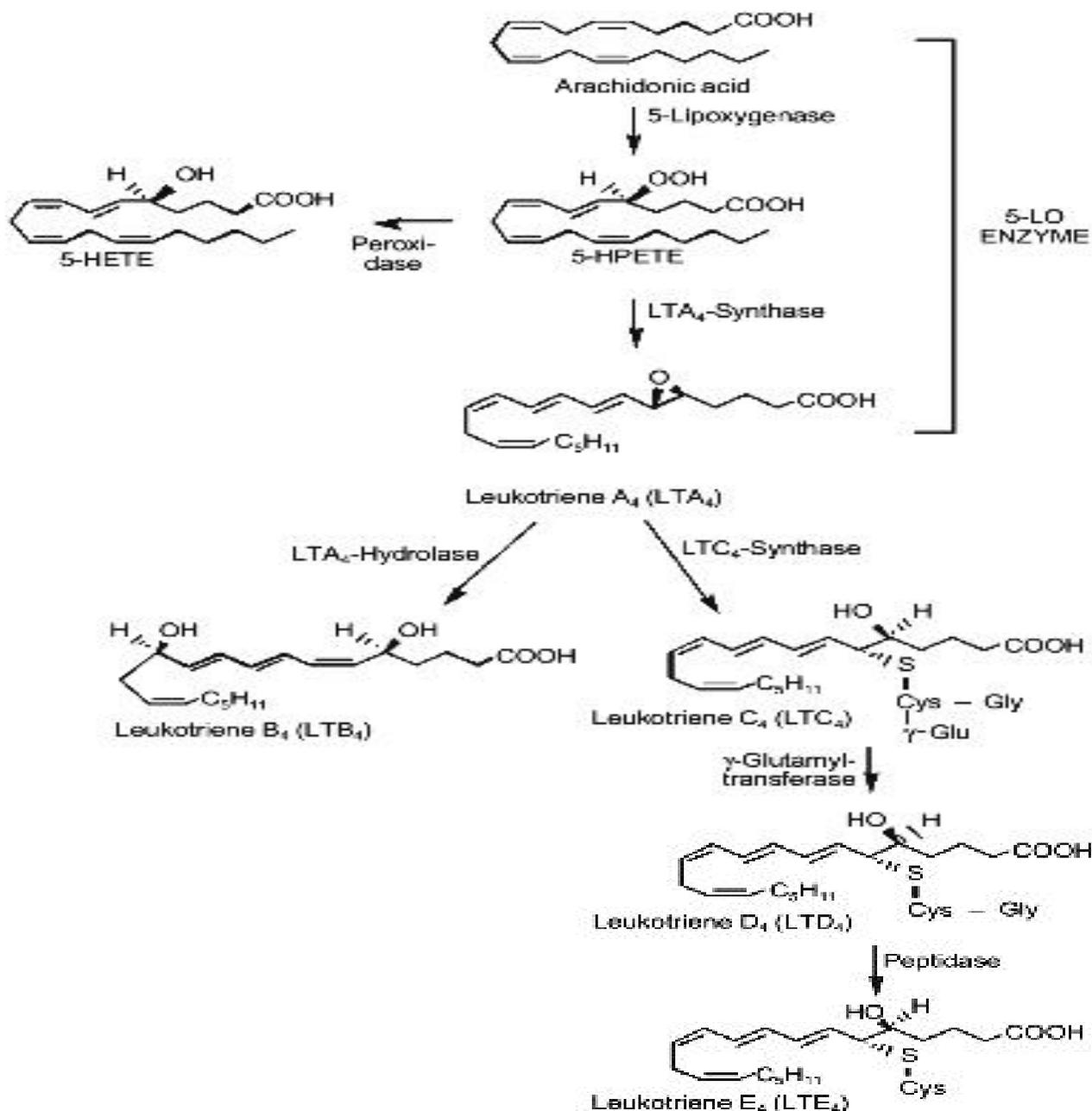
Unlike cyclooxygenase, another important enzyme catalyzing oxygenation of AA that is present in constitutive or inducible form in most cell types, 5-LO presents a more limited distribution. 5-lipoxygenase is an iron-containing enzyme consisting 673 amino acids. It is activated by Ca^{2+} and adenosine triphosphate, and translocates from the cytosol to the cell membrane and begins to form leukotrienes.²² Also 5-lipoxygenase activating factor (FLAP) is necessary for LT synthesis. The mechanism of FLAP action is however is not completely clear although it has been suggested that FLAP acts by presenting arachidonic acid to 5-LO.²¹ All leukotriene molecules are derived from a common precursor, leukotriene A_4 (LTA_4). This leukotriene, however, formed only after an intermediate step, in which 5-hydroperoxyeicosatetraenoic acid (HPETE), is formed through oxidising action of 5-lipoxygenase acting on arachidonic acid in the presence of adenosine triphosphate, calcium ions,³⁰ and the 5-lipoxygenase-activating protein.³¹

c. Biosynthesis of Leukotriene B₄ and the Cysteinyl leukotriene

The formation of LTA₄ is the last common step in the synthesis of LTB₄ on the one hand and of the sulfidopeptide or cysteinyl leukotrienes (C₄, D₄, and E₄) on the other side. Leukotriene B₄ is a dihydroxy acid formed from LTA₄ through the action of LTA₄ hydrolase, a cytosolic protein by addition

of net water molecule.³² The sequence of cysteinyl leukotriene formation started when LTC₄ synthase, a glutathione-S-transferase enzyme, converts LTA₄ to the glutathione-containing sulfidopeptide LTC₄.²⁴ Leukotriene C₄ may then be converted to LTD₄ by γ -glutamyltranspeptidase by removing glutamic acid from LTC₄ and LTD₄ may be subsequently metabolized to LTE₄ by cysteinylglycine dipeptidase by removing of glycine from LTD₄.³³

Fig.1: Metabolism of the arachidonic acid by 5-lipoxygenase enzyme and biosynthesis of leukotriene.



Heritable deficiencies of enzymes in the leukotriene synthetic pathway are rare, but there are allelic variants of the coding and promoter regions of the genes for 5-lipoxygenase, FLAP, LTA₄ hydrolase, and LTC₄ synthase. Moreover, the transcription of these genes can be regulated by cytokines, transforming growth factor β, leptin, endothelin, vitamin D₃, endotoxin, and corticosteroids. Expression of LTC₄ synthase, for example, is up-regulated by interleukin-4 and down-regulated by endotoxin.³¹

Sites of Leukotriene Biosynthesis

The locations at which the leukotrienes synthesized are determined by the cellular distribution of the enzymes controlling each stage of the biosynthetic pathway. Because 5-lipoxygenase is only found in cells of myeloid lineage, the synthesis of LTA₄ is limited to these cells.³³ The distribution of 5-lipoxygenase is limited to a specific number of myeloid cells: neutrophils, eosinophils, monocytes, macrophages, mast cells, basophils, and B lymphocytes.³⁴ Among the myeloid cells, considerable variation exists in both the type and quantity of leukotriene secreted. Most of these cells produce appreciable quantities of either LTB₄ or LTC₄ but not both, with the exception of human monocytes and macrophages. All other cells identified as leukotriene secretors have been shown to release either LTB₄ or C₄ almost exclusively. Neutrophils have been shown to synthesize large amounts of LTB₄, possessing 5-LO and LTA₄-hydrolase,³⁵ while eosinophils and mast cells preferentially synthesize LTC₄, according to the presence of 5-LO and LTC₄-synthase within these cells.³⁶

Although nonleukocyte cells generally do not have sufficient 5-lipoxygenase and FLAP to synthesize appreciable amounts of leukotrienes from arachidonate, such cells expressing distal LTA₄-metabolizing enzymes can take up

leukocyte-derived LTA₄ and metabolize it into bioactive leukotrienes, a process that is termed “transcellular biosynthesis”.³¹ Because LTA₄ hydrolase and LTC₄ synthase enzymes are widely distributed among different cell types, not all components of the arachidonic acid cycle must be in the same cellular location for leukotriene biosynthesis to proceed. The secretion of LTA₄ could provide a means for marked amplification of leukotriene production at inflammatory sites. For example, capillary endothelial cells, platelets and vascular smooth muscle cells contain LTC₄ synthase and can generate LTC₄ from neutrophil-derived LTA₄.³⁷ Erythrocytes lack both phospholipase A₂ and 5-lipoxygenase and thus lack the appropriate cellular machinery to produce LTA₄. However, erythrocytes containing LTA₄ hydrolase can use neutrophil-derived LTA₄ to synthesize LTB₄.³⁸ These findings point out to the important role in leukotriene biosynthesis of cells that are devoid of 5-lipoxygenase activity.

Leukotriene Receptors

The classification and nomenclature of LT receptors have been proposed by an ad hoc committee appointed by the International Union of Pharmacology (IUPHAR).³⁹ Leukotrienes act by binding to specific heptahelical receptors of the rhodopsin class that are located on the outer plasma membrane of structural and inflammatory cells. Once ligated by the leukotriene, these receptors interact with G proteins in the cytoplasm, thereby eliciting increases in intracellular calcium and reductions in intracellular cyclic AMP. These proximal signals activate downstream kinase cascades in ways that alter various cellular activities, ranging from motility to transcriptional activation.³¹ The relative synthesis property of various cell to produce LTB₄ and Cys LTs, their receptor expression is summarized in Table 3.

Table 3: Leukotriene Synthesis and Receptor Expression in Leukocyte Subgroups³¹

Types of Cell	Relative Synthetic Capacity			Receptor Expression			
	LTB ₄	Cyst LTs		BLT ₁	BLT ₂	CystLT ₁	Cyst LT ₂
Neutrophil	+++	-	+	+	±		±
Macrophage or monocyte	++	++	+	+	+		+
Eosinophil	-	+++	+	+	+		+
Basophil	-	+++	+	-	+		+
Mast cell	+	+++	+	+	+		+
B lymphocyte	-	-	ND	+	+		ND
CD4 T lymphocyte	-	-	+	+	+		ND
CD8 T lymphocyte	-	-	+	+	ND		ND
Dendritic cell	++	+	+	+	ND		ND
Hematopoic progenitor cell	-	-	ND	+	+		ND

Relative synthetic capacity is expressed by the number of plus (+) signs; a minus sign (-) denotes no or negligible synthetic capacity. Receptor expression is classified as positive (+), negative (-), minimal (±), or not determined (ND).

a. Receptors for Leukotriene B₄:

Two receptors for leukotriene B₄ (LTB₄) have been molecularly identified: BLT₁ and BLT₂. Both receptors are G protein-coupled seven transmembrane domain receptors, are member of the rhodopsin-like receptor superfamily,⁴⁰ whose genes are located in very close proximity to each other in the human and mouse genomes. The two receptors differ in their affinity and specificity for LTB₄: BLT₁ is a high-affinity receptor specific for LTB₄ (K_d 0.15-1 nM), whereas BLT₂ is a low-affinity receptor for LTB₄ (K_d 23 nM) that also binds other eicosanoids. The two receptors also differ in their pattern of expression with BLT₁ being expressed primarily in leukocytes and on neutrophils thereby induces their chemotaxis and adhesion in response to LTB₄,⁴¹ whereas BLT₂ is expressed more ubiquitously. By mediating the activities of LTB₄, these receptors participate both in host immune responses and in the pathogenesis of inflammatory diseases. Reduced disease severity in animal inflammatory models seen with LTB₄ receptor antagonists and in mice with targeted deletion of BLT₁ have revealed important roles for LTB₄ and its receptors in regulating pathologic inflammation.⁴²

b. Receptors for Cysteinyl Leukotrienes:

Two receptors for the Cys-LTs, CysLT₁ receptor and CysLT₂ receptor, have been cloned for the human⁴³ and for mouse.⁴⁴ But a CysLT₃ receptor has been hypothesized as responsible for the effect observed in guinea pig lung.⁴⁵ The cysteinyl leukotriene (CysLT) receptors are putative 7 transmembrane spanning G protein-coupled receptors (GPCRs) of the rhodopsin subfamily of GPCRs.⁴⁶ CysLTs trigger a variety of tissue responses, including increases in vascular permeability. CysLT₁ receptor is expressed on airway smooth muscle, alveolar macrophages, peripheral blood monocytes, eosinophils,⁴⁷ and endothelial cells.⁴⁸ CysLT₂ receptor is expressed on alveolar macrophages, airway smooth muscle, cardiac purkinje cells, adrenal medulla cells, peripheral blood leukocytes, and brain cells.⁴⁴ The expression of CysLT₁ can be influenced at the transcriptional level by type 2 helper T (Th2)-cell type cytokines.³¹

The order of affinities of the CysLTs for the CysLT₁ receptor and CysLT₂ receptor defined with transfected cells is LTD₄ > LTC₄ > LTE₄ LTB₄ and LTD₄ = LTC₄ LTE₄ LTB₄, respectively.⁴⁹ The affinity of LTD₄ for the receptor is about 350 fold higher than that of LTC₄ and 200 fold higher than that of LTE₄. LTD₄ is about 10 fold more potent than LTC₄ in activating the receptor while LTE₄ is a weak agonist.⁵⁰ The CysLT₂ receptor is functionally defined as the receptor

responsible for a CysLT response resistant to CysLT₁ receptor antagonists.²¹

Metabolism of leukotriene

a. Cysteinyl leukotrienes.

Bioconversion of LTC₄ into LTD₄ and LTE₄ does not appear as a catabolic inactivation because LTD₄ is at least as potent as LTC₄ with respect to most biological activities, and LTE₄ appears to be only slightly less potent. Infusion of radio labeled LTC₄ and LTE₄ in normal subjects results in rapid disappearance from the bloodstream, associated with the detection of fractional amounts of LTE₄ in urine during the first 2 hour.⁵¹ Substantial amounts of *omega*- and *beta*-oxidized metabolites of LTE₄ are detected in urine at later times.⁵² Several-fold increase in urinary LTE₄ excretion observed in patients with important liver dysfunction suggests that the liver may represent the site of catabolism of Cys-LTs.⁵³

b. Leukotriene B₄.

On the contrary to what is observed for Cys-LTs, no urinary metabolites of LTB₄ have been identified so far. LTB₄ undergoes rapid metabolism in purified polymorphonuclear leukocytes preparations to give 20-hydroxy LTB₄ and 20-carboxy LTB₄.⁵⁴ This conversion is catalyzed by a specific cytochrome P-450 enzyme, but occurs mainly after release of intact LTB₄ and re-uptake by neighboring cells.⁵⁵ In light of the lower biological activity of the 20-carboxy metabolite, *omega*-oxidation may represent a mechanism to locally inactivate the LTB₄. On the other end, this metabolism is not observed in monocytes, eosinophiles, and macrophages, and there are limited evidences that it may occur *in vivo*.

Biological Activities of Leukotrienes

Cysteinyl Leukotrienes

The sulfidopeptide leukotrienes were first identified as the constituents of the slow-reacting substance of anaphylaxis.²² The sulfidopeptide leukotrienes play a complicated role within the inflammatory process, inducing vasoconstriction, increasing vasopermeability, enhancing mucous secretion, and acting as immunomodulatory agents.^{18,56} LTC₄ and LTD₄ are among the most potent bronchoconstricting agents. LTC₄ and LTD₄ may relaxed pulmonary arteries in guinea pig.⁵⁷ LTE₄ is relatively less potent than LTC₄ and LTD₄ in this respect, but substantial hyper reactivity toward LTE₄ has been observed in asthmatic patients.⁵⁸ In addition Cys-LTs are able to induce mucous secretion from human bronchial mucosa,⁵⁹ that may contribute to the obstruction of airway lumen in asthma. Leukotrienes C₄ and D₄ are potent mucous secretagogues in human bronchial explants *in vitro* and in the trachea of dogs and cats *in vivo*.²²

LTC₄ and LTD₄ cause contraction of both venous and arterial vascular smooth muscle preparation⁶⁰ and Cys-LTs synthesize through neutrophil endothelial cell results in marked coronary vasoconstriction, impaired functions and morphological modifications of isolated rabbit heart preparations.⁶¹ In isolated human pulmonary veins and arteries, LTD₄ induces contraction above basal tone but also nitric oxide-dependent relaxation of vessel previously contracted with noradrenaline.⁶²

The sulfidopeptide leukotrienes are also intimately involved in changes within the vasculature. The increase in the permeability of the venular endothelium that allows proinflammatory cells to migrate to the site of inflammation is thought to be driven by the sulfidopeptide leukotrienes.⁶³ The sulfidopeptide leukotrienes may also modulate the activity of several components of the immune system.²²

In the microcirculation, Cys-LTs promote plasmatic exudation with a direct action on the endothelial cells. Intradermal injection of LTC₄ and LTD₄ induces vasodilation in man but may cause vasoconstriction in the guinea pig;⁶⁴ this latter effect may relate to a secondary production of thromboxane, as observed for the bronchoconstriction both *in vivo* and *in vitro* in this species.⁶⁵

Leukotriene B₄

Leukotriene B₄ is probably the most potent neutrophil chemotactic agent produced by the arachidonic acid cascade. LTB₄ exerts substantially stronger chemokinetic effects on human cells.⁶⁶ Subcutaneous injection of LTB₄ into humans causes neutrophils to accumulate rapidly in the affected tissue.⁶⁷ Leukotriene B₄ may play a crucial role in the induction of neutrophil-endothelial cell adherence.⁶⁸ Leukotriene B₄ induced endothelial cell hyperadhesiveness for neutrophils depends on increased CD11/CD18 expression on the neutrophil surface and possibly a specific domain of the adhesion molecule CD54 found on endothelial cells.⁶⁹

At nanomolar concentrations, LTB₄ causes the release of substantial quantities of glucuronidase and lysozyme from neutrophils, although less effectively than the chemotactic fragment of the complement component C₅. Leukotriene B₄-induced enzyme secretion is mediated by LTB₄ recognition of a surface receptor of substantially lower affinity than that which mediates neutrophil aggregation, adherence to endothelium, and chemotaxis.²²

Microvascular microscopy showed rapid adhesion of leukocytes upon superfusion with LTB₄, followed by progressive diapedesis into extravascular tissues.⁷⁰ In addition to effects on leukocyte adhesion and migration, LTB₄ stimulates secretion of superoxide anion and release of different

granular constituents from leukocytes.⁷¹ Adhesion and migration of leukocytes was accompanied by increased microvascular permeability that was totally leukocyte dependent.

In vitro, LTB₄ stimulates myelopoiesis, a phenomenon that may be linked to the secretion of significant quantities of LTB₄ by human bone marrow cells.²²

LTB₄ and to a lesser extent other lipoxygenase products, is able to induce neutrophil aggregation and degranulation,⁷² and it has been demonstrated that the secretion of azurophilic granules in human polymorphonuclear leukocytes is largely mediated

through an autocrine effect of LTB₄.⁷³

There is complex role of LTB₄ and the two G-protein coupled receptors, BLT-1 and BLT-2, in atherosclerosis. LTB₄ may play a critical role in atherosclerosis. High levels of all components involved in LTB₄ biosynthesis, i.e., 5-lipoxygenase, 5-lipoxygenase activating protein, and LTA₄ hydrolase, were detected in human atherosclerotic lesions.⁷⁴

Leukotriene Release In Inflammation

Leukotrienes are potent lipid bioeffectors and their synthesis is tightly controlled in 5-LO bearing cells. Since their structural characterization, their biological activities suggested a potential involvement of both Cys-LTs and LTB₄ in inflammatory responses.¹⁹ Urinary excretion of LTE₄ has been widely used as an index of systemic production of Cys-LTs and increased urinary LTE₄ has been reported after antigen challenge of atopic asthmatics⁷⁵ and either oral or inhaled aspirin challenge of aspirin-sensitive subjects.⁷⁶

LTC₄ and LTD₄ have been detected in pulmonary lavages from children showing essential pulmonary hypertension.⁷⁷ Elevated levels of LTB₄ and sulfidopeptide leukotrienes have been recovered in the bronchoalveolar lavage fluid of patients with the adult respiratory distress syndrome (ARDS).⁵⁸ Thus, release of LTB₄ may promote the influx of large numbers of neutrophils into the airways and sulfidopeptide leukotrienes may be important mediators of the hypoxemia, permeability pulmonary edema, and reduced pulmonary compliance observed in patients with ARDS.⁷⁷ Much less information is available concerning the involvement of LTB₄ in inflammatory reactions. LTB₄ is generated and released *in vitro* from colonic mucosa obtained from patients with ulcerative colitis or Crohn's disease and *in vivo*.⁷⁷

LTB₄ has also been found to be present in high concentrations in psoriatic scales. An important inflammatory feature of psoriasis is neutrophil infiltration of the epidermal skin lesions. Higher levels of LTB₄ are found in both acute and chronic psoriatic skin lesions than in normal skin.⁷⁸

Sulfidopeptide leukotriene levels are also increased in psoriatic skin lesions; a four fold increase in urinary LTE₄ levels has been found in patients with psoriasis compared with healthy controls.⁷⁹

Elevated levels of LTB₄ and sulfidopeptide leukotrienes are found in the bronchoalveolar lavage fluid²², sputum and urine⁸⁰ of patients with cystic fibrosis disease. Thus, LTB₄ may be important in mediating the neutrophil infiltration of the airways observed in cystic fibrotic patients.

Patients with allergic rhinitis experience both early- and late-phase periods of mucosal inflammation after nasal instillation of a specific allergen such as ragweed pollen; LTC₄ is recovered in their nasal lavage fluids during both the early and late phases of inflammation after allergen challenge.⁸¹

LTB₄ release promoting leukocyte infiltration and degranulation in the glomeruli, which are characteristic features of glomerular immune injury. High basal synthesis of LTB₄ by isolated glomeruli has been observed in rats with cationic bovine gammaglobulin induced glomerulonephritis.⁸² The renal plasma flow and glomerular filtrate rates decrease and the number of glomerular neutrophils increases after the *in vivo* intrarenal infusion of LTB₄ in rats with nephrotoxic serum-induced glomerulonephritis.⁸³

Sulfidopeptide leukotrienes may reduce the glomerular filtration rate in glomerular immune injury by stimulating contraction of mesangial smooth-muscle cells. Urinary LTE₄ levels directly correlate with disease activity in patients with and with a marked increase in LTE₄ levels observed during periods of active disease.⁷⁹

The blood and synovial fluids of patients with rheumatoid arthritis contain higher levels of LTB₄ than those of normal persons.⁸⁴ Synovial fluid levels of LTB₄ and sulfidopeptide leukotrienes are substantially higher in patients with rheumatoid arthritis than in patients with osteoarthritis; synovial fluid levels of leukocytes, immune complexes, and rheumatoid factor directly correlate with LTB₄ levels in patients with rheumatoid arthritis. In inflamed joints, infiltrating neutrophils are the probable source of LTB₄ because the synovial lining cells from patients with rheumatoid arthritis generate little LTB₄.⁷⁹

Human colonic epithelial cells synthesize LTB₄,⁸⁵ which may promote the infiltration by neutrophils of injured colonic mucosa in patients with inflammatory bowel disease. The colonic mucosa of patients with ulcerative colitis and Crohn disease contains substantially elevated levels of LTB₄ compared with similar tissue from normal persons and LTB₄ levels are substantially increased in the rectal dialysate fluid of these patients.⁸⁶

DISEASES THAT HAVE ROLE OF LEUKOTRIENE^{31, 87}

Allergic diseases: Asthma, Allergic rhinitis, Rhino sinusitis, Atopic dermatitis, Urticaria, Allergic fungal sinusitis.

Fibrotic diseases: Airway remodeling in asthma, Bronchiolitis obliterans after lung transplantation, Idiopathic pulmonary fibrosis, Scleroderma, Asbestosis.

Other pulmonary syndromes: Acute lung injury or adult respiratory distress syndrome, Viral bronchiolitis, Obstructive sleep apnea, Chronic obstructive pulmonary disease, Cystic fibrosis and other forms of bronchiectasis, Bronchopulmonary dysplasia.

Other local inflammatory diseases: Arthritis (including osteoarthritis and gout), Glomerulonephritis, Interstitial cystitis, Psoriasis, Inflammatory bowel disease.

Systemic inflammatory diseases: Rheumatoid arthritis, Vasculitides (systemic lupus erythematosus, Churg–Strauss syndrome, Henoch–Schonlein purpura), Transplant rejection.

Cancer: Solid tumors (including melanoma, mesothelioma, and pancreatic, lung, esophageal, prostate, and colon cancers), Leukemias, Lymphomas, etc.

Cardiovascular disease: Atherosclerosis, Aortic aneurysm, Sick cell crisis, Ischemia–reperfusion injury, Pulmonary arterial hypertension, Sepsis.

Anti Leukotriene Therapy

Two basic modes of action are available for inhibition of leukotriene effects: 1) inhibition of synthesis; and 2) antagonism of leukotriene receptors. Many different approaches are available for inhibiting leukotriene synthesis, including antagonism of FLAP, iron chelation, redox-activity, and inhibition of 5-LO active site. Inhibitors of 5-LO have the added advantage of also preventing the synthesis of LTB₄ in addition to that of cysteinyl leukotrienes. Antagonism of leukotriene receptors is mainly achieved by using specific cysteinyl leukotriene receptor antagonists and blocking the actions of cysteinyl leukotrienes.⁸⁸

Leukotriene Synthesis Inhibitors

One design hypothesis was to devise a pharmacophore that would bind the Fe⁺³ atom in the active site of 5-LO and thus block oxidative catalysis. Corey and coworkers first reported hydroxamate containing lipophilic compounds which effectively inhibited 5-LO *in vitro*. Zileuton became the first 5-LO inhibitor to demonstrate anti-LT activity in man and efficacy in the treatment of asthmatics.⁸⁹

Inhibitors of 5-lipoxygenase reactions can act through a number of mechanisms, which include trapping of radical intermediates, chelation or reduction of iron, reversible binding

at an active or a regulatory site, as well as combinations of these mechanisms.⁸⁸

Direct inhibition of 5-LO, partly through an iron-catalysed redox mechanism, has been achieved with compounds such as benzofurans (L-670,630 and L-650,224), hydroxamates (BWA4C), N-hydroxyurea derivatives (A-64077 or zileuton) and indazolinones (ICI 207,968), with good selectivity and potency. Zileuton had similar *in vitro* potency and selectivity to acetohydroxamates and inhibited leukotriene synthesis *ex-vivo*.⁹⁰ Zileuton inhibit airway microvascular leakage and bronchoconstriction induced by inhaled allergen in the sensitized guinea-pig model, in addition to inhibiting leucocyte accumulation.⁸⁸

A new series of non redox 5-lipoxygenase inhibitors, devoid of iron-chelating properties, the methoxyalkylthiazoles, such as ICI D2138, are most potent and selective inhibitors of 5-lipoxygenase.⁸⁸

FLAP Inhibitors

Inhibitors of FLAP such as MK-886 and MK-591 which is a structural analogue of MK-886, have no direct activity on 5-LO but antagonizes FLAP thus preventing the translocation of the enzyme to the membrane.⁹¹ MK886 is a highly selective compound with no effects of prostaglandin synthesis. MK886 inhibits antigen induced bronchoconstriction in *Ascaris*-sensitive squirrel monkeys. MK-591 inhibits LTB₄ synthesis *ex-vivo* by up to 90% and urinary LTE₄ by >80% at 24 hour. Although FLAP antagonists REV5091 and WY50295 were shown to be active *in vitro* and in animals, they were inactive in inhibiting leukotriene synthesis in volunteers. BAY-X-1005 inhibits anti-immunoglobulin E (IgE) challenge in human airways *in vitro*.⁸⁸

One FLAP inhibitor, DG031 is being reformulated for use in phase 3 trials for the prevention of myocardial infarction.³¹

Estimation of the potency of 5-LO inhibitors and FLAP antagonists has been based on inhibition of *ex vivo* LTB₄ production from whole blood leukocytes. Following administration of therapeutic doses, zileuton, BAY x1005, and MK 886 each produced approximately 90% inhibition of *ex vivo* LTB₄ production. Based upon the clinical activity of these agents, this degree of LT inhibition appears sufficient for meaningful efficacy.^{91, 92}

Leukotriene Receptor Antagonists

There are two classes of receptors for leukotrienes, those for the dihydroxy-leukotrienes, LTB₄, termed BLT receptors, and those for cysteinyl leukotrienes, CysLT receptors. Although few synthetic agonists for CysLT-receptors now exist, many antagonists have been produced.

Two broad subgroups of Cys LT-receptors have been recognized, those blocked by known antagonists (CysLT1-receptors) and those that are resistant to blockade (CysLT2-receptors). One recent antagonist appears to have activity both for CysLT1-receptors and CysLT2-receptors.⁸⁷ In human airway smooth muscle, LTC₄, LTD₄ and LTE₄ all activate a CysLT1-receptor, although a subclass of CysLT1-receptor may be activated specifically by LTE₄ alone. In human pulmonary vasculature, a CysLT2-receptor has been identified. CysLT1-receptor is likely to be G-protein coupled, leading to calcium mobilization on activation.^{88, 93}

Early compounds in the development of receptor antagonists were relatively weak in activity. The first leukotriene receptor antagonist of the hydroxyacetophenone class described was FPL-55712, which exhibited poor bioavailability and a short half-life. Other compounds within the same class, *e.g.* LY 171883 (tomelukast), L-649,923, and YM-16638, were synthesized, but did not possess sufficient potency to act effectively as an LTD₄ receptor antagonist. In addition to having no effect on allergen induced responses, L-649,923 was poorly-tolerated, with a high incidence of gastrointestinal effects.^{88, 91}

The newer generation of leukotriene antagonists, such as ICI 204,219 (or Accolate), the quinolones MK-571 and RG-12,525, ONO-1078 (pranlukast) and SK&F 104,353 are more promising. The efficacy and safety of potent leukotriene receptor antagonists against leukotriene-induced bronchoconstriction in normal and asthmatics has been shown in several studies.⁸⁸

Although a great number of leukotriene CysLT1 receptor antagonists have been developed, only three have reached the market, montelukast, pranlukast and zafirlukast. With regard to CysLT2 antagonists, there are only early preclinical candidate substances and it is unknown if the CysLT2 receptor is a relevant target for treatment of asthma.⁸⁹

CONCLUSION

Inflammatory diseases like RA, asthma and COPD are the major health hazards globally. Recently, the leukotrienes have become a great interest of the scientific community which is working in this area. Taken together, the evidences reported above underline the potential role of leukotrienes in the inflammatory responses that characterizes asthma and other pathological conditions. These potent lipid bioeffectors are synthesized during the course of inflammatory reactions and their pharmacological modulation is able to significantly attenuate the clinical manifestations associated with different inflammatory pathologies.

The accumulating evidence that the secretion of leukotrienes may initiate a chain of biochemical events that amplify inflammatory responses possess a challenge for those attempting to devise appropriate pharmacologic interventions because the complex of reactions may have both pathologic and homeostatic consequences. The more specific our knowledge of the biochemical changes, the more likely it is that specific interventions producing more benefit than harm in reducing leukotriene-induced inflammation, vasodilation, and edema will be found.

The development of new leukotriene receptor antagonists or synthesis inhibitors possessing higher potencies and good safety profiles, as well as novel therapeutic approaches to different targets, such as the leukotriene C₄-synthase or nuclear transcription factors of corresponding enzymes, represent an important task that might help to provide a better understanding of the role of these lipids in physiology and pathology.

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