Leukotrienes, Antileukotrienes and Asthma

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Abstract: Leukotrienes (LTs), including LTB_4 and cysteinyl-LTs (CysLTs) (LTC_4, LTD_4, and LTE_4), are potent inflammatory lipid mediators which are derived from 5–lipoxygenase activity. CysLTs, which stimulate $CysLT_1$ and $CysLT_2$ receptor subtypes, are functionally involved in the pathophysiology of asthma. Selective $CysLT_1$ receptor antagonists are effective anti-asthmatic drugs. $CysLT_1$ receptor antagonists have been developed from leukotriene structural analogs, analogs of FPL 55712, a chromone carboxylic acid, and by random screening of corporate compound banks. This review will examine the biosynthesis, metabolism and mechanism of action of leukotrienes, their role in asthma, the therapeutic implications of the leukotriene pathway inhibition for asthma, and the medicinal chemistry strategies that have been exploited in the design of potent and selective $CysLT_1$ receptor antagonists.

Key Words: Leukotrienes, asthma, leukotriene receptor antagonists, airway inflammation, pharmacological therapy.

INTRODUCTION

Leukotrienes (LTs), including LTB_4 and cysteinyl-LTs (CysLTs) (LTC_4, LTD_4, and LTE_4), are potent inflammatory lipid mediators which are derived from 5–lipoxygenase (5-LO) activity [1]. Specific pathways for the synthesis of LTs from arachidonic acid are present in several types of leukocytes in the airways and become activated during allergic airway inflammation [1,2]; however, other cell types like platelets and endothelial cells which lack 5-LO activity, can produce LTs by taking up the intermediate LTA_4 synthesized and released by leukocytes and metabolizing it into bioactive LTs, a process known as transcellular biosynthesis [2].

CysLTs induce pathophysiological responses similar to those associated with asthma such as bronchoconstriction, vasodilatation and increased microvascular permeability, increased mucus secretion, decreased mucociliary clearance, eosinophil migration, and increased eosinophil survival [1,3-5]. Elevated CysLT concentrations have been detected in broncho-alveolar lavage fluid [6], sputum [7], and exhaled breath condensate (EBC) in patients with asthma [8]. The CysLTs play a pivotal role in airway remodeling which characterizes persistent asthma [5].

Two G-protein coupled receptor subtypes for CysLTs ($CysLT_1$ and $CysLT_2$) have been identified [9-12] and pharmacological evidence suggests the presence of a third receptor subtype [13]. The effects of CysLTs that are relevant to the pathophysiology of asthma are mostly mediated through activation of the $CysLT_1$ receptor which is expressed in several airway inflammatory and structural cells [1,5].

The most convincing evidence for a causative role of Cys-LTs in asthma comes from the clinical effectiveness of $CysLT_1$ receptor antagonists (e.g., montelukast, zafirlukast, pranlukast) and 5-LO inhibitors (e.g., zileuton) in patients with asthma [3]. These drugs are effective in preventing asthmatic responses induced by allergen-challenge [14], exercise and cold-air hyperventilation [15,16], and aspirin [17]. Moreover, $CysLT_1$ receptor antagonists have a therapeutic role in chronic stable asthma as they improve pulmonary function, symptoms and quality of life, and reduce β-agonist use, eosinophilia, asthma exacerbations, and the required dose of inhaled glucocorticoids in patients with asthma [18-20].

$CysLT_1$ receptor antagonists have significant anti-airway remodeling effects in an animal model of asthma [21] and inhibitory effects on myofibroblasts in patients with a asthma [22].

LTB_4, a potent chemoattractant for neutrophils, is functionally involved in severe asthma and asthma exacerbations [5], but its role in less severe chronic stable asthma is less known. Elevated LTB_4 concentrations in EBC have been reported in adults and children with stable asthma [23,24]. LTB_4 could be also functionally involved in airway hyperresponsiveness (AHR) [1]. This review will examine the biosynthesis, metabolism and mechanism of action of LTs, their role in asthma and the therapeutic implications of the LT pathway inhibition for asthma.

BIOSYNTHESIS OF LEUKOTRIENES

LTs are conjugated triene metabolites containing a linear-20 carbon chain that are formed from esterified arachidonic acid on plasma membrane phospholipids derived through the 5-LO activity (Fig. **1**) [25]. Arachidonic acid is cleaved by the action of different phopsholipase A_2 (PLA_2) enzymes, particularly cytosolic $PLA_2\alpha$ [26]. 5-LO is located in the nucleoplasm or the cytoplasm in resting leukocytes and move to the external on internal nuclear membrane upon cell activation [5]. Apart from leukocytes [1,4,5], 5-LO is also expressed in hemopoietic stem cells [27] and fibroblasts [28]. Interaction of arachidonic acid with 5-LO produces LTA_4 [5(*S*)5,6-oxido-7,9,11,14-(*E,E,Z,Z*)-eicosatetraenoic acid] through two enzymatic steps: abstraction of a hydrogen atom from C-7 of arachidonic acid followed by the addition of

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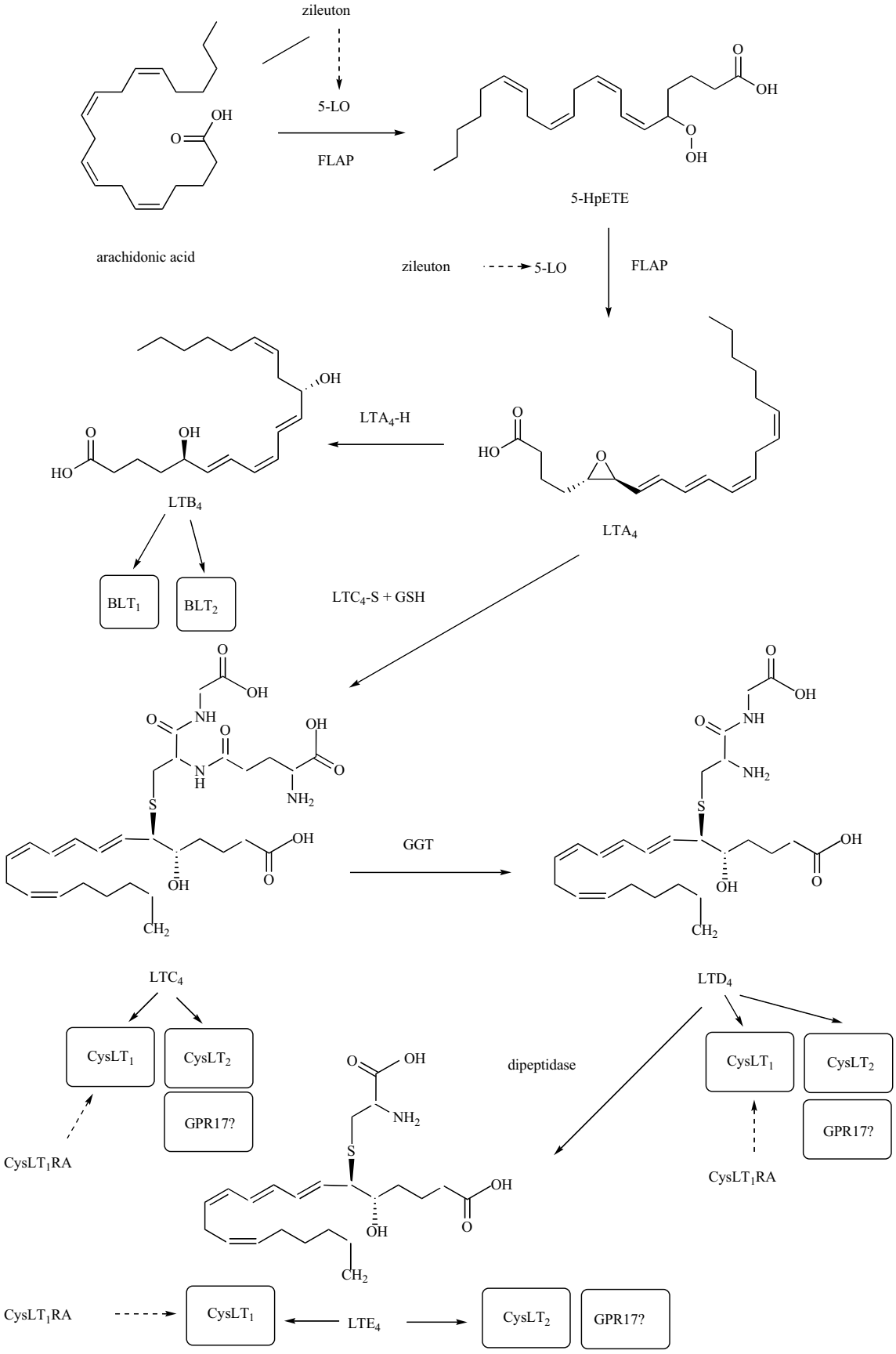

Fig. (1). Biosynthesis of leukotrienes [2,5,25], leukotriene receptors [9,11,13], and mechanisms of antileukotriene drugs [1,3-5].

molecular oxygen to form 5-hydroperoxyeicosatetraenoic acid (5-HPETE); removal of a hydrogen atom from C-10 with formation of the epoxide conjugated triene LTA_4 (Fig. **1**) [25]. LTA_4 is a chemically reactive intermediate which is subsequently metabolized by LTA_4 hydrolase (LTA_4-H) into LTB_4 [5(*S*)12(*R*)-dihydroxy-6,8,10,14-(*Z,E,E,Z*)-eicosatetraenoic acid] and by LTC_4 synthase (LTC_4-S), or different members of the membrane-associated proteins in eicosanoid and glutathione metabolism superfamily such as microsomal glutathione-S-transferase 2 (MGST2) [2], into LTC_4 [5(*S*)-hydroxy-6(*R*)-S-glutathyionyl-7,9,11,14-(*E,E,Z,Z*)-eicosatetraenoic acid] [25]. LTC_4-S is an integral membrane protein that is present at the nuclear envelope, whereas LTA_4-H is cytosolic [5,25]. In the LTA_4-H-catalyzed reaction, a molecule of water is added to C-12 of LTA_4 to form LTB_4, whereas in the LTC_4-S-catalyzed reaction the chemical structure of LTA_4 is opened at C-6 by the thiol anion of glutathione to form LTC_4 [25]. Biosynthesis of LTs requires cellular activation such as IgE receptor cross-binding on mast cell surface and involves $cPLA_2\alpha$ and five-lipoxygenase activating protein (FLAP) which assemble at the perinuclear region before production of LTA_4 [25]. After their intracellular formation, CysLTs and LTB_4 are released to the extracellular space through specific carrier-proteins that are potential targets for future antileukotriene drugs [1]. In the extracellular space, cleavage of the γ-glutamic acid amide peptide bond of LTC_4 by a γ-glutamyl transpeptidase (GGT) results in the formation of LTD_4 (Fig. **1**) [25]. GGT is a heterodimeric glycosylated enzyme located on the external side of the cell plasma membrane [29]; LTD_4 is then metabolized into LTE_4 by dipeptidases with hydrolysis of the Cys-Gly amide bond [25]. At least two membrane-bound dipeptidases (MBD-1 and MBD-2) have a role in the metabolism of LTD_4 [25]. LTC_4, LTD_4 and LTE_4, are designated CysLTs due to the common cysteine in the side chain. The regulation of leukotriene production occurs at various levels, including expression of 5-LO, translocation of 5-LO to the perinuclear region and phosphorylation to either enhance or inhibit the activity of 5-LO [5,25]. Cells which do not express 5-LO, including platelets, red blood cells, endothelial cells and epithelial cells, can also produce CysLTs and/or LTB_4 through the transcellular metabolism of LTA_4 synthesized and released by activated neutrophils [2].

METABOLISM OF LEUKOTRIENES

LTB_4 is metabolized by ω-oxidation carried out by specific cytochrome P450s (CYP4F3A) in the human neutrophil to form 20-hydroxy-LTB_4 (20-OH-LTB_4) that can be oxidized further either by CYP4F3A or by alcohol dehydrogenase and aldehyde dehydrogenase to form 20-carboxy-LTB_4 (20-COOH-LTB_4) [25]. After CoA ester formation, 20-carboxy-LTB_4 can undergo β-oxidation leading to 18-carboxy-dinor-LTB_4 and 14,15-dihydro-17,18,19,20-tetranor-LTB_3 which have been found as urinary metabolites [25]. Other specific pathways of LTB_4 metabolism include the 12-hydroxydehydrogenase/15-oxo-prostaglandin-13-reductase that forms a series of conjugated diene metabolites that are excreted into urine [25].

Methyl terminal ω-oxidation of LTE_4 is a major metabolic process [30] and may be followed by sequential β-oxidation reactions within the peroxisome [31].

Genetic variation in 5-LO, FLAP, LTA_4-H and LTC_4-S affects the response to antileukotrienes [32].

MECHANISM OF ACTION OF LEUKOTRIENES

CysLTs activate two receptor subtypes ($CysLT_1$ and $CysLT_2$) belonging to the class of the G protein-coupled receptors (GPCRs) [9,11]. A third receptor subtype that responds to both CysLTs and uracil nucleotides has been identified and described as GPCR17 [13]. $CysLT_1$ and $CysLT_2$ receptor activation involves interaction with the Gq and/or Gi class of G proteins, resulting in modulation of intracellular calcium and/or cAMP levels [5,9,12,33]. Activation of the $CysLT_1$ receptor account for most of the effects of CysLTs which are relevant to the pathophysiology of asthma [1,5]. This receptor subtype is expressed in monocytes and macrophages, eosinophils, basophils, mast cells, neutrophils, T cells, B lymphocytes, pluripotent hematopoietic stem cells (CD34+), interstitial cells of the nasal mucosa, airway smooth muscle cells, bronchial fibroblasts and vascular endothelial cells (Table **1**) [4,5,9,10,28]. $CysLT_2$ receptor is expressed in human peripheral basophils [34], endothelial cells [35], and cultured mast cells (Table **1**) [11]. Although its role in the pathophysiology of asthma is largely unknown [35], $CysLT_2$ receptor might be involved in asthma exacerbations as $CysLT_2$ activation induces IL-8 generation by human cultured mast cells, potentially leading to neutrophilic inflammation [11], which characterizes acute and severe asthma. Interestingly, this effect is calcium-insensitive, indicating the possibility of other activation pathways [11]. $CysLT_2$ receptor expression on eosinophils is increased in patient with asthma exacerbation, and is upregulated by interferon-γ in asthmatic patients [36]. In human vascular endothelial cells, interferon-γ induces $CysLT_2$ receptor expression and enhances CysLT-induced inflammatory responses [37]. In human monocytes, $CysLT_1$ and $CysLT_2$ receptor expression is increased by CysLTs themselves [38].

LTB_4 act by binding to two receptor subtypes, the high affinity BLT_1 receptor and the low affinity and BLT_2 receptor [39], which are expressed in a human mast cell line (HMC-1) [39] and bone marrow-derived dendritic cells [40]. BLT_1 receptors, which are GPCRs, are also expressed in human bronchial fibroblasts [28], human neutrophils [41] and alveolar macrophages [42], and in a subset of effector memory interleukin (IL)-13-producing CD8+ T cells in bronchoalveolar lavage fluid of asthmatic patients (Table **1**) [43]. Activation of BLT_1 receptor is required for mast cell-dependent AHR induced by effector CD8+ T cells in mice [43], indicating a possible role for LTB_4 in AHR.

BIOLOGICAL EFFECTS OF LEUKOTRIENES IN THE AIRWAYS

The biological effects of CysLTs are relevant for the pathophysiology of asthma (Table **2**) [1,4,5]. CysLTs are the most potent endogenous bronchoconstrictors known. Asthmatic patients are hyperresponsive to LTC_4, LTD_4 and LTE_4 inhalation [1]. CysLTs increase microvascular permeability in the lungs in experimental animals and mucus secretion in isolated animal and human airways [1]. Inhalation of CysLTs in asthmatic patients induces recruitment of eosinophils into the airway mucosa and increases the number of sputum eosi-

Table 1. Expression of Leukotriene Receptor Subtypes* in Different Cell Types

	$CysLT_1$	$CysLT_2$	BLT_1	BLT_2
Eosinophils	+	+	+	+
Basophils	+	+	+	-
Neutrophils	+	-	+	+
Mast cells	+	+	+	+
Macrophages	+	-	+	+
Monocytes	+	-	+	+
T lymphocytes	+	-	+	-
B lymphocytes	+	-	-	+
Smooth muscle cells	+	-	-	-
Dendritic cells	+	-	+	+
Fibroblasts	+	-	+	-
Hematopoietic stem cells	+	+	-	-
Endothelial cells	+	+	+	+

*A third receptor subtype that responds to both CysLTs and uracil nucleotides has been identified and described as G protein-coupled receptor 17 (GPCR17). +, presence of receptor subtype; -, absence or lack of evidence of receptor subtype.

nophils [44]. However, the mechanism(s) of the eosinophil chemotactic effect induced by CysLTs has not been established.

Table 2. Biological Effects of Leukotrienes in the Airways

Leukotriene	Effects
Cysteinyl-leukotrienes	airway smooth muscle contraction and hyperplasia
	increased AHR
	eosinophil chemotaxis, activation and decreased apoptosis
	increased T cell recruitment and Th2 responses
	myofibroblast accumulation
	plasma leak
	increased mucus secretion
	increased collagen deposition
LTB_4	neutrophil chemotaxis
	increased AHR
	plasma leak
	increased mucus secretion
	increased T cell recruitment and Th2 responses
	increased mast cell recruitment

AHR: airway hyperesponsiveness; LT: leukotriene

Apart from their local effect in the airways, CysLTs are multifunctional mediators with a central role in the inflammatory process that characterizes asthma [3,45]. CysLTs: prime progenitor cells to differentiate into mature blood cells and modulate leukopoiesis induced by granulocyte-macrophage-colony-stimulating factor, IL-5 and IL-3; promote leukocyte migration from the bone marrow into the circulatory system; increase chemotaxis of eosinophils, their cellular adhesion and transendothelial migration into the airways; increase eosinophil survival in response to mediators released from mast cells and lymphocytes; and activate eosinophils, monocytes, basophils, mast cells and T lymphocytes [3,45].

Allergen-induced lung inflammation is reduced in LTC_4 synthase-null mice [43], indicating that CysLTs regulate the Th2 cell-dependent inflammatory response [46], which has a central role in the pathophysiology of asthma.

CysLTs contribute to airway remodeling, which includes the eosinophil cell inflammatory response, mucus gland hyperplasia, mucus hypersecretion, airway smooth muscle cell hyperplasia and migration, and collagen deposition beneath the epithelial layer and in the lung interstitium at sites of leukocyte infiltration [5]. Montelukast reduces allergen-induced lung inflammation and fibrosis in an animal model of the airway remodeling changes observed in patients with persistent asthma [21].

LTB_4 has no direct bronchoconstrictor effect in healthy or asthmatic subjects and the lack of effect of LTB_4 receptor antagonists in allergen-induced early or late phase airway obstruction in patients with asthma [47] argues against an important role for LTB_4 in acute bronchoconstriction. However, LTB_4 might contribute to airway narrowing, producing

local edema and increasing mucus secretion in asthma (Table **2**). As a potent chemoattractant for neutrophils, LTB_4 might be functionally involved in the neutrophilic inflammatory process that characterizes severe asthma or asthma exacerbations [5], but its pathophysiological role in intermittent and mild-to-moderate persistent asthma is less clear. LTB_4 may have a role in AHR [1]. By activating BLT_1 receptors on a subset of effector CD8+ T cells, LTB_4 triggers allergic responses in the lungs of mice [43]. The absence or antagonism of BLT_1 receptors on these cells markedly reduces AHR and airway inflammation induced by allergen challenge [43]. CD8+/BLT_1+ T cells expressing BLT_1 receptors have been identified in BAL fluid and lung tissue from patients with asthma but not from healthy subjects [43]. Patients with glucocorticoid-resistant asthma have higher number of this subset of CD8+ T cells compared with patients with glucocorticoid-sensitive asthma [43], but the biological significance of LTB_4-induced activation of effector CD8+ T cells in asthmatic patients needs to be established. A role for LTB_4 in AHR in asthma is also indicated by the fact that 5-LO inhibitors, which decrease both CysLT and LTB_4 production, significantly reduce AHR in asthmatic patients [48,49] concomitant with a reduction in ex vivo LTB_4 production [49], whereas selective $CysLT_1$ antagonists have a modest effect [3,50].

5-LO inhibition improves nasal function in aspirin-sensitive asthma (ASA) patients at baseline [50], whereas $CysLT_1$ receptor antagonists, which significantly reduce bronchospastic reactions, have only minor effects on ASA-induced upper airway reactions, indicating a central role for LTB_4 in nasal symptoms [51].

LEUKOTRIENES IN BIOLOGICAL FLUIDS IN ASTHMA

LTs have been measured in the urine [52], sputum [7], BAL fluid [6], and EBC [8,23] in asthmatic patients. LTE_4 is excreted into urine as a major CysLT metabolite accounting for about 4% of total LTC_4 synthesis in healthy subjects [53]. Urinary measurement of LTE_4 is generally used for assessing the systemic synthesis of CysLTs as circulating LT concentrations are below the detection limit of most assays [52]. No or small differences have generally been reported in urinary LTE_4 levels between atopic asthmatic patients and healthy subjects under basal conditions [52]. However, urinary LTE_4 excretion is elevated after allergen challenge in atopic asthmatics [1,52], in aspirin-sensitive asthmatics under basal conditions [54], in patients with nocturnal asthma [6], during asthma exacerbations [55], and in severe asthma [56]. Urinary metabolites of LTB_4 are excreted in low amounts in healthy subjects [57]. Using high performace liquid chromatography purification followed by an enzyme immunoassay, urinary LTB_4-glucuronide concentrations were detected in healthy subjects and found elevated in patients with aspirin-tolerant atopic asthma [58]. Urinary LTB_4-glucuronide levels were further increased in patients with ASA after intravenous aspirin challenge [58], but not in patients with aspirin-tolerant atopic asthma after allergen challenge [59]. Measurement of LTs in BAL fluid, sputum and EBC is more likely to reflect pulmonary synthesis of LTs. Sputum CysLT concentrations are elevated in patients with asthma, reflecting disease severity [7] and LT concentrations are increased in BAL fluid in patients with nocturnal asthma [6]. Several studies reported increased LT levels in EBC in both adults and children with asthma [8,23,24,60-62], but the methodology is new and further studies are required [61].

LEUKOTRIENE RECEPTOR ANTAGONISTS: DRUG DESIGN AND STRUCTURE-ACTIVITY RELATIONSHIPS

Medicinal chemistry strategies that have been exploited in the design of potent and selective $CysLT_1$ receptor antagonists include leukotriene structural analogs, analogs of FPL 55712, a chromone carboxylic acid, and random screening of corporate compound banks [63]. FPL 55712 is a prototype antagonist of slow-reacting substance of anaphylaxis that was then identified as a mixture of LTC_4, LTD_4 and LTE_4.

Early analysis of the structure of LTD_4 and FPL 55712, based on the hypothesis that the hydroxyacetophenone region of FPL 55712 was mimicking the olefinic region of LTD_4 and the chromone carboxylic acid segment of FPL 55712 was mimicking either the backbone C1-C5 carboxylic acid region or the peptidic component, led to the synthesis of an aliphatic acid structure that exhibited slight CysLT antagonist activity [63]. Compared to FPL 55712, four-fold increase in potency and 2.5-fold increase in selectivity was obtained by incorporating an aryl group into the structure and by the addition of a methoxy group to the aryl moiety [64]. The replacement of a carboxylic acid group with a biomimetic tetrazole results in increased potency, as shown by tomelukast, a compound that was withdrawn from clinical study for safety reasons [65]. Random screening of compounds banks led to the discovery of pranlukast, the first CysLT receptor antagonist approved for marketing, which is sold in Japan. Pranlukast derives from a series of carboxylic acids (Fig. **2**) [66]. Restricting conformational flexibility by incorporation of the chromone carboxylic acid structure of FPL 55712 resulted in 90-fold increase in potency. Replacement of the carboxylic acid with tetrazole improved bioavailability and further increased potency. Optimization of the lipid backbone led to pranlukast (Fig. **2**) [66].

Another strategy for identifying CysLT receptor antagonists is based on changes of leukotriene structure. This strategy, that led to the development of montelukast, requires replacing the highly unstable triene-containing chain and converting CysLT agonist activity into antagonist activity [63]. Montelukast was identified starting from a quinoline-containing structure that mimick the olefin backbone of CysLTs (Fig. **3**) [67]. Addition of a dithioacetal linkage led to a compound with increased *in vitro* potency, but low oral bioavailability. One of the carboxylic acids was then replaced by an amide group resulting in greater potency and high oral bioavailability. The enantiomers were resolved to yield verlukast, a compound which was withdrawn because of toxicity. Further structure-activity relationship analysis led to the development of montelukast, a well tolerated $CysLT_1$ receptor antagonist (Fig. **3**).

Zafirlukast was developed from lipid acid analogs that incorporated structural components from both FPL 55712 and leukotrienes [63, 64]. The starting point in the identifica-

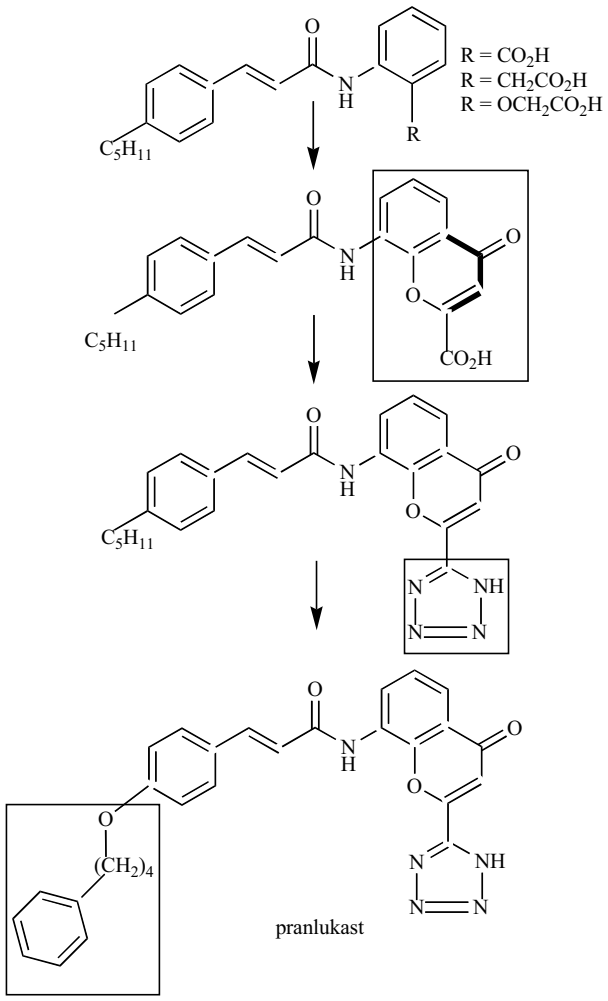

Fig. (2). Development of pranlukast from a series of carboxylic acids [63,66].

tion of zafirlukast was an indole-containing structure (Fig. **4**). This molecule was modified in the lipid-like tail, the acidic head region, and the indole-backbone (Fig. **4**). Substitution with carbonyl-containing groups in the lipid-like tails results in increased selectivity, potency and oral efficacy in animal models [63]. Replacement of the carboxylic acid head group with phenylsulfonimide results in a further increase in potency of 100-fold. Changes in the indole group led to a potent and selective $CysLT_1$ receptor antagonist, although oral bioavailability was lower than 1%. Incorporation of an inverted indole template and substitution with a 2-methyl group in the sulfonamide structure considerably increased oral bioavailability leading to zafirlukast (Fig. **4**) [63].

Chloroquinolylvinylphenyl derivatives based on the molecular structures of the thromboxane A_2 antagonist daltroban and the $CysLT_1$ receptor antagonist montelukast are under development. These compounds are orally active and show potent TxA_2 and LTD_4 antagonist activity [68].

ANTILEUKOTRIENES IN ASTHMA

Anti-LTs that have been approved for asthma include $CysLT_1$ receptor antagonists (montelukast, zafirlukast and pranlukast) and zileuton, a 5-LO inhibitor. Montelukast is the most prescribed $CysLT_1$ receptor antagonist in Europe and the USA, whereas pranlukast is only marketed in Japan and other Asian countries. Montelukast and pranlukast are also approved for allergic rhinitis [1]. Zafirlukast was the first anti-LT to be approved in Europe, but, unlike montelukast, has possible food and drug interactions, and requires twice daily administration [1]. The fact that $CysLT_1$ receptor antagonists and 5-LO inhibitors have similar efficacy indicates that most of the antiasthmatic effects of anti-LTs are due to $CysLT_1$ antagonism [1]. At present, the use of zileuton, which is commercially available in the USA, is limited by its hepatotoxic effects and four daily administration regimen required by its short half-life [1]. However, a twice-daily controlled-release formulation of zileuton has recently been approved by the Food and Drug Administration (FDA) [5]. Despite their disadvantages, 5-LO inhibitors might be useful for reducing AHR in asthmatic patients [48,49], which is slightly affected by $CysLT_1$ antagonists [3] and for treating rhinitis and rhinopolyposis [48].

$CysLT_1$ receptor antagonists improve lung function and symptoms, and reduce the use of rescue bronchodilators, exacerbation rate, and airway and blood eosinophilia in adults and children with asthma [1-5]. $CysLT_1$ receptor antagonists rapidly improve asthma control, although the efficacy of their group mean effects after a few weeks of therapy is lower than that observed with inhaled glucocorticoids [18]. Intravenous montelukast can be effectively added to standard therapy in adults with acute asthma, suggesting a possible indication for $CysLT_1$ receptor antagonists in severe asthma exacerbations [69]. $CysLT_1$ receptor antagonists inhibit early and late asthmatic responses induced by allergen challenge [14,70]. In contrast to budesonide, montelukast reduces the maximal early asthmatic response, whereas both drugs are effective on the late asthmatic response [14]. $CysLT_1$ receptor antagonists are also effective in allergen-induced asthma in children [71]. However, inhaled glucocorticoids are more effective than $CysLT_1$ receptor antagonists in reducing allergen-induced AHR [14]. Treatment with montelukast protects against exercise-induced bronchoconstriction over a 12-week period in asthmatic adults [15]. Reduction in exercise-induced bronchoconstriction is observed as soon as two hours after a single oral dose of montelukast (10 mg) and persist up to 24 hours [72]. Montelukast is more effective and tolerated than salmeterol in the chronic treatment of exercise-induced bronchoconstriction over a period of eight weeks in adults with mild asthma [73]. Likewise, $CysLT_1$ receptor antagonists are effective in exercise-induced bronchoconstriction in children [74]. $CysLT_1$ receptor antagonists and 5-LO inhibitors are particularly effective in patients with ASA as they prevent the fall in FEV_1 in response to aspirin challenge [1] and improve asthma control over and above the therapeutic response to glucocorticoids [17].

The following aspects of the clinical pharmacology of $CysLT_1$ receptor antagonists should be pointed out: their role as monotherapy in asthmatic patients; their efficacy as add-on therapy; the possibility to reduce the dose of inhaled glucocorticoids in combination therapy; the variability in their therapeutic response; their potential effect on airway remodeling; their safety.

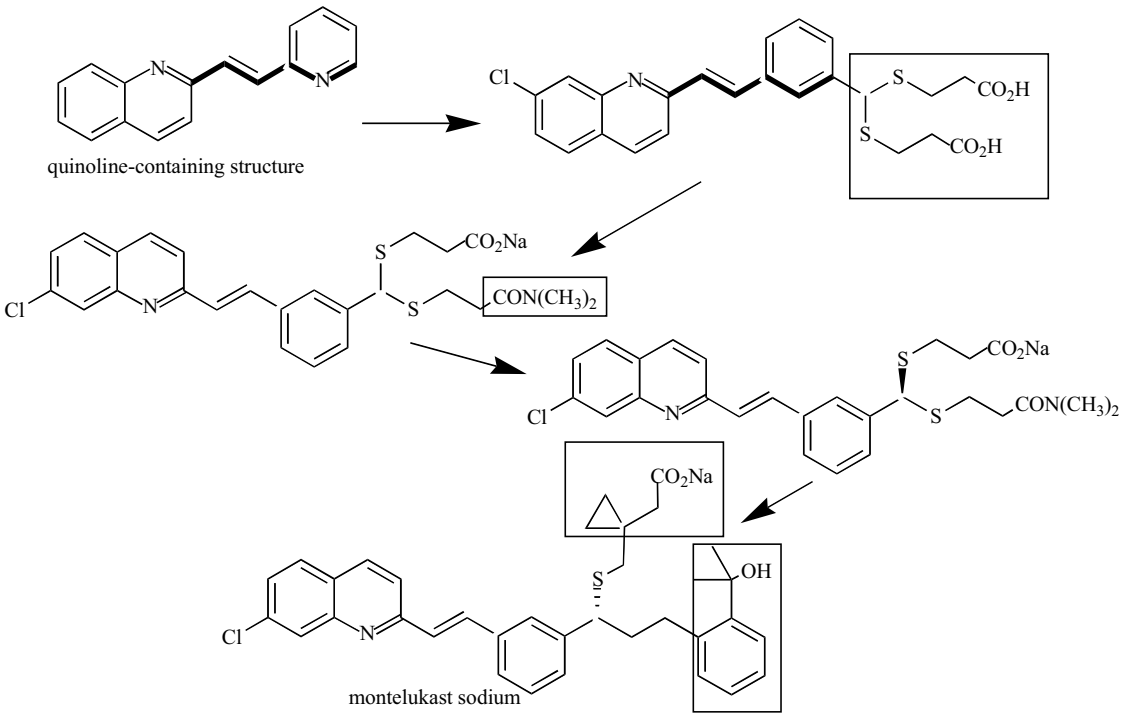

Fig. (3). Development of montelukast sodium from a quinoline-containing structure [63,67].

Although they are less effective than inhaled glucocorticoids as first-line agents [75], $CysLT_1$ receptor antagonists can be used as monotherapy in patients with mild persistent asthma [76].

$CysLT_1$ receptor antagonists are indicated for preventing exercise-induced bronchoconstriction and as add-on therapy in asthmatic patients not sufficiently controlled by inhaled glucocorticoids alone [1]. In these patients, the addition of montelukast to a constant dose of inhaled budesonide improves asthma control [77], with a similar efficacy of doubling the dose of budesonide [18]. Combination therapy might reduce the risk of side effects due to long-term administration of high-dose inhaled glucocorticoids [18]. In asthmatic patients who are not adequately controlled with inhaled fluticasone, the addition of montelukast is effective [78], although add-on therapy with a long-acting β_2-agonist is generally superior to add-on therapy with a $CysLT_1$ receptor antagonist [79].

Add-on therapy with $CysLT_1$ receptor antagonists enables a reduction in the dose of inhaled glucocorticoids required to control asthma [18,80]. The rationale for combining inhaled glucocorticoids and $CysLT_1$ receptor antagonists in asthmatic patients is based on the relative steroid resistance of the LT pathway [81]. AHR to LTD_4, and urinary LTE_4 concentrations in adults with mild asthma are not affected by inhaled fluticasone (500 μg b.i.d. for two weeks) [81], whereas after treatment with inhaled fluticasone (100 μg b.i.d. for four weeks), there is a reduction of 18% in LTE_4 concentrations in EBC in children with intermittent and mild persistent asthma [60], indicating that neither the biosynthesis nor the actions of LTs seem to be sensitive to inhaled glucocorticoids [81]. There is variability in the therapeutic response to LT receptor antagonists in both adults and children with asthma [20,75]. Identification of responders to LT receptor antagonists might have important clinical implications, given the importance of considering an individualized approach to asthma management and assessment rather than a strategy directed to the best outcome in a group of patients [75]. Some phenotypic characteristics, including younger age, shorter disease duration [75] and, possibly, elevated LTE_4 levels in EBC [8], are associated with a favorable response to montelukast. Identification of biomolecule profiles in biological fluids and studies on genetic polymorphisms of 5-LO cascade and CysLT receptors could help to predict the therapeutic response to antileukotrienes.

In an animal model of asthma, montelukast not only prevent allergen-induced airway changes, but also reverse structural changes such as airway smooth muscle cell layer thickening and subepithelial fibrosis, which are resistant to glucocorticoids [21]. These findings could contribute to a better understanding of the pathophysiology of airway remodeling and be relevant for the management of asthmatic patients as they might clarify the role of $CysLT_1$ receptor antagonists in asthma therapy. A reduction in basal membrane thickening [82] and subepithelial collagen deposition [83] has also been reported with inhaled glucocorticoids, although these effects seem to have little impact on the clinical evolution of asthma [84]. Treatment with montelukast at a dose of 10 mg once daily for eight weeks reduces myofibroblast accumulation in the airways of asthmatic subjects following low-dose allergen challenge [22]. However, clinical studies in patients with asthma are required to determine the preventive effect on airway remodeling and/or the reversal of established airway structural changes by $CysLT_1$ receptor antagonists.

LT receptor antagonists are well tolerated, with headache and gastric discomfort being the most common side effects

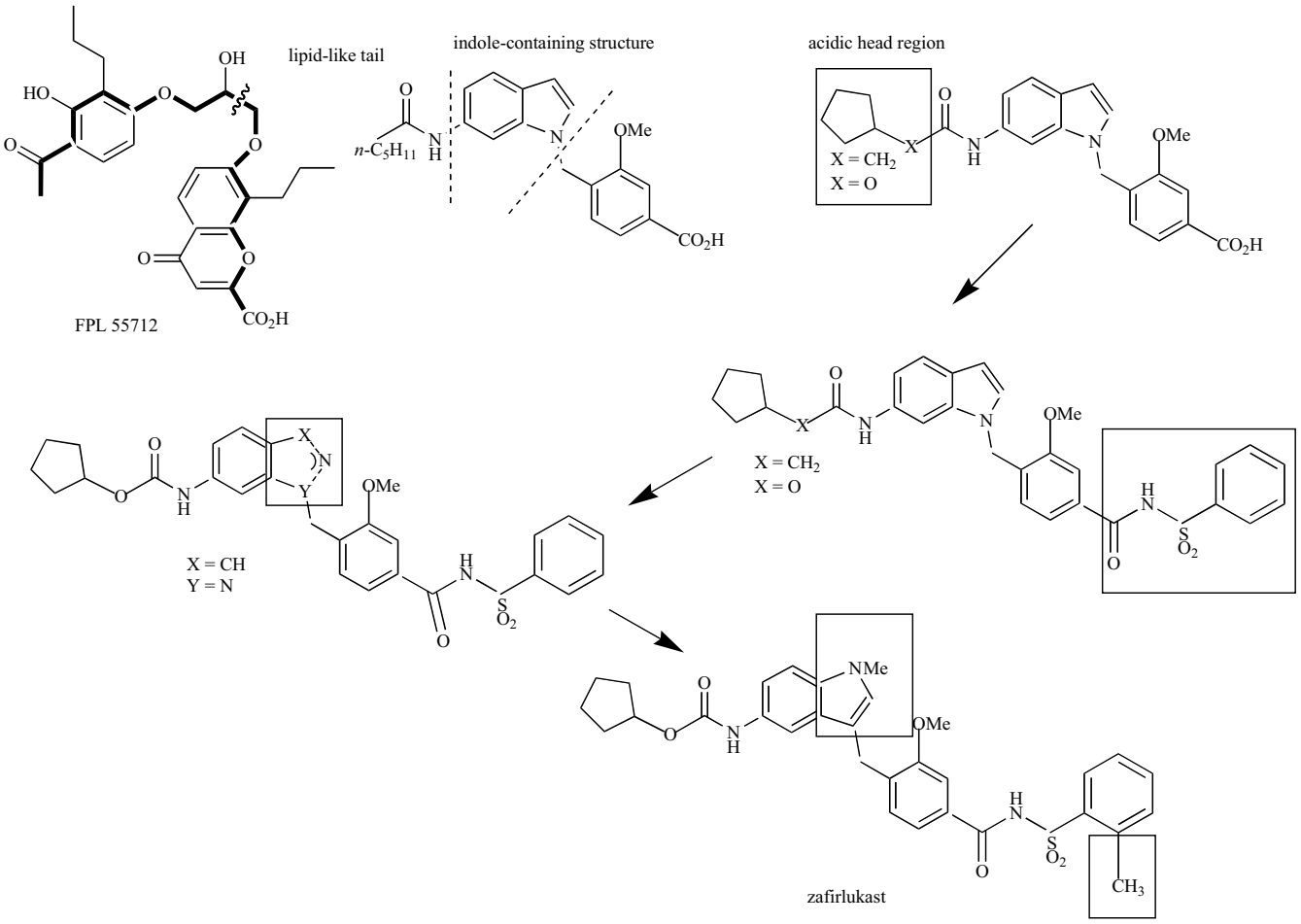

Fig. (4). Development of zafirlukast from lipid acid analogs derived from both FPL 55712 and leukotrienes. The starting point in the identification of zafirlukast was an indole-containing structure [63, 64].

[1]. An association between treatment with $CysLT_1$ receptor antagonists and Churg–Strauss syndrome has been reported, although a causative link has been excluded [1]. By antagonizing the effects of relevant pathophysiological mediators in the airways, oral administration of antileukotrienes provides a single therapeutic approach to asthma and allergic rhinitis, potentially increasing the efficacy and tolerability compared with increasing the dose of inhaled glucocorticoids alone [18]. The fact that a combination of oral montelukast and inhaled budesonide is more effective than doubling the dose of inhaled budesonide alone in asthmatic patients with concomitant allergic rhinitis supports the validity of this therapeutic strategy [85].

CONCLUSIONS

Most of our knowledge of the pathophysiological role of LTs in asthma is currently limited to $CysLT_1$ receptor-mediated effects, whereas the role of the $CysLT_2$ receptor has not yet been established. $CysLT_1$ receptor antagonists provide a therapeutic alternative to inhaled glucocorticoids in patients with mild persistent asthma. In combination with inhaled glucocorticoids, $CysLT_1$ receptor antagonists improve asthma control and enable steroid tapering while maintaining similar asthma control.

Identifying those subjects who are more likely to respond to $CysLT_1$ receptor antagonists might be relevant for a more rational therapy of patients with asthma. The potential effect of $CysLT_1$ receptor antagonists in preventing and reversing airway remodeling, as well as the role of LTB_4 in asthma, requires further study.

ACKOWLEDGEMENTS

This work was supported by Catholic University of the Sacred Heart, academic grant 2005-2007.

Abbreviations

AHR	=	Airway hyperresponsiveness
ASA	=	Aspirin-sensitive asthma
BAL	=	Bronchoalveolar lavage
CysLTs	=	Cysteinyl-LTs
EBC	=	Exhaled breath condensate
FLAP	=	Five-lipoxygenase activating protein
GGT	=	γ-glutamyl transpeptidase
GPCR	=	G protein-coupled receptor

GSH = Reduced glutathione

5-HPETE = 5-hydroperoxyeicosatetraenoic acid

IL = Interleukin

HMC = Human mast cell

LT = Leukotriene

LTA_4-H = LTA_4 hydrolase

LTC_4-S = LTC_4 synthase

LO = Lipoxygenase

MBD = Membrane-bound dipeptidases

MGST = Microsomal glutathione-S-transferase

PLA_2 = Phopsholipase A_2

Tx = Thromboxane

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Received: 29 November, 2007 Revised: 25 February, 2008 Accepted: 26 February, 2008