The role of cysteinyl leukotrienes in asthma: From the molecule to the bedside

BEA Lams and TH Lee
Department of Allergy and Respiratory Medicine, Guy's Hospital, London, UK

ABSTRACT
There is increasing interest in the role played by the cysteinyl leukotrienes in the pathogenesis of bronchial asthma. They have been demonstrated to have a bronchoconstrictor effect both in vitro and in vivo and have been isolated from bronchoalveolar lavage fluid and urine from stable asthmatics and from asthmatics during exacerbations and after endobronchial challenge. Pharmacological intervention has been studied through antagonism of leukotriene receptors and inhibition of leukotriene synthesis. Both have been shown to have an effect on the asthmatic response after challenge with allergen, exercise and inhalation of cold air and to have an effect on aspirin sensitive asthmatics and on the symptoms and markers of severity of chronic asthma. The differences between receptor antagonism and synthesis inhibition are discussed.

Key words: asthma, leukotriene, leukotriene antagonist, 5-lipoxygenase

INTRODUCTION
There is increasing evidence for the role played by the cysteinyl leukotrienes LTC₄, LTD₄ and LTE₄ in the pathogenesis of bronchial asthma. Evidence is also accumulating that pharmacological manipulation through antagonism or inhibition of their synthesis might be clinically useful in the treatment of asthma.

The cysteinyl leukotrienes are generated by the enzyme 5-lipoxygenase (5LO; Fig. 1) which acts on arachidonic acid in conjunction with 5LO associated protein (FLAP) to generate the unstable epoxide LTA₄. This is then either converted to LTB₄ or, via LTC₄ synthase, to the cysteinyl leukotriene LTC₄ which is subsequently converted by γ-glutamyltranspeptidase to LTD₄ and by a dipeptidase to LTE₄.

BRONCHOCONSTRICCTOR EFFECT OF THE LEUKOTRIENES
In vitro studies with LTC₄ have demonstrated a contractile action on isolated human bronchus² and tracheal smooth muscle³ with a potency of 1000 times that of histamine. Isolated human bronchi of diameter 3–12 mm have a high degree of baseline bronchomotor tone equivalent to 50% of maximal bronchoconstriction induced by BaCl₂. This resting tone is reduced by preincubation with the LTD₄ antagonists ICI 204,219 and SKF 104,353 and this reduction is additive to that induced by the addition of histamine H₁ antagonists suggesting that this resting tone is mediated by the continuous production of histamine and leukotrienes.⁴

In vivo studies have revealed a bronchoconstrictor effect of inhaled nebulized solutions of leukotrienes in both normal and asthmatic individuals. In normal subjects, LTC₄ is 600–9500 times as potent as histamine in causing a 30% fall in expiratory flow at a lung volume of 30% of baseline vital capacity above residual volume.⁵ LTD₄ was found to be 6000 times more potent than histamine.⁶ LTE₄ appears to be only 40–60 times as potent as histamine but has a longer duration of action when compared to the other cysteinyl leukotrienes.⁷⁸

In asthmatic individuals the leukotrienes also have a bronchoconstrictor effect. However, in asthmatics LTC₄ is only 40 times as potent as histamine in inducing bronchoconstriction⁹ and LTD₄ only 140 times as potent.¹⁰ Further studies have confirmed that the relative (to histamine) potencies of LTC₄ and LTD₄ are reduced in asthmatics when compared to non-asthmatics.¹¹

A further study has shown that when compared to normal individuals, asthmatics have a 14-fold greater response to histamine, a 15-fold greater response to methacholine, a 6-fold greater response to LTC₄, a 9-fold greater response to LTD₄, and a 219 times greater response to LTE₄. Furthermore, as airways...

Correspondence: Professor TH Lee, Department of Allergy and Respiratory Medicine, Guy's Hospital, 4th Floor, Hunt's House, Guy's Hospital, London SE1 9RT, UK.
Received 10 August 1996.
became more hyperresponsive as judged by their response to histamine and methacholine, so the relative potency of LTE4 increases when compared to LTC4 and LTD4.\textsuperscript{12}

**Aspirin-sensitive asthma**

Studies in asthmatics whose asthma is precipitated by aspirin have shown that these individuals are exquisitely sensitive to LTE4. In aspirin-sensitive asthmatics, LTE4 is 1870 times more potent than histamine in inducing bronchospasm whereas in non-aspirin-sensitive asthmatics, LTE4 is only 145 times as potent as histamine.\textsuperscript{13} Furthermore, desensitization of these patients to aspirin leads to a fall in the relative potency of LTE4 by 20-fold, suggesting that LTE4 plays a part in the induction of bronchospasm in this group. This hyperresponsiveness is specific to LTE4 and is not seen with LTC4.\textsuperscript{14}

These studies suggest that LTC4 and LTD4 appear to have less relative potency in asthmatics when compared to normal individuals but that asthmatic airways, and especially those of aspirin-sensitive asthmatics, have a disproportionate responsiveness to LTE4. This implies that LTE4 may play an important role in bronchial asthma, perhaps because of its stability and the fact that it persists for the longest time at its site of release.\textsuperscript{15}

**ISOLATION OF LEUKOTRIENES FROM ASTHMATICS**

Various investigators have looked at the concentrations of leukotrienes in the bronchoalveolar lavage fluid (BALF) and urine of asthmatics. A study of 17 subjects with mild to severe asthma revealed that 15 had detectable levels of LTE4 in BALF whereas no LTE4 was detected in nine controls.\textsuperscript{14} Similarly, Wardlaw et al. found increased levels of LTB4 and LTC4 in the BALF of eight symptomatic asthmatics when compared to 14 controls.\textsuperscript{16} Drazin et al. looked at 72 patients attending accident and emergency departments with asthma and found that urinary LTE4 levels were higher in those who responded to a nebulized bronchodilator when compared to those who did not, suggesting that cysteinyl leukotrienes may have a bronchospastic role in acute asthma.\textsuperscript{17}

**ENDOBRONCHIAL CHALLENGE**

Wenzel et al. investigated a group of atopic asthmatics with endobronchial allergen challenge and found that LTC4 was the predominant leukotriene and increased 9-fold after challenge.\textsuperscript{18} In addition, whereas LTC4 was detected in 9 of 11 atopic asthmatics at baseline, it was detected in only one out of seven atopic non-asthmatics and one out of six non-asthmatics. A further study by the same group\textsuperscript{19} measured levels of the mast cell derived products PGD2, TXB2, LTC4 and histamine before and 5 min after endobronchial allergen challenge in seven non-asthmatics, six asthmatics and six asthmatics with a late asthmatic response (LAR), all of whom were atopic.\textsuperscript{19} Detectable levels of LTC4 were found in 9 out of 12 of the asthmatics and in only one out of seven of the non-asthmatics. An increase in all four mediators after challenge was found in the asthmatics only. In addition, the asthmatic group without a LAR had a greater increase in mediator levels when compared with asthmatics with a LAR. In a study of allergen provocation sufficient to cause 25–59% fall in FEV1 (forced expiratory volume in one second) in 17 allergic asthmatics, urinary LTE4 levels were found to increase from 46 to 92 ng/24 h. No increase in urinary LTE4 was detected after methacholine challenge caused a similar fall in FEV1. Whereas a correlation existed between urinary LTE4 and the decrease in FEV1 in the early asthmatic response (EAR), there was no correlation with the fall in FEV1 in the LAR.\textsuperscript{20}

Manning et al. looked at 18 asthmatics and found an increase in urinary LTE4 in those with an early asthmatic response and that the degree of bronchoconstriction correlated with the level of urinary LTE4.\textsuperscript{21} Those with a dual asthmatic response had a lesser but still significant increase in urinary LTE4 and those with an isolated LAR had no increase. Cysteinyl leukotrienes, therefore, appear to be important in the EAR and contribute significantly to the degree of bronchoconstriction.

**Aspirin-sensitive asthma**

Christie et al. have demonstrated a 6-fold increase in urinary LTE4 in aspirin-sensitive asthmatics when compared to normal individuals and non-aspirin-sensitive asthmatics.\textsuperscript{22} Six aspirin-sensitive asthmatics had a 4-fold increase in urinary LTE4 after oral aspirin challenge which led to a 21% fall in FEV1, whereas a control group had no fall in FEV1, and no increase in urinary LTE4 levels after aspirin ingestion.

**LEUKOTRIENE ANTAGONISTS**

The evidence for the role of the cysteinyl leukotrienes in asthma has been supported by studies involving both leukotriene antagonists and agents which inhibit their synthesis. Several studies suggest that a number of LTD4 antagonists developed for use in human trials have a blunting effect on the fall in FEV1 seen in asthmatics when experimentally challenged with allergen, exercise, inhalation of cold dry air and aspirin.

The first generation antagonists LY 171,883 and LY 649,923 produce a slight fall in the EAR after allergen challenge but have no effect on baseline FEV1, or the LAR.\textsuperscript{23,24} Ten asthmatics pre-treated with the selective and potent second generation oral agent ICI 204,219 showed a reduction of 80% in their EAR and of 50% in their LAR (Fig. 2).\textsuperscript{25} Treatment also suppressed an increase in bronchial reactivity 6h post challenge. Inhaled ICI 204,219 was not as effective and although it reduced the EAR, it had no effect on the LAR.\textsuperscript{26} In addition, a single dose of inhaled ICI 204,219 produced a 10-fold rightward shift in the dose response curve to inhaled cat allergen.\textsuperscript{27} Inhaled ICI 204,219 also led to a shortening of the recovery time of the EAR from 60 to 40 min.\textsuperscript{28} The quinolone derivative MK571 has also been shown to inhibit the EAR by 88% and the LAR by 63%.\textsuperscript{29}
Studies in exercise-induced asthma have shown that SK&F 104,353, a weak LTD₄ antagonist, led to a reduction from 29 to 20% in the exercise-induced bronchoconstrictor response, this being of a similar degree to that seen with sodium cromoglycate. In a model for exercise-induced asthma, oral treatment with ICI 204,219 resulted in a reduction in the fall in FEV₁ after inhalation of cold dry air from 36 to 21% in eight asthmatics. A further study on the same compound revealed a halving of the fall in FEV₁, after exercise in nine asthmatics. MK571 also led to a decrease in the fall in FEV₁, and a shortening in the recovery time from 33 to 8 min in 12 asthmatics after exercise (Fig. 3). However, despite their potency, LTD₄ antagonists were unable to abolish the bronchospastic response to exercise completely, suggesting that other mediators such as mast cell histamine and prostaglandins are important in this reaction.

Aspirin-sensitive asthma is probably the most leukotriene-dependent model for the investigation of anti-leukotriene therapy. The weak LTD₄ antagonist SK&F 104,353 reduced the response to ingested aspirin by a mean of 47% in five out of six subjects. The quinolone derivative MK679 was found to improve baseline function in eight aspirin-sensitive asthmatics and to block the airways obstruction caused by inhaled lysine aspirin, producing a 4.4-fold rightward shift in the dose response curve.

Bronchodilator effect

Several studies have shown that leukotriene antagonists have a bronchodilator effect on asthmatics. Intravenous treatment with MK679 led to an increase in baseline FEV₁ of up to 15.8% in a group of nine asthmatics with resting FEV₁ of between 40 and 80% of that predicted by age and height. Oral administration of ICI 204,219 led to an increase in resting FEV₁, compared to placebo with the effect of salbutamol being additive. This additive effect was confirmed in a study of 12 asthmatics treated with intravenous MK571, with the degree of bronchodilation...
being inversely proportional to the baseline FEV₁ and additive to that produced by albuterol (Fig. 4).³⁸ MK679 also led to an 18% increase in FEV₁ in eight aspirin-sensitive asthmatics with the degree of bronchodilation again strongly correlating with the severity of asthma³⁹ suggesting that cysteinyl leukotrienes play a major role in determining resting tone. Treatment with MK476 led to a 12% increase in FEV₁ in a group of moderate asthmatics.⁴⁰ Addition of the LTD₄ antagonist RG 12525 to existing steroid treatment led to an increase in FEV₁ in stable asthmatic subjects.⁴¹

Chronic asthma

The LTD₄ antagonists also appear to provide symptomatic benefit in chronic asthma. Treatment with oral LY 171,883 for 6 weeks in a group of 138 asthmatics led to an increase in FEV₁ from 73.8 to 83.3% of that predicted by age and height, an improvement in symptoms of wheeze and dyspnea and a decrease in the use of β₂ agonists, yet no change in cough or chest tightness.⁴² Oral MK571 treatment for 6 weeks led to an 8–14% increase in FEV₁ and a 30% reduction in salbutamol usage.⁴³ Six weeks of oral ICI 204,219 in mild to moderate asthma led to an improvement in their daytime asthma score, nocturnal awakenings and morning peak flow recordings.⁴⁴ A study of varying doses of the antagonist ICI 204,219 in 266 moderate asthmatics found a reduction in nocturnal awakening (by 46%), a decrease in first morning asthma symptoms, daytime asthma scores (by 26%) and salbutamol use (by 30%), and an increase in FEV₁ (by 11%) and evening peak expiratory flow rates (PEFR).⁴⁵

5-LIPOXYGENASE (5LO) INHIBITORS

There are a number of theoretical arguments to suggest that an inhibition of 5LO would be more effective than antagonism of a specific leukotriene receptor. First, inhibition of 5LO leads to a reduction in not only the cysteinyl leukotrienes LTC₄, LTD₄ and LTE₄, but also LTB₄. In addition, there is evidence that leukotriene receptor antagonism alone may prolong the half lives of some of the leukotrienes by interfering with their elimination, thereby possibly potentiating their action.⁴⁶ Studies on the 5LO inhibitors have addressed their effect on the asthmatic response to challenge with allergen, cold air, exercise and aspirin.

In a study of nine asthmatics, the 5LO inhibitor, zileuton, led to complete inhibition of ex vivo whole blood LTB₄ generation and a reduction of 50% in the rise of urinary LTE₄ post-allergen challenge. However, there was no reduction in the EAR or LAR and no reduction in airway responsiveness post-challenge.⁴⁷ The potent and selective inhibitor ZD2138 led to an 82% fall in ex vivo whole blood LTB₄ generation and a 72% decrease in the rise in urinary LTE₄, but again had no effect on the EAR or the LAR.⁴⁸ A study of eight atopic men with the translocation

![Fig. 4](image_url) Mean change in FEV₁ in asthmatic patients with existing airways obstruction after infusion of (x) MK-571 or (○) placebo and subsequent response to inhaled albuterol. Reproduced with permission from Gaddy JN, Margolseed DJ, Bush RK, Williams VC, Busse WW. American Review of Respiratory Disease 1992; 146: 358–63.

![Fig. 5](image_url) Mean percentage change in FEV₁ after aspirin challenge after premedication with the (●) 5-lipoxygenase (5LO) inhibitor ZD2138 or (○) placebo. Reproduced with permission from BMJ Publishing Group (Ref. 49).

inhibitor MK886 revealed a fall in the EAR of 58% and a fall in the LAR of 44%. This was accompanied by a 54% fall in the A23187 stimulated whole blood LTB₄ generation and a 51.5% inhibition in the rise of urinary LTE₄ in the EAR and an 80% inhibition during the LAR.⁴⁹ Administration of the FLAP inhibitor MK0591 prior to allergen challenge led to a 96% inhibition of LTB₄ and an 84% inhibition of urinary LTE₄ production 24 h post-allergen challenge. In addition, there was a fall of 79% in the EAR and the LAR was delayed by 3 h.⁵₀ BAYx1005, a FLAP inhibitor of similar potency to MK0591, was administered to atopic asthmatics and led to a reduction of 68% in the EAR to inhaled allergen and an 87% reduction in urinary LTE₄.⁵¹
Thirteen asthmatics premedicated with a single dose of zileuton had a reduction in ex vivo LTB4 generation of 74% and an increase of 47% in the amount of cold air required to cause a fall in FEV1 of 10%. Twenty-four asthmatics treated with a 2 day course of zileuton had a 40% fall in the amount of exercise induced bronchospasm.

Treatment with zileuton in eight aspirin-sensitive asthmatics led to a fall in urinary LTE4 of 70% and prevented a drop in FEV1 after aspirin challenge. Similarly, ZD2138 protects against aspirin-induced asthma with a 20.3% fall after challenge and treatment with placebo, and a fall of 4.9% after treatment with ZD2138 was associated with a reduction in whole blood LTB4 generation of 72% and of urinary LTE4 of 74% at 6 h (Fig. 5).

**Chronic asthma**

Studies have been conducted to assess the effect of the 5LO inhibitors on baseline FEV1 and symptoms in chronic asthma. Treatment with zileuton led to an increase in baseline FEV1 of 15% after 1 h in a group of chronic asthmatics treated with salbutamol only. Treatment of a group of aspirin-sensitive asthmatics with ZD2138 led to a 10% increase in baseline FEV1. In a study of 139 asthmatics with a baseline FEV1 of between 40 and 75% of that predicted by age and height and not taking steroids, zileuton led to a 13.6% increase in FEV1, a decrease in asthma symptom score, a decrease in β-2 agonist use and a decrease in excreted urinary LTE4. In a group of 398 asthmatics treated with salbutamol only and with a mean baseline FEV1 of 61%, treatment with zileuton for 13 weeks led to an increase in FEV1 of 0.251 compared with 0.081 with placebo. For 4 weeks, 109 asthmatics with baseline FEV1 ranging from 50 to 75% of predicted were treated with inhaled steroids with MK0591 or placebo. The group treated with MK0591 had an increase in their FEV1 of 6.8% whereas placebo had an increase of 0.6%. Increases in morning and evening PEFR of 19 and 135% respectively, were also noted as was a decrease in β-2 agonist use.

**CONCLUSION**

It appears that despite theoretical advantages over the leukotriene antagonists the 5-lipoxygenase (5LO) antagonists do not suppress leukotriene mediated processes any more than the leukotriene antagonists. This may be because, although 5LO inhibitors are effective in reducing ex vivo LTB4 generation and urinary LTE excretion, they are not potent enough to inhibit intra-airways leukotriene generation and that even small amounts of leukotrienes in this area may be able to exert pathological effects. In addition, SLO inhibition may decrease the amount of lipoxin A4 generated which may itself have a modulating effect on leukotriene action. Similarly, inhibition of SLO may lead to shunting of arachidonic acid derivatives down the cyclooxygenase pathway with the generation of proinflammatory products.

Further details are needed to investigate whether these compounds have steroid sparing effects and their effect on long-term symptoms. Similarly, biopsy studies to investigate the effects on airways inflammation of these compounds are needed. Finally, although there is evidence that leukotrienes are implicated in the pathogenesis of asthma and that pharmacological modification of their action has been shown to be effective in both models of acute asthma and on chronic asthma, it should be noted that leukotrienes are just one of many proinflammatory compounds active in asthma.

**References**


