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REVIEW

Leukotriene receptor antagonists and biosynthesis inhibitors: potential breakthrough in asthma therapy

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Leukotriene receptor antagonists and biosynthesis inhibitors: potential breakthrough in asthma therapy. K.F. Chung. ©ERS Journals Ltd 1995.

ABSTRACT: Cysteinyl leukotrienes are potent bronchoconstrictors, inducers of airway microvascular leakage and oedema, and of mucus secretion, in addition to causing an eosinophilic airway infiltration. Increased urinary excretion of the cysteinyl leukotriene E_4 (LTE_4) has been demonstrated following allergen challenge and during acute asthma attacks.

Strategies for inhibition of cysteinyl leukotriene effects include antagonism of cysteinyl leukotriene receptors and inhibition of 5-lipoxygenase activity. In experimental challenge studies in asthmatic patients, these compounds can inhibit bronchoconstriction in response to exercise, aspirin and allergen. Results from clinical studies using receptor antagonists, such as ICI 204,219 and MK-571, and synthesis inhibitors, such as zileuton, demonstrate beneficial effects, with improvement in symptoms and forced expiratory volume in one second (FEV_1), and a reduction in the use of β_2-adrenergic relief medication. Further studies are needed to clarify the exact mechanisms by which these compounds provide beneficial effects.

Cysteinyl leukotrienes are important mediators of asthma, and inhibition of their effects may represent a potential breakthrough in the therapy of asthma.
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In recent years, there have been major advances in asthma both in the understanding of the pathophysiological processes in the airways and in the effective use of anti-inflammatory therapy [1, 2]. Examination of bronchial mucosal biopsies obtained from the proximal large airways reveals a cellular infiltrate typically of eosinophils and lymphocytes, and epithelial damage and desquamation [3], with the expression of several cytokines, such as interleukins (IL-) 3, 4 and 5, and granulocyte-macrophage colony stimulating factor (GM-CSF), particularly by lymphocytes and other cells types [4–6]. Several mediators, such as histamine, cysteinyl leukotrienes, kinins, and eosinophil products such as eosinophil cationic protein (ECP), have been detected in the asthmatic airway. Priming for release of mediators, in particular cysteinyl leukotrienes, especially from the eosinophil, may occur [7]. Mediators may act on target cells within the airways to induce features typical of asthma, such as bronchoconstriction, mucus plugging, airway wall oedema through microvascular leakage, eosinophil infiltration and bronchial hyperresponsiveness [8].

Although many mediators are likely to play a role in inducing these features, there is now compelling evidence for an important role for cysteinyl leukotrienes in asthma. This will be reviewed and the potential therapeutic importance of inhibiting the effects of leukotrienes in asthma will be discussed.

Generation of leukotrienes (fig. 1)

Leukotrienes are synthesized from arachidonic acid, a normal constituent of the phospholipid bilayer, which is liberated by the action of phospholipases in responses to various stimuli. Leukotrienes are formed by the activation of 5-lipoxygenase (5-LO) enzyme on arachidonic acid to form an unstable intermediate, 5-hydroperoxyeicosatetraenoic acid (5-HPETE) which is converted to epoxide leukotriene (LT)A_4 [9]. 5-LO is a member of a family of lipoxygenases, and is an iron-containing enzyme consisting of 673 amino acids, which is dependent on Ca^{++}, adenosine triphosphate and several cofactors for maximal activity [10]. 5-LO translocates from the cytosol to the nuclear cell membrane to initiate leukotriene biosynthesis. 5-HPETE is formed through the action of 5-LO and the 5-lipoxygenase-activating protein (FLAP), a nuclear membrane protein to which 5-LO binds to make a stable complex [11].

LTA_4 is the pivotal intermediate from which all other leukotrienes are synthesized. LTA_4 hydrolase is a zinc-containing cytosolic metalloproteinase possessing intrisic aminopeptidase activity [12], with considerable homology to the aminopeptidase N family of enzymes. LTA_4 enzymatic activity can be inhibited by metallohydrolase inhibitors, such as bestatin. LTA_4 is unstable and may be hydrolysed to the dihydroxyacid LTB_4 by LTA_4

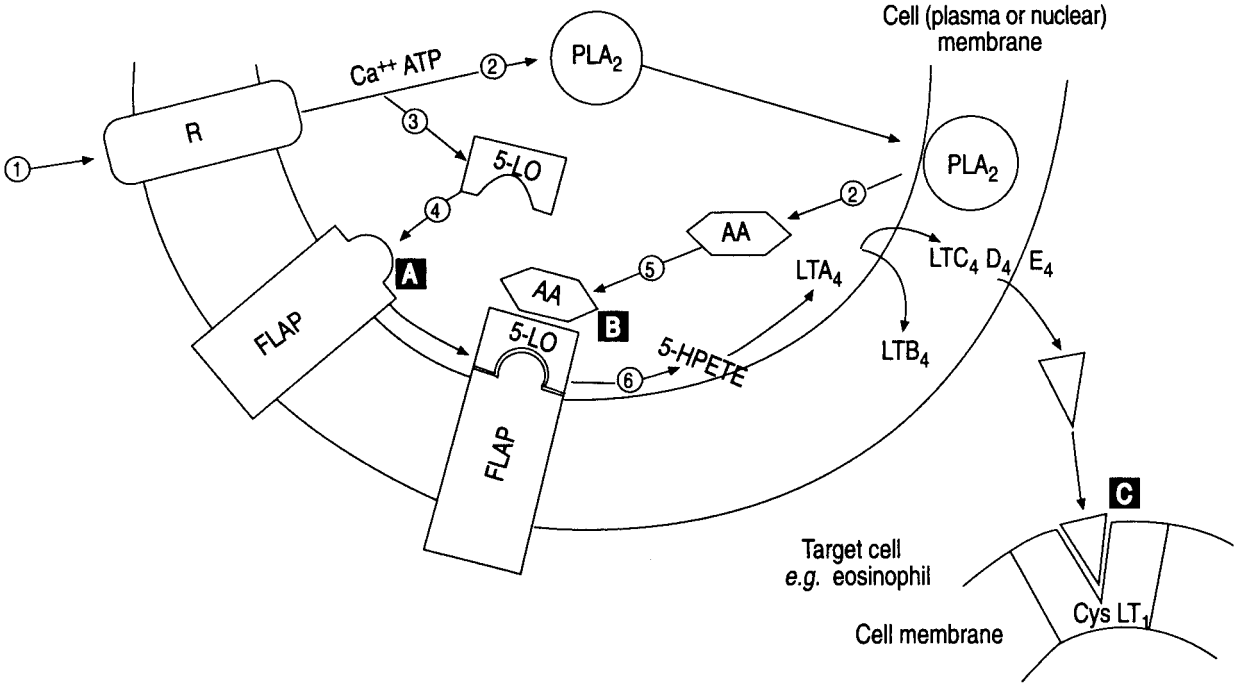

Fig. 1. – Proposed mechanism for the involvement of 5-LO and FLAP in the regulation of cellular leukotriene biosynthesis, and for various ways of inhibiting the effects of cysteinyl-leukotrienes. An inflammatory stimulus (R) leads to receptor-mediated intracellular influx of calcium (Ca^{++}) ions ①, altowing cytosolic PLA_2 and 5-LO to translocate from the cytosol to the cell membrane ② and ③. 5-LO binds to FLAP in the cell membrane to make a stable complex ④. PLA_2 cleaves AA from membrane phospholipids, and AA is converted by 5–LO in the presenc of FLAP to 5-HPETE ⑤ and ⑥. 5-HPETE is converted by 5-LO to LTA_4 which is converted by LTA_4 hydrolase to LTB_4, or by LTC_4 synthas to LTC_4, and subsequently to LTD_4 and LTE_4. Three sites of action for drugs are illustrated: A FLAP inhibitors which prevent 5-LO binding with FLAP; B 5-LO inhibitors which inhibits 5-LO activity and C cysteinyl-leukotriene receptor antagonists which inhibit the effects of cysteinyl-leukotrienes on other cells. R: receptor; ATP: adenosine triphosphate; PLA_2: phospholipase A_2; FLAP: 5-lipoxygenase-activating protein; 5-LO: 5-lipoxygenase; AA: arachidonic acid; 5-HPETE: 5-hydroperoxy-eicosatetraenoic acid; LT: leukotriene; $CysLT_1$: cysteinyl leukotriene receptor.

hydrolase, or glutathione is incorporated to form the peptidoleukotriene LTC_4 by the enzyme LTC_4 synthase. LTC_4 synthase has been recognized as an 18 kDa integral microsomal membrane protein and has recently been cloned [13]. The nucleotide and deduced amino acid sequences of its complementary deoxyribonucleic acid (cDNA) show no significant homology to glutathione S transferases but share amino acid identity with FLAP [13]. Interestingly, MK-886, a FLAP inhibitor, inhibits LTC_4 synthase activity.

The subsequent conversion of LTC_4 to LTD_4 a cysteinyl glycinyl derivative, is *via* the action of α-glutamyl transpeptidase. LTD_4 is further metabolized to the cysteinyl derivative, LTE_4, by the action of a dipeptidase. Leukotrienes are rapidly metabolized and removed from the circulation. Peptidoleukotrienes undergo oxidation, resulting in biliary and urinary elimination of biologically less active and inactive metabolites. LTE_4 is an important urinary metabolite that can be used to monitor the production of leukotrienes in man [14, 15].

The location of leukotriene synthesis is determined by the cellular distribution of the enzymes controlling each step of the pathway. The distribution of 5-lipoxygenase is limited to myeloid cells, including neutrophils, eosinophils, monocytes, macrophages, mast cells and basophils. LTC_4 synthase has been identified not only in mast cells and eosinophils but also in endothelial cells and platelets. LTA_4 hydrolase has been found in human plasma, human erythrocytes, inflammatory cells, bronchoalveolar lavage fluid and airway epithelial cells. Because these enzymes are distributed among different cell types, various inflammatory cells, in concert with noninflammatory cells, such as endothelial cells or epithelial cells, can participate in the transcellular synthesis of leukotrienes [16, 17]. Monocytes and macrophages release both LTB_4 and LTD_4 after stimulation, while most other cells produce significant quantities of either LTB_4 or LTC_4 but not both. Cellular sources of cysteinyl leukotrienes include mast cells, macrophages and eosinophils. The predominant product of 5-LO eosinophil is LTC_4 rather than LTD_4, and cytokines such as IL-3, IL-5 and GM-CSF, which have been localized in asthmatic airways [4], are capable of enhancing leukotriene (LTC_4) synthesis [18, 19].

Actions of leukotrienes relevant to asthma

In *in vitro* and *in vivo* studies on human isolated bronchus, and in normal or asthmatic subjects following aerosol administration, respectively, LTC_4 and LTD_4 are approximately 1,000 times more potent than histamine in contracting human isolated bronchus [20–23]. Both large and small airways of normal and asthmatic patients are constricted by cysteinyl leukotrienes [22, 23]. Inhaled LTC_4 and LTD_4 are 1,000–5,000 times more potent than histamine, with a long duration of action [22, 24].

Although LTE_4 is equipotent with LTC_4 and LTD_4 on isolated human bronchi, *in vivo* LTE_4 is approximately one tenth as potent as LTD, but with a longer duration of action [25].

LTC_4 and LTD_4 are also potent stimulants of mucous glycoproteins from human airways *in vitro* [26, 27]. *In vivo,* they enhance secretion of mucus [28], and stimulate secretion of chloride across the epithelium in dog trachea [29]. LTC_4, D_4 and E_4 cause vascoconstriction and increase microvascular permeability in the airways of guinea-pigs [30, 31], being at least 100-1,000 times more active than histamine. In human skin, LTC_4 and LTD_4 are potent vasodilators, producing wheal and flare responses at low concentrations [32, 33]. Maximal airway narrowing induced by methacholine is augmented by LTD_4 in normal subjects, an effect attributed to induction of airway oedema [34]. Inhalation of LTE_4 by asthmatic subjects led to an increase in the number of eosinophils, and to a lesser extent of neutrophils, in bronchial mucosal biopsies 4 h after inhalation [35].

Production of leukotrienes in asthma

Increased production of leukotrienes can be demonstrated in asthmatic patients. Measurement of LTE_4 excretion in urine is a convenient method for examining leukotriene production *in vivo*. Thus, increased urinary leukotrienes have been demonstrated following allergen challenge, during acute asthma, and aspirin-induced asthma [36–41]. However, no increase in LTE_4 excretion was shown after exercise-induced asthma [42], despite the fact that leukotriene antagonists inhibit this response [43]. Raised levels of leukotrienes, particularly LTE_4 have been found in bronchoalveolar lavage fluid of asthmatic volunteers [44–46], with further increases after endobronchial allergen challenge [47].

Strategies for inhibition of leukotriene effects

In view of the properties of cysteinyl leukotrienes in mimicking several features of asthma, and of the strong evidence for their production in asthma, it was reasonable to hypothesize that inhibition of leukotriene effects would provide clinical benefit. Two basic modes of action are available for inhibition of leukotriene effects: 1) inhibition of synthesis; and 2) antagonism of leukotriene receptors [48] (fig. 1). 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 LTB_4 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.

Inhibitors of 5-lipoxygenase

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

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 [49]. ICI 207,968 and BWA4C, which are orally active, produced dose-dependent inhibition of the cysteinyl-leukotriene component of antigen-induced bronchoconstriction in sensitized guinea-pigs [50]. BWA4C also exhibited *ex-vivo* LTB_4 synthesis when dosed orally to volunteers. Zileuton had similar *in vitro* potency and selectivity to acetohydroxamates and inhibited leukotriene synthesis *ex-vivo* [49]. Zileuton inhibited airway microvascular leakage and bronchoconstriction induced by inhaled allergen in the sensitized guinea-pig model [51], in addition to inhibiting leucocyte accumulation [51, 52]. Zileuton produced dose-dependent inhibition of leukotriene synthesis *ex-vivo* following oral administration to volunteers, with a duration of action of 6 h at doses of 600–800 mg [53].

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 [54]. ICI D2138 exhibits prolonged inhibition of *ex-vivo* leukotriene synthesis when administered orally to volunteers, with a half-life of around 12 h.

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 [55]. MK886 is a highly selective compound with no effects of prostaglandin synthesis. MK886 inhibits antigen-induced bronchoconstriction in Ascaris-sensitive squirrel monkeys [56, 57]. MK-591 [58] inhibits LTB_4 synthesis *ex-vivo* by up to 90% and urinary LTE_4 by >80% at 24 h, with a half-life of 6 h [59]. 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 [60, 61]. BAY-X-1005 inhibits anti-immunoglobulin E(IgE) challenge in human airways *in vitro* [62].

Effects in asthma (table 1)

Inhibition of bronchial challenges. Zileuton has been the most extensively evaluated in human studies *in vivo*. It inhibits bronchoconstriction induced by cold, dry air and aspirin-induced asthma [63, 64]. In addition, nasal, gastrointestinal and dermal reactions were also inhibited, together with urinary LTE_4 excretion [64]. Similarly, ZD-2138 was also effective in inhibiting aspirin-induced asthma [65]. The effects of several 5-LO inhibitors on allergen-induced early and late responses have been more variable [66–71]. Whilst zileuton and ZD-2138 did not inhibit early and late asthmatic responses [66, 68], the inhibitors, MK-886, MK-0591 and BAY-X-1005, have

Table 1. – Effect of leukotriene biosynthesis inhibitors in asthma

Challenge/ type of asthma	Compound and dose	Effect	First author	Year	[Ref]
Cold, dry air	Zileuton 800 mg, oral	↑ 47% of cold, dry air needed for 10% ↓ in FEV1	Israel	1990	[63]
Allergen	Zileuton 800 mg, oral	No effect on EAR and LAR	Hui	1991	[66]
	MK-886 500 mg 1 h before and 250 mg 2 h after, oral	↓ EAR 58% FEV1* ↓ LAR 44% FEV1 No effect on AHR	Friedman	1993	[67]
	ZD-2138 350 mg 4 h before, oral	No effect on EAR and LAR	Shuaib Nasser	1994	[68]
	MK-0591 250 mg, 24, 12 and 1.5 h before, oral	↓ EAR 79% ↓ LAR 39% (LAR reappeared 3 h later)	Diamant	1995	[69]
		No effect on AHR	Dahlen	1993	[70]
	BAY-X-1005 750 mg, 4 h before, oral	↓ EAR 68% FEV1	O'Byrne	1994	[71]
	500 mg *b.i.d.*, 3 days before and on day	↓ EAR 79% ↓ LAR 53%			
Aspirin	Zileuton 600 mg *q.i.d.* for 7 days, oral	Complete blockade	Israel	1993	[64]
	ZD-2138 350 mg, 4 h before, oral	↓ fall in FEV1 20%	Shuaib Nasser	1994	[65]
Clinical asthma (139 patients)	Zileuton 2.4 gm·day^{-1}	↑ 13.4% FEV1 ↓ 24% β-agonist use	Israel	1993	[72]
	1.6 gm·day^{-1}	↑ 10.9% FEV1 17% ↓ β-agonist use			

* Indicates attenuation of the induced fall in FEV1. EAR: early asthmatic response; LAR: late asthmatic response; AHR: airway hyperresponsiveness after allergen challenge; FEV1: forced expiratory volume in one second; ↑: increase; ↓: decrease.

been reported to inhibit both responses [67, 69–71]. MK-886 and MK-0591 protected against the late asthmatic response between 3–8 h, but this was subsequently lost [67, 69]. These compounds had no effect on allergen-induced airway hyperresponsiveness, despite effective blockade of LTB_4 biosynthesis and LTE_4 excretion at the time of measurement of airway responsiveness. In the study with a single oral dose of 800 mg zileuton [66], there was an almost complete blockade of LTB_4 biosynthesis *ex-vivo*, and a nearly 50% inhibition of urinary LTE_4, whilst in the MK-886 studies, LTB_4 biosynthesis *ex-vivo* was reduced by 54%, with an 80% reduction in LTE_4 urinary excretion at 3–9 h postchallenge. Almost complete blockage of LTB_4 biosynthesis and LTE_4 urinary excretion were observed with MK-0591. Zileuton also reduced allergen-induced nasal congestion, and selectively blocked leukotriene release in nasal lavage fluid in patients with allergic rhinitis [73].

Clinical asthma

Despite the relative lack of effect of zileuton on allergen responses, in a 4 week placebo-controlled trial in patients with mild to moderate asthma, it improved airway function and symptoms. At the highest dose of 2.4 gm·day^{-1}, there were a mean increase in FEV1 of 13.4%, a decrease in β-agonist usage by 24%, an improvement in morning peak expiratory flow rate of 10%, a decrease in overall symptom scores of 37%, and a decrease in urinary leukotriene excretion by 39% [72]. There were no significant side-effects reported. Another long-term study with zileuton examined its protective effect against cold, dry air-induced bronchoconstriction after 13 weeks of pretreatment. The protection observed was found to persist for up to 10 days after discontinuation of zileuton, which has a half-life of only 2.3 h [74].

Leukotriene receptor antagonists

There are two classes of receptors for leukotrienes, those for the dihydroxy-leukotrienes, LTB_4, termed BLT-receptors, and those for cysteinyl leukotrienes, CysLT-receptors, according to the recent International Union of Pharmacology (IUPHAR) receptor nomenclature. Specific membrane CysLT-receptors have been described, using functional receptor assays on isolated smooth muscle preparations and receptor ligand-binding studies in mammalian lung tissues [75]. 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 (Cys-LT_1-receptors) and those that are resistant to blockade (Cys-LT_2-receptors). One recent antagonist appears to have activity both for Cys-LT_1-receptors and Cys-LT_2-receptors [76]. In human airway smooth muscle, LTC_4, LTD_4 and LTE_4 all activate a Cys-LT_1-receptor, although a subclass of Cys-LT_1-receptor may be activated specifically by LTE_4 alone. In human pulmonary vasculature, a Cys-LT_2-receptor has been identified. Cys-LT_1-receptor is likely to be G-protein-coupled, leading to calcium mobilization on activation [77].

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 [78], which exhibited poor bioavailability and a short half-life. Other compounds within the same class, *e.g.* LY 171883, L-649,923, and YM-16638, were synthesized, but did not possess sufficient potency to act effectively as an LTD_4 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 [79].

The newer generation of leukotriene antagonists, such as ICI 204,219 (or Accollate), the quinolones MK-571 and RG-12,525, ONO-1078 (prankulast) and SK&F 104,353 are more promising. SK&F 104,353 has little oral activity and has been studied *via* the inhaled route. These compounds are at least 200 fold more potent than earlier leukotriene antagonists in [^{3}H]-LTD_4 binding assays [80]. The efficacy and safety of potent leukotriene receptor antagonists against leukotriene-induced bronchoconstriction in normals and asthmatics has been shown in several studies. ICI 204,219, at a single oral dose of 40 mg, shifted the LTD_4-induced bronchoconstriction dose-response curve by 100-fold and provided significant antagonism for at least 24 h in normal subjects, with no apparent side-effects [81]. MK-571 provided a shift of greater than 88 fold in asthmatic patients [82]. The introduction of these potent antagonists has been critical in defining the role of LTD_4 in bronchial asthma.

Inhibition of bronchial challenges (table 2). A number of studies have now been carried out showing that leukotriene receptor antagonists inhibit several experimental challenges that provoke airway narrowing in patients

Table 2. – Effect of leukotriene receptor antagonists on clinical challenges in asthma

Challenge	Compound	Effect	First author	Year	[Ref]
Exercise	ICI 204,219 20 mg, oral	↓ by 40% FEV1*	FINNERTY	1992	[83]
	ICI 204,219 400 μg, inhaled	↓ by 49% FEV1	MAKKER	1993	[84]
	SK&F 104,353 800 μg, inhaled	↓ by 50% FEV1	ROBUSCHI	1992	[85]
	MK-571 160 mg, oral	↓ by 68% FEV1	MANNING	1990	[43]
Allergen	ICI 204,219 40 mg, oral	↓ EAR 81% FEV1 ↓ LAR 55% FEV1 ↓ AHR	TAYLOR	1991	[86]
	ICI 204,219 1,600 μg, inhaled	↓ EAR No change in LAR	O'SHAUGHNESSY	1993	[90]
	ICI 204,219 40 mg, oral	↑ 10 fold of cat allergen to cause 20% fall in FEV1	FINDLAY	1992	[87]
	MK-571 450 mg, infused	↓ EAR 88% FEV1 ↓ LAR 68% FEV1	RASMUSSEN	1994	[88]
	ICI 204,219 20 mg, oral	↑ 5.5 fold in allergen dose	DAHLÉN	1994	[89]
Aspirin	SK&F 104,353 893 μg, inhaled	↓ 43–74% of FEV1	CHRISTIE	1991	[91]
	MK-0679 750 mg, oral	↑ aspirin dose by 4.4 fold	DAHLÉN	1993	[92]
Dipyrone	ONO-1078 225 mg, oral	↑ 14 fold in dipyrone dose	YAMAMOTO	1994	[93]
PAF (normal subjects)	ICI 204,219 40 mg, oral	↓ 59% of fall in sGaw after PAF	KIDNEY	1993	[94]
	SK&F 104,353 1.2 mg, inhaled	↓ 12.6% of fall in sGaw after PAF	SPENCER	1991	[95]

* Indicates attenuation of the induced fall in FEV1. sGaw: Specific airways conductance; PAF: platelet-activating factor. For further abbreviations see legend to table 1.

with asthma, such as exercise, allergen, and aspirin. Exercise-induced asthma is partially inhibited by approximately 50% in most studies [43, 83–85], despite the likelihood that the degree of leukotriene receptor antagonism achieved in each study could have been different. The studies using allergen challenge are of interest because both the early and late phase responses are inhibited by the leukotriene antagonists, ICI 204,219 (40 mg orally) and MK-571 [86–88]. ICI 204,219 also inhibited airway hyperresponsiveness to histamine at 6 h after allergen challenge [86]. ICI 204,219 inhibited the airway response to cumulative allergen challenge by 5.5 fold increase in allergen dose, associated with a shorter recovery time [89]. When administered by inhalation, ICI 204,219 (1.6 mg) reduced the early but not the late asthmatic response [90]. Inhaled L-648051 administered over 7 days attenuated the early and late responses to inhaled allergen [96]. In normal subjects, PAF-induced bronchoconstriction was inhibited by the LTD_4 leukotriene antagonists, ICI 204,219 [94] and SK&F 104,353 [95], in accordance with the observation that platelet-activating factor (PAF)-induces an increase in urinary LTE_4 excretion [97].

The profile of activities demonstrated by the leukotriene antagonists is different from that observed with single doses of inhaled or oral corticosteroids, which do not inhibit exercise or the acute response to allergen challenge. Aspirin-induced bronchoconstriction in aspirin-sensitive asthmatics was inhibited by the leukotriene receptor antagonists MK-069 and SK&F 104,353 [91, 92]. These results contrast with those obtained with potent receptor antagonists of PAF, which demonstrate little or no significant activity against allergen- or exercise-induced bronchoconstriction [98–100], and in trials involving moderately severe asthmatic patients [101].

Oral prankulast (ONO-1078), after one week of treatment, causes a small (half of one doubling dilution of methacholine provocative concentration producing a 20% decrease in forced expiratory volume in one second (PC_{20})) improvement in bronchial hyperresponsiveness in stable asthmatic patients [102]. Inhaled L-648051 also improved bronchial hyperresponsiveness by 1.5 doubling dilutions of methacholine, after 9 days of treatment [96]. These studies indicate that, as after inhaled steroids, bronchial hyperresponsiveness improves after treatment with leukotriene antagonists. They are also in agreement with studies showing that LTC_4 interacts synergistically with histamine and prostaglandin D_2 [103], and that LTE_4 increases histamine airway responsiveness in asthmatics but not in normal subjects [104, 105]. It is possible that further improvement may occur with longer periods of treatment with leukotriene antagonists.

Studies in clinical asthma (table 3)

Single dosing. Results from several studies show that leukotriene antagonists, like synthesis inhibitors, produce significant improvement in airways function, together with a reduction in symptoms. In a study of 10 patients with mild-to-moderate asthma, a single oral dose of ICI 204,219 induced significant bronchodilation, with a mean increase of 8% in FEV_1 (range 2–14%) [106]. However, inhaled ICI 204,219 (1,600 μg dose) did not induce bronchodilatation [107], whilst SK&F 104,353 by inhalation was effective (5% mean increase in FEV_1) [108]. In 12 moderately severe asthmatics, infusion of MK-571 resulted in a mean 20% increase in FEV_1 noticed 20 min after the start of infusion, and persisting for the 5 h observation period [109, 115]. The bronchodilator properties of LT antagonists and of the β_2-adrenergic agonist, salbutamol, appear to be additive. In addition, the degree of baseline airway obstruction was correlated with the degree of bronchodilation achieved with MK-571. Similarly, in eight patients with aspirin-sensitive asthma, MK-679, the (R)-enantiomer of MK-571, by oral administration, induced a 5–34% (mean 18%) improvement in FEV_1, lasting for at least 9 h [110]. These studies suggest that persistent activation of leukotriene receptors to increase airway tone is present in patients with chronic asthma. The bronchodilator response correlated strongly with the severity of asthma and with aspirin sensitivity. It is interesting to note that leukotriene receptor antagonists do not induce bronchodilatation in normal volunteers [81, 82].

Multiple dosing. The earlier relatively weak leukotriene receptor antagonist LY 171,883 (600 mg *b.i.d.* for 6 weeks) caused a small improvement in basal lung function, with some reduction in the use of β_2-andrenergic agonist relief medication [111]. It is possible that this beneficial effect may have resulted from its other properties, such as phosphodiesterase inhibition. Studies using more potent and specific antagonists have recently been completed. MK-571 administered orally for 6 weeks resulted in a mean increase in FEV_1 of 8–14%, a decrease in daytime symptom scores by 30%, a decrease in β-agonist inhaler use by 30% and improved diurnal variation in peak expiratory flow rate [112]. Development of MK-571 has been suspended, in favour of the (R)-enantiomer, MK-679. However, this has also been abandoned because of hepatotoxicity, which is unrelated to its leukotriene receptor antagonism.

In a 6 week study of ICI 204,209 (5, 10 or 20 mg *b.i.d.*) in patients with mild to moderate asthma, a dose-dependent improvement in symptoms was observed. The 40 $mg \cdot kg^{-1}$ dosage led to a significant improvement of evening peak expiratory flow, rescue β-agonist inhaler use (reduced by 30%), nocturnal awakenings (reduced by 46%), morning asthma symptoms and daytime symptoms (reduced by 26%) [113]. ICI 204,219 was more effective in subjects who had the lowest predicted FEV_1 at entry to the study, and a linear response was observed with increasing doses of the antagonist [113].

In general, leukotriene receptor antagonists and inhibitors were well-tolerated in studies where the treatment period was over one week. Only mild adverse events have been reported, such as headaches and gastrointestinal disturbances. Some patients on zileuton have reported dyspepsia [72], and LY-171,883 was associated with mild diarrhoea in some patients [111]. However, in many studies, the incidence of reported adverse effects was similar to that reported in the placebo group [72, 111, 113].

Table 3. – Effect of leukotriene receptor antagonists in clinical asthma

Compound	Type of asthma	Effects	First author	Year	[Ref]
Single dosing					
ICI 204,219 40 mg, oral	Mild-moderate (n=10)	↑ 8% FEV1* (2–14%)	Hui	1991	[106]
ICI 204,219 1,600 μg, inhaled	Moderate (n=10)	Nil	Kips	1993	[107]
SK&F 104,353 800 μg, inhaled	(n=10)	↑ 5% FEV1	Joos	1991	[108]
MK-571 776 mg over 6 h, infused	Moderately severe (n=12)	↑ 22% FEV1	Gaddy	1992	[109]
MK-0679 825 mg, oral	Aspirin-sensitive (n=8)	↑ 18% FEV1 (5–34%)	Dahlén	1993	[110]
Multiple dosing					
ICI 204,219 5, 10 or 20 mg *b.i.d.* for 6 weeks	Mild-moderate (n=276)	At 40 mg·day^{-1} ↓ rescue β-agonist (30%) ↓ nocturnal awakening (46%) ↓ day symptom (26%) ↑ FEV1 (11%)	Spector	1994	[113]
MK-571 75 mg *t.i.d.*, 2 weeks, then 50 mg *t.i.d.* for 4 weeks	(n=43)	↑ 8–14% FEV1 ↓ 30% symptom scores	Margolskee	1991	[112]
LY-171,883 600 mg *b.i.d.*, 6 weeks	Mild-moderate (n=138)	↑ Mean weekly FEV1 of 4.5% ↓ use of β-agonist	Cloud	1989	[111]
RG 12525 50 mg *q.i.d.* 10 days	(n=29)	↑ 7.9% FEV1	Wahedna	1992	[114]

* Indicates change in FEV1 from baseline.

Inhibition of leukotriene: effects in asthma therapy

Although it is likely that many mediators are involved in the pathogenesis of the asthmatic diathesis, inhibition of the effects of leukotrienes by receptor antagonism or inhibition of synthesis leads to improvement in symptoms and bronchodilation in patients with asthma. Although leukotriene receptor antagonists appear to be more effective than the synthesis inhibitors in blocking the late asthmatic response, the limited clinical trials that have been published show that both classes have similar effects in clinical improvement. It is too early to say whether there will be an advantage of one class over the other, despite the theoretical advantage of leukotriene 5-LO inhibitors in blocking leukotriene B_4 synthesis.

Further clinical studies of inhibitors of leukotriene effects should clarify their exact place in asthma therapy, but it is important to consider the mechanism of action of these drugs in asthma. Some of the improvement may be secondary to inhibition of airway microvascular leakage and oedema. Their mechanism of bronchodilator effect in asthma would be similar to that provided by anti-inflammatory agents, such as corticosteroids, in that these effects are "indirect". Whether leukotriene receptor antagonists or inhibitors improve bronchial responsiveness to bronchoconstrictor stimuli, such as histamine or methacholine, on a longer term basis as do topical corticosteroids needs investigation, as do their potential effects on the cellular influx of inflammatory cells, in particular, eosinophils in the airway. They may also prevent the long-term consequences of asthma, such as worsening decline in lung function, as supported by results in a rat model [116].

Current guidelines for the therapy of asthma emphasize the central role of inhaled corticosteroid therapy with the use of bronchodilators, mainly short-acting β-adrenergic agonists on an as-needed basis [2]. Inhibitors of leukotriene effects may be considered at all stages of asthma therapy. Firstly, they could be introduced at Step 1 of the guidelines as regular therapy in patients with symptoms or β-agonist usage of more than 3–4 times per week. Secondly, they could be introduced, together with inhaled steroid therapy, for a potential synergistic or additive effect, to limit or reduce the dose of inhaled steroid therapy. In this respect, it would be important to determine any steroid-sparing properties of these compounds. Leukotriene receptor antagonists or synthesis inhibitors appear to provide useful bronchodilation over and above what β_2-adrenergic agonists may provide. Most of these drugs are active by the oral route, and this may possess the advantage of improved patient compliance with once or twice daily oral dosing over

inhaled therapy. With a relatively safe profile of unwanted side-effects so far, compliance may be better than with the use of regular twice daily inhaled steroid therapy [117].

The introduction of leukotriene antagonists and synthesis inhibitors in the therapy of asthma will represent an important breakthrough in asthma therapy. These drugs represent the first class of mediator antagonists that have provided clinical benefit in asthma, in contrast to the disappointing results seen with very potent histamine antagonists. Although further work needs to be done, one should be optimistic as to their future contribution in the management of asthma; they are likely to become an established class of anti-asthma drugs.

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References

1. Barnes PJ. New concepts in the pathogenesis of bronchial hyperresponsiveness and asthma. *J Allergy Immunol* 1989; 83: 1013–1025.
2. British Thoracic Society. Guidelines for the management of asthma: a summary. *Br Med J* 1993; 306: 776–782.
3. Djukanovic R, Roche WR, Wilson JW, *et al*. Mucosal inflammation in asthma. *Am Rev Respir Dis* 1990; 142: 434–457.
4. Hamid Q, Azzawi M, Ying S, *et al*. Expression of mRNA for interleukins in mucosal bronchial biopsies from asthma. *J Clin Invest* 1991; 87: 1541–1546.
5. Broide DH, Firestein GS. Endobronchial allergen challenge: demonstration of cellular source of granulocyte macrophage colony-stimulating factor by *in situ* hybridization. *J Clin Invest* 1991; 88: 1048–1053.
6. Broide DH, Lotz M, Cuomo AJ, Coburn DA, Federman EC, Wasserman SI. Cytokines in symptomatic asthmatic airways. *J Allergy Clin lmmunol* 1992; 89: 958–967.
7. Weller PF, Lee CW, Foster DW, Corey ET, Austen KF, Lewis RA. Generation and metabolism of 5-lipoxygenase pathway leukotrienes by human eosinophils: predominant production of leukotriene C_4. *Proc Natl Acad Sci USA* 1983; 80: 7626–7630.
8. Chung KF, Barnes PJ. Role of inflammatory mediators in asthma. *Br Med Bull* 1992; 48: 135–148.
9. Rouzer CA, Matsumoto T, Samuelsson B. Single protein from human leukocytes possesses 5-lipoxygenase and leukotriene A synthase activities. *Proc Natl Acad Sci USA* 1986; 83: 857–861
10. Rouzer CA, Samuelsson B. On the nature of the 5- lipoxygenase reaction in human leukocytes: enzyme purification and requirement for multiple stimulatory factors. *Proc Natl Acad Sci USA* 1985; 82: 6040–6044.
11. Miller DK, Gillard JW, Vickers PJ, *et al*. Identification and isolation of a membrane protein necessary for leukotriene production. *Nature* 1990; 343: 278–281.
12. Haeggstrom JZ, Wetterholm A, Vallee BL, Samuelsson B. Leukotriene A_4 hydrolase: an epoxide hydrolase with peptidase activity. *Biochem Biophys Res Commun* 1990; 173: 431–437.
13. Lam BK, Penrose JF, Freeman GJ, Austen KF. Expression cloning of a cDNA for human leukotriene C_4 synthase, an integral membrane protein conjugating reduced glutathione to leukotriene A_4. *Proc Nat Acad Sci USA* 1994; 91: 7663–7667.
14. Sala A, Voelkel N, Maclouf J, Murphy RC. Leukotriene E_4 elimination and metabolism in normal human subjects. *J Biol Chem* 1990; 265: 21771–21778.
15. Maltby NH, Taylor GW, Ritter JM, Moore K, Fuller RW, Dollery CT. Leukotriene C_4 elimination and metabolism in man. *J Allergy Clin Immunol* 1990; 85: 3–9.
16. Feinmark SJ, Cannon PJ. Endothelial cell leukotriene C_4 synthesis results from intercellular transfer of leukotriene A_4 synthesized by polymorphonuclear leukocytes. *J Biol Chem* 1986; 261: 16466–16472.
17. Bigby TD, Lee DM, Meslier N, Gruenert DC. Leukotriene A_4 hydrolase activity of human airway epithelial cells. *Biochem Biophys Res Commun* 1989; 164: l–7.
18. Silberstein DS, Owen WF, Gasson JC, *et al*. Enhancement of human eosinophil cytotoxicity and leukotriene synthesis by biosynthetic (recombinant) granulocyte-macrophage colony-stimulating factor. *J Immunol* 1986; 137: 3290–3294.
19. Takafuji S, Bischoff SC, De Weck AL, Dahinden CA. IL-3 and IL-5 prime normal human eosinophils to produce leukotriene C_4 in response to soluble agonists. *J Immunol* 1991; 147: 3855–3861.
20. Dahlén S-E, Hedqvist P, Hammarström S, Samuelsson B. Leukotrienes are potent constrictors of human bronchi. *Nature* 1980; 288: 484–486.
21. Piper PJ, Samhoun MN. Comparison of the actions of leukotriene E_4 with those of leukotrienes B_4, C_4 and D_4 on guinea-pig lung and ileal smooth muscle *in vitro*. *In:* Samuelsson B, Paoletti R, Ramwell P, eds. Advances in Prostaglandin, Thromboxane and Leukotriene Research. Vol. 12. New York, Raven Press, 1983; pp. 127–131.
22. Barnes NC, Piper PJ, Costello JF. Comparative effects of inhaled leukotriene C_4, leukotriene D_4, and histamine in normal human subjects. *Thorax* 1984; 39: 500–504.
23. Smith LJ, Greenberger PA, Patterson R, Krell RD, Bernstein PR. The effect of inhaled leukotriene D_4 in humans. *Am Rev Respir Dis* 1985; 131: 368–372.
24. Weiss JW, Drazen JM, McFadden ERJ, *et al*. Airway constriction in normal humans produced by inhalation of leukotriene D: potency, time course, and effect of aspirin therapy. *J Am Med Assoc* 1983, 249: 2814–2817.
25. Davidson AB, Lee TH, Scanlon PD, *et al*. Bronchoconstrictor effects of leukotriene E_4 in normal and asthmatic subjects. *Am Rev Respir Dis* 1987; 135: 500–504.
26. Marom Z, Shelhamer JH, Bach MK, Morton DR, Kaliner M. Slow-reacting substances, leukotrienes C_4 and D_4, increase the release of mucus from human airways *in vitro*. *Am Rev Respir Dis* 1982; 126: 449–451.
27. Coles SJ, Neill KH, Reid LM, Austen KF, Corey EJ, Lewis RA. Effects of leukotrienes C_4 and D_4 on glycoprotein and lysozyme secretion by human bronchial mucosa. *Prostaglandins* 1982; 25: 155–170.
28. Johnson HG, McNee ML. Secretagogue responses of leukotriene C_4, D_4: comparison of potency in canine trachea *in vivo*. *Prostaglandins* 1983; 25: 237–243.
29. Leikauf GD, Ueki IF, Widdicombe JH, Nadel JA. Alteration of chloride secretion across canine tracheal epithelium by lipoxygenase products of ovaclindonic acid. *J Appl Physiol* 1986; 250: F47–F53.
30. Woodward DF, Wasserman MA, Weichmann BM. lnvestigation of leukotriene involvement in the vasopermeability response associated with guinea-pig tracheal anaphylaxis: comparison with cutaneous anaphylaxis. *Eur J Pharmacol* 1983; 93: 9–19.

31. Hua XY, Dahlen SE, Lundberg JM, Hammerstrom S, Hedqvist P. Leukotrienes C_4 and E_4 cause widespread and extensive plasma extravasation in the guinea-pig. *Naunyn Sch Arch Pharmacol* 1985; 330: 136–141.
32. Bisgaard H, Kristensen J, Sondergaard J. The effect of leukotriene C_4 and D_4 on cutaneous blood flow in humans. *Prostaglandins* 1982; 23: 797–801.
33. Camp RDR, Coutts AA, Greaves MW, Kay AB, Walport MJ. Response of human skin to intradermal injection of leukotrienes C_4, D_4 and B_4. *Br J Pharmacol* 1983; 80: 497–502.
34. Bel EH, Van der Veen H, Kramps JA, Dijkman JH, Sterk PJ. Maximal airway narrowing to inhaled leukotriene D_4 in normal subjects: comparison and interaction with methacholine. *Am Rev Respir Dis* 1987; 136: 979–984.
35. Laitinen LA, Laitinen A, Haahtela T, Vilkka V, Spur BW, Lee TH. Leukotriene E_4 and granulocytic infiltration into asthmatic airways. *Lancet* 1993; 341: 989–990.
36. Kumlin M, Dahlen B, Björck T, Zetterstrom O, Granstrom E, Dahlén S-E. Urinary excretion of leukotriene E_4 and 11-dehydro-thromboxane B_2 in response to bronchial provocations with allergen, aspirin, leukotriene D_4 and histamine in asthmatics. *Am Rev Respir Dis* 1992; 146: 96–103.
37. Drazen JM, O'Brien J, Sparrow D, *et al*. Recovery of leukotriene E_4 from the urine of patients with airways obstruction. *Am Rev Respir Dis* 1992; 146: 104–108.
38. Taylor GW, Taylor I, Black P, *et al*. Urinary leukotriene E_4 after antigen challenge and in acute asthma and allergic rhinitis. *Lancet* 1989; i: 584–588.
39. Smith CM, Christie PE, Hawksworth RJ, Thien F, Lee TH. Urinary leukotriene E_4 levels following allergen and exercise challenge in bronchial asthma. *Am Rev Respir Dis* 1991; 144: 1411–1413.
40. Smith CM, Hawksworth RJ, Thien FCK, Christie PE, Lee TH. Urinary leukotriene E_4 in bronchial asthma. *Eur Respir J* 1992; 5: 693–699.
41. Christie PE, Tagari P, Ford-Hutchinson AW, *et al*. Urinary leukotriene E_4 concentrations increase after aspirin challenge in aspirin-sensitive asthmatic subjects. *Am Rev Respir Dis* 1992; 145: 65–69.
42. Taylor IK, Wellings R, Taylor GW, Fuller RW. Urinary leukotriene E_4 excretion in exercise induced asthma. *J Appl Physiol* 1992; 73: 743–748.
43. Manning PJ, Watson RM, Margolskee DJ, Williams VC, Schwartz JJ. Inhibition of exercise-induced bronchoconstriction by MK-571, a potent leukotriene D_4 receptor agonist. *N Engl J Med* 1990; 323: 1736–1739.
44. Lam S, Chan H, Leriche JC, Chan Yeung M, Salari H. Release of leukotrienes in patients with bronchial asthma. *J Allergy Clin Immunol* 1988; 81: 711–717.
45. Wardlaw AJ, Hay H. Cromwell O, Collins JV, Kay AB. Leukotrienes, LTC_4 and LTB_4, in bronchoalveolar lavage in bronchial asthma and other respiratory diseases. *J Allergy Clin Immunol* 1989; 84: 19–26.
46. Crea AEG, Nakhosteen JA, Lee TH. Mediator concentrations in bronchoalveolar lavage fluid of patients with mild asymptotic bronchial asthma. *Eur Respir J* 1992; 5: 190–195.
47. Wenzel SE. Larsen GL, Johnston G, Voelkel NF, Westcott JY. Elevated levels of leukotriene C_4 in bronchoalveolar lavage fluid from atopic asthmatics after endobronchial allergen challenge. *Am Rev Respir Dis* 1990; 142: 112–119.
48. Ford-Hutchinson AW. Activation of the 5-lipoxygenase pathway of arachidonic acid metabolism. *In:* Chung KF, Barnes PJ, eds. Pharmacology of the Respiratory Tract: Experimental and clinical Research. New York, USA. Marcel Dekker, 1993; pp. 375–414.
49. McMillan RM, Girodeau JM, Foster SJ. Selective chiral inhibitors of 5-lipoxygenase with anti-inflammatory activity. *Br J Pharmacol* 1990; 101: 501–503.
50. Foster SJ, Bruneau P, Walker ER, McMillan RM. Two substituted indazolinones: orally active and selective 5-lipoxygenase inhibitors with anti-inflammatory activity. *Br J Pharmacol* 1990; 99: 113–118.
51. Hui KP, Lotvall J, Chung KF, Barnes PJ. Attenuation of inhaled allergen-induced airway microvascular leakage and airflow obstruction in guinea-pigs by a 5-lipoxygenase inhibitor (A-63162). *Am Rev Respir Dis* 1991; 143: 1015–1019.
52. Carter GW, Young PR, Albert DH, *et al*. 5-lipoxygenase inhibitory activity of zileuton. *J Pharmacol Exp Ther* 1991; 256: 929–937.
53. Rubin P, Dube L, Braeckman R, *et al*. Pharmacokinetics, safety, and ability to diminish leukotriene synthesis by zileuton, an inhibitor of 5-lipoxygenase. *In:* Acherman NR, Bonney RJ, Doherty N, eds. *Prog Inflam Res Ther* 1991; 103–112.
54. McMillan RM, Spruce KE, Crawley GC, Walker ER, Foster SJ. Preclinical pharmacology of ICI D2138, a potent orally-active non-redox inhibitor of 5-lipoxygenase. *Br J Pharmacol* 1992; 107: 1042–1047.
55. Brideau C, Chan C, Charleson S, *et al*. Pharmacology of MK-0591 (3-[l-(4–chlorobenzyl)-3-(t-butyl thio)-5-(quinolin-2-yl-methoxy)-indol-2-yl]-2,2–dimethylpropanoic acid), a potent, orally active leukotriene biosynthesis inhibitor. *Can J Physiol Pharmacol* 1992; 70: 799–807.
56. Depre M, Friedman B, Tanaka W, Van Hecken A, Buntinx A, DeSchepper PJ. Biochemical activity, pharmacokinetics, and tolerability of MK-886, a leukotriene biosynthesis inhibitor, in humans. *Clin Pharmacol Ther* 1993; 53: 602–607.
57. Gillard J. Ford-Hutchinson AW, Chan C. *et al*. L-663, 536 (MK-886) (3-[1-(4chlorobenzyl)-3-t-butyl-thio-5-isopropylindol-2-yl]-2,2-dimethylpropanoic acid), a novel, orally active leukotriene biosynthesis inhibitor. *Can J Physiol Pharmacol* 1989; 67: 456–464.
58. Prasit P, Belley M, Blouin M, *et al*. A new class of leukotriene biosynthesis inhibitor: the development of MK-0591. *J Lipid Mediators* 1993; 6: 239–244.
59. Depre M, Friedman B, Van Hecken A. *et al*. Pharmacokinetics and pharmacodynamics of multiple oral doses of MK-0591, a 5-lipoxygenase-activating protein inhibitor. *Clin Pharmacol Ther* 1994; 56: 22–30.
60. Grimes D, Sturm RJ, Marinari LR, *et al*. WY-50,295 tromethamine, a novel, orally active 5-lipoxygenase inhibitor: biochemical characterization and antiallergic activity. *Eur J Pharmacol* 1993; 236: 217–228.
61. Evans JF, Leville C, Mancini JA, *et al*. 5-lipoxygenase-activating protein is the target of a quinoline class of leukotriene synthesis inhibitors. *Mol Pharmacol* 1991; 40: 22–27.
62. Gorenne I, Labat C, Gascard JP, *et al*. (R)-2-[4-(quinolin-2-yl-methoxy)phenyl]-2cyclopentyl] acetic acid (BAY-X-1005), a potent leukotriene synthesis inhibitor: effects on anti-IgE challenge in human airways. *J Pharmacol Exp Ther* 1994; 268: 868–872.
63. Israel E, Dermarkarian R, Rosenberg M, et al. The effects of a 5-lipoxygenase inhibitor on asthma induced by cold, dry air. *N Engl J Med* 1990; 323:1740–1744.
64. Israel E, Fischer AR, Rosenberg MA, *et al*. *The* pivotal role of 5-lipoxygenase products in the reaction of aspirin-sensitive asthmatics to aspirin. *Am Rev Respir*

Dis 1993; 148: 1447–1451.
65. Shuaib Nasser SM, Bell GS, *et al.* Effects of the 5-lipoxygenase inhibiter ZD2138 on aspirin-induced asthma. *Thorax* 1994; 49: 749–756.
66. Hui KP, Taylor IK, Taylor GW, Rubin P, Kesterson J, Barnes NC. Effect of a 5-lipoxygenase inhibitor on leukotriene generation and airway responses after allergen challenge in asthmatic patients. *Thorax* 1991; 46: 184–189.
67. Friedman BS, Bel EH, Buntinx A, *et al.* Oral leukotriene inhibitor (MK-886) blocks allergen-induced airway responses. *Am Rev Respir Dis* 1993; 147: 839–844.
68. Shuaib Nasser SM, Bell GS, Hawksworth RJ, *et al.* Effect of the 5-lipoxygenase inhibiter ZD2138 on allergen-induced early and late asthmatic responses. *Thorax* 1994; 49: 743–748.
69. Diamant Z, Timmers MC, Van der Veen H. The effect of MK-0591, a novel 5-lipoxygenase activating protein (FLAP) inhibitor, on leukotriene biosynthesis and allergen-induced airway responses in asthmatic subjects *in vivo*. *J Allergy Clin Immunol* 1995; 95: 42–51.
70. Dahlen SE, Dahlen B, lhre E, *et al.* The leukotriene biosynthesis inhibiter BAY-X-1005 is a potent inhibitor of allergen-induced airway obstruction and leukotriene formation in man. *Am Rev Respir Dis* 1993; 147: A837.
71. O'Byrne PM, Watson RM, Strong HA, Wyile G. The effect of treatment with a 5-lipoxygenase inhibitor, BAY-X-1005, on allergen-induced asthmatic responses in human subjects. *Am J Respir Crit Care Med* 1994; 149: A532.
72. Israel E, Rubin P, Kemp JP, *et al.* The effect of inhibition of 5-lipoxygenase by zileuton in mild-to-moderate asthma. *Ann Intern Med* 1993; 119: 1059–1066.
73. Knapp HR. Reduced allergen-induced nasal congestion and leukotriene synthesis with an orally active 5-lipoygenase inhibitor. *N Engl J Med* 1990; 323: 1745–1748.
74. Fischer AR, McFadden CA, Frantz R, Cohn J, Drazen JM, Israel E. Chronic inhibition of 5-lipoxygenase decreases airway reactivity to cold, dry air dependent on the acute inhibition of 5-lipoxygenase. *Am J Respir Crit Care Med* 1994; 149: A1056.
75. Synder DW, Fleisch JH. Leukotriene receptor antagonists as potential therapeutic agents. *Ann Rev Pharmacol Toxicol* 1989: 29: 123–143.
76. Gardiner PJ, Norman P, Cuthbert NJ, Tudhope SR, Abram TS. Characterisation of the peptidoleukotriene receptor PL2 on the ferret spleen strip. *Eur J Pharmacol* 1993; 238: 19–26.
77. Crooke ST, Mattern M, Sarau HM, *et al.* The signal transduction system of the leukotriene D_4 receptor. *Trends Pharmacol Sci* 1989; 10: 103–107.
78. Augstein J, Farmer JB, Lee TB, Sheard P, Tattersall ML. Selective inhibitor of slow reacting substance of anaphylaxis. *Nature (New Biol)* 1973; 245: 215–217.
79. Britton JR, Hanley SP, Tattersfield AE. The effect of an oral leukotriene D_4 antagonist L-649,923 on the response to inhaled antigen in asthma. *J Allergy Clin Immunol* 1987; 79: 811–816.
80. Cheng JB. Early efficacy data with a newer generation of LTD_4 antagonists in antiasthma trials: early promise for a single mediator antagonist. *Pulm Pharmacol* 1992; 5(2): 77–80.
81. Smith LJ, Geller S, Ebright L, Glass M, Thyrum PT. Inhibition of leukotriene D_4-induced bronchoconstriction in normal subjects by the oral LTD_4 receptor antagonist ICI 204,219. *Am Rev Respir Dis* 1990; 141: 988–992.
82. Kips JC. Joos GF, Delepeleire I, *et al.* MK-571: a potent antagonist of LTD_4-induced bronchoconstriction in the human. *Am Rev Respir Dis* 1991; 144: 617–621.
83. Finnerty JP, Wood-Baker R, Thomson H, Holgate ST. Role of leukotrienes in exercise-induced asthma. *Am Rev Respir Dis* 1992; 145: 746–749.
84. Makker HK, Lau LC, Thomson HW, Binks SM, Holgate ST. The protective effect of inhaled leukotriene D_4 receptor antagonist ICI 204,219 against exercise-induced asthma. *Am Rev Respir Dis* 1993; 147: 1413–1418.
85. Robuschi M, Riva E, Fuccella LM, *et al.* Prevention of exercise-induced bronchoconstriction by a new leukotriene antagonist (SK&F) 104,353. *Am Rev Respir Dis* 1992; 145: 1285–1288.
86. Taylor IK, O'Shaughnessy KM, Fuller RM, Dollery CT. Effect of cysteinyl-leukotriene receptor antagonist ICI 204,219 on allergen-induced bronchoconstriction and airway hyperreactivity in atopic subjects. *Lancet* 1991; 337: 691–694.
87. Findlay SR, Barden JM, Easley CB, Glass M. Effect of the oral leukotriene antagonist ICI 204,219 on the antigen-induced bronchoconstriction in subjects with asthma, *J Allergy Clin Immunol* 1992; 89: 1040–1045.
88. Rasmussen JB, Eriksson LO, Margolskee DJ, Tagari P, Williams VC, Andersson KE. Leukotriene D_4 receptor blockade inhibits the immediate and late bronchoconstrictor responses to inhaled antigen in patients with asthma. *J Allergy Clin lmmunol* 1992; 90: 193–201.
89. Dahlén B, Zetterström O, Björck T, Dahlén S-E. The leukotriene-antagonist ICI 204,219 inhibits the early airway reaction to cumulative bronchial challenge with allergen in atopic asthmatics. *Eur Respir J* 1994; 7(2): 324–331.
90. O'Shaughnessy KM, Taylor IK, O'Connor B, O'Connell F, Thomson H, Dollery, CT. Potent leukotriene D_4 receptor antagonist ICI 204,219 given by the inhaled route inhibits the early but not the late phase of allergen-induced bronchoconstriction. *Am Rev Respir Dis* 1993; 147: 1431–1435.
91. Christie PE, Smith CM, Lee TH. The potent and selective sulfidopeptide leukotriene antagonist, SK&F 104, 353, inhibits aspirin-induced asthma. *Am Rev Respir Dis* 1991; 144: 957–958.
92. Dahlén B, Kumlin M, Margolskee DJ, *et al.* The leukotriene-receptor antagonist MK-0679 blocks the airway obstruction induced by inhaled lysine-aspirin in aspirin-sensitive asthmatics. *Eur Respir J* 1993; 6: 1018–1026.
93. Yamamoto H, Nagata M, Kuramitsu K, et *al.* Inhibition of analgesic-induced asthma by leukotriene receptor antagonist ONO-1078. *Am J Respir Crit Care Med* 1994; 150: 254–257.
94. Kidney J, Ridge S, Chung KF, Barnes PJ. Inhibition of PAF-induced bronchoconstriction by the oral leukotriene D_4 receptor antagonist, ICI 204,219. *Am Rev Respir Dis* 1993; 147: 215–217.
95. Spencer DA, Evans JM, Green SE, Piper PJ, Costello JF. Participation of the cysteinyl leukotrienes in the acute bronchoconstrictor response to inhaled platelet-activating factor in man. *Thorax* 1991; 46: 441–415.
96. Bel EH, Timmers MC, Dijkman JH, Stahl EC, Sterk PJ. The effect of an inhaled leukotriene antagonist, L-648,051, on early and late asthmatic reactions and subsequent increase in airway responsivenss in man. *J Allergy Clin Immunol* 1990; 85: 1067–1075.
97. O'Connor BJ, Uden S, Carty TJ, Eskra JD, Barnes PJ, Chung KF. Inhibitory effects of UK 74505, a potent and specific oral platelet-activating factor (PAF) receptor

antagonist, on airway and systemic responses to inhaled PAF in man. *Am J Respir Crit Care Med* 1994; 150: 35–40.
98. Kuitert LM, Hui KP, Uthayarkumar S, *et al*. Effect of a platelet-activating factor (PAF) antagonist UK-74,505 on allergen-induced early and late response. *Am Rev Respir Dis* 1993; 147: 82–86.
99. Freitag A, Watson RM, Matsos G, Eastwood C, O'Byrne PM. The effect of an oral platelet-activating factor antagonist, WEB 2086, on allergen-induced asthmatic responses. *Thorax* 1993; 48: 594–598.
100. Bel EH, De Smet M, Rossing TH, Timmers MC, Dijkman JH, Sterk PJ. The effect of a specific oral PAF-antagonist, MK-287, on antigen-induced early and late asthmatic reactions in man. *Am Rev Respir Dis* 1991; 143: A811 (Abstract).
101. Kuitert LM, Angus RM, Barnes NC, *et al*. Effect of a novel potent PAF antagonist, Modipafant, in clinical asthma. *Am J Respir Crit Care Med* 1995; 151: 1331–1336.
102. Fujimura M, Sakamoto S, Kamio Y, Matsuda T. Effect of a leukotriene antagonist, ONO-1078, on bronchial hyperresponsiveness in patients with asthma. *Respir Med* 1993; 87: 133–138.
103 Phillips DC, Holgate ST. Interaction of inhaled LTC_4 with histamine and PGD_2 in airway calibre in asthma. *J Appl Physiol* 1989; 66: 304–312.
104. Arm JP, Spur BW, Lee TH. The effects of inhaled leukotriene E_4 on the airway responsiveness to histamine in subjects with asthma and normal subjects. *J Allergy Clin Immunol* 1988; 82: 654–660.
105. Arm JP, O'Hickey SP, Hawksworth RJ, *et al*. Asthmatic airways have a disproportionate hyperresponsiveness to LTE_4 as compared with normal airways, but not to LTC_4. LTD_4, methacholine and histamine. *Am Rev Respir Dis* 1990; 142: 1112–1118.
106. Hui KP, Barnes NC. Lung function improvement in asthma with a cysteinyl-leukotriene receptor antagonist. *Lancet* 1991; 337: 1062–1063.
107. Kips JC, Joos GF, Felman EA, Pauwels RA. The effect of inhaled ICI 204,219 on baseline lung function in moderate asthma. *Am Rev Respir Dis* 1993; 147 (Suppl 2): A297 (Abstract).
108. Joos GF, Kips JC, Pauwels RA, Van Der Straeten ME. The effect of aerosolized SK&F 104,353-Z2 on the bronchoconstrictor effect of leukotriene D_4 in asthmatics. *Pulm Pharmacol* 1991; 4: 37–42.
109. Gaddy JN, Margolskee DJ, Bush RK, Williams VC, Busse WW. Bronchodilation with a potent and selective leukotriene D_4 (LTD_4) receptor antagonist (MK-571) in patients with asthma. *Am Rev Respir Dis* 1992; 146: 358–363.
110. Dahlén B, Margolskee DJ, Zetterstrom O, Dahlen SE. Effect of leukotriene receptor antagonist MK-0679 on baseline pulmonary function in aspirin-sensitive asthmatic subjects. *Thorax* 1993; 48: 1205–1210.
111. Cloud ML, Enas GC, Kemp J, *et al*. A specific LTD_4/LTE_4-receptor antagonist improves pulmonary function in patients with mild, chronic asthma. *Am Rev Respir Dis* 1989; 140: 1336–1339.
112. Margolskee D, Bodman S, Dock R, *et al*. The therapeutic effects of MK-571, a potent and selective leukotriene (LT) D_4 receptor antagonist in patients with chronic asthma. *J Allergy Clin Immunol* 1991; 87: 309. (Abstract).
113. Spector SL, Smith LJ, Glass M. Effects of six weeks of therapy with oral doses of ICI 204,219, a leukotriene D_4, receptor antagonist, in subjects with bronchial asthma. *Am J Respir Crit Care Med* 1994, 150: 618–623.
114. Wahedna J, Wisniewski AFZ, Wong GS, Tattersfield AE. Effect of multiple doses of RG 12525, an oral leukotriene D_4 antagonist in chronic asthma. *Am Rev Respir Dis* 1992; 145: A16 (Abstract).
115. Gaddy J, Bush RK, Margolskee D, Williams VC, Busse W. The effects of a leukotriene D_4 (LTD_4) antagonist (MK-571) in mild to moderate asthma. *J Allergy Clin Immunol* 1990; 85: 197A.
116. Wang CG, Du T, Xu LJ, Martin JG. Role of leukotriene D_4 in allergen-induced increases in airway smooth muscle in the rat. *Am Rev Respir Dis* 1993; 148: 413–417.
117. Yeung M, O'Connor SA, Parry DT, Cochrane GM. Compliance with prescribed drug therapy in asthma. *Respir Med* 1994; 88: 31–35.