

# Pathophysiology of the cysteinyl leukotrienes and effects of leukotriene receptor antagonists in asthma

Cysteinyl leukotrienes, synthesized *de novo* from cell membrane phospholipids, are proinflammatory mediators that play an important role in the pathophysiology of asthma. These mediators are among the most potent of bronchoconstrictors and cause vasodilation, increased microvascular permeability, exudation of macromolecules and edema. The cysteinyl leukotrienes also have potent chemoattractant properties for eosinophils, causing an influx of eosinophils into the airway mucosa, which further fuels the inflammatory process. In addition, the cysteinyl leukotrienes are potent secretagogues and reduce ciliary motility, which may hinder mucociliary clearance. Asthmatic patients demonstrate increased production of cysteinyl leukotrienes during naturally occurring asthma and acute asthma attacks as well as after allergen and exercise challenge. The leukotriene receptor antagonists montelukast, zafirlukast and pranlukast inhibit bronchoconstriction in asthmatic patients undergoing allergen, exercise, cold air or aspirin challenge. They attenuate the hallmarks of asthmatic inflammation, including eosinophilia in the airway mucosa and peripheral blood. Moreover, exhaled nitric oxide concentrations, another correlate of airway inflammation, are decreased during montelukast treatment in children. Cysteinyl leukotriene synthesis is not blocked by corticosteroid therapy. This important observation suggests that the leukotriene receptor antagonists represent a novel therapeutic approach, one that may provide benefits that are additive with corticosteroid therapy. This supposition is supported by clinical observations that treatment with leukotriene receptor antagonists significantly improve asthma control when added to inhaled corticosteroid therapy. Moreover, the bronchodilator properties of the leukotriene receptor antagonists are additive with those of  $\beta$  agonists. These data provide strong support for the use of leukotriene receptor antagonists for treating asthma.

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The cysteinyl leukotrienes, LTC<sub>4</sub>, LTD<sub>4</sub>, and LTE<sub>4</sub>, originally described as the slow-reacting substance of anaphylaxis (SRS-A), are proinflammatory mediators that play an integral role in the pathophysiology of asthma. In addition to functioning as potent bronchoconstrictors, these mediators contribute to the airway inflammation that is seen during naturally occurring asthma, acute asthma attack and allergen challenge (1).

The leukotrienes are eicosanoids that are produced *de novo* from the cell membrane phospholipid arachidonic acid by the activity of 5-lipoxygenase in conjunction with 5-lipoxygenase-activating protein (Fig. 1). This reaction generates the unstable leukotriene A<sub>4</sub>, which is, in turn, converted either to the chemotaxin LTB<sub>4</sub> or the cysteinyl leukotrienes, LTC<sub>4</sub>, LTD<sub>4</sub>, and LTE<sub>4</sub> (2).

## Role of the cysteinyl leukotrienes in asthma

A growing body of evidence demonstrates that the

cysteinyl leukotrienes play a critical role in most components of an asthma attack (Fig. 2). In humans, the cysteinyl leukotrienes are at least 100–1000 times more potent bronchoconstrictors than histamine, identifying these mediators as among the most potent bronchoconstrictors known (3). They also increase airway blood flow and vascular permeability, allowing the exudation of plasma macromolecules and contributing to the airway edema that characterizes asthma (4–6). In addition, the cysteinyl leukotrienes induce mucus secretion (7) and reduce respiratory ciliary motility, hampering mucociliary clearance (8).

It has also been observed that LTD<sub>4</sub> and LTE<sub>4</sub> are potent and specific chemoattractants for eosinophils, which are recognized as the predominant cell type in asthma-related inflammation (9). Laitinen et al. (10) compared the effects of methacholine with those of LTE<sub>4</sub> on the numbers of several different cell types in the lamina propria of the airway mucosa of patients

with bronchial asthma. Although inhalation of each drug produced a similar decrease in airway conductance,  $\text{LTE}_4$ , but not methacholine, induced a significant increase in eosinophil numbers. Neutrophils were also increased after provocation with  $\text{LTE}_4$ , but there were no consistent changes in the numbers of mast cells, lymphocytes, plasma cells or macrophages.

#### Effects of cysteinyl leukotriene blockade in asthma

Perhaps the most convincing evidence of the pivotal role that leukotrienes play in the pathogenesis of asthma stems from the effects of agents that block the 5-lipoxygenase pathway (see Fig. 1) (11). Zileuton, a direct inhibitor of 5-lipoxygenase activity, reduces cysteinyl leukotriene synthesis and ameliorates a range of inflammatory symptoms in asthmatic patients. The use of this agent, however, appears to be limited by its relatively low potency and short half-life *in vivo* (12).

A second, apparently more effective, approach to inhibiting the 5-lipoxygenase pathway involves specific antagonism of the Type 1 leukotriene receptor ( $\text{CysLT}_1$ ). The  $\text{CysLT}_1$  receptor is a G-protein-linked receptor found on bronchial smooth muscle. This receptor was recently cloned (13). Several  $\text{CysLT}_1$  receptor antagonists (leukotriene receptor antagonists [LTRA]) are available by prescription in many countries worldwide; these include montelukast, zafirlukast and pranlukast. The biological role of the  $\text{CysLT}_2$  receptor, found primarily on pulmonary vasculature, is unknown.

There is now substantial evidence that treatment with the LTRA attenuates many of the important hallmarks of asthma pathophysiology. The LTRA inhibit bronchoconstriction in normal subjects provoked with inhaled cysteinyl leukotrienes, as well as in asthmatics undergoing allergen, exercise, cold air or aspirin challenge (12).

The bronchodilator properties of the LTRA are additive with those of  $\beta$  agonists (11, 12). In contrast with  $\beta$  agonists, however, LTRA do not induce bronchodilation in normal subjects (14, 15). This suggests that persistent activation of the  $\text{CysLT}_1$  receptor is an integral component of asthmatic inflammation.

#### Leukotriene receptor antagonists decrease eosinophilia in asthma

Eosinophils play a critical role in the pathogenesis of asthma. Growing evidence supports a scenario that the LTRA work in part by decreasing recruitment of eosinophils. In a placebo-controlled, double-blind, crossover study, Calhoun et al. (16) measured the numbers of eosinophils in bronchoalveolar lavage fluid after segmental antigen challenge in 11 allergic asth-

matic adults who received the LTRA zafirlukast 160 mg or placebo twice daily. Zafirlukast therapy resulted in significantly ( $P < 0.05$ ) reduced numbers of eosinophils in these patients as compared with patients who received placebo. Similarly, adult asthmatics treated with montelukast 10 mg once daily exhibited significantly ( $P < 0.05$ ) decreased numbers of airway eosinophils after allergen challenge in two placebo-controlled studies of 4 and 6 weeks' duration. For these studies, airway eosinophils were measured in sputum (17) and in lower airway biopsy (18), respectively.

Montelukast treatment reduces the numbers of eosinophils in peripheral blood as well. In six clinical studies in adult asthmatics (17, 19–23), treatment with montelukast for 3–12 weeks (most patients received once-daily dose of 10 mg) reduced peripheral blood eosinophils by a mean of 15% (Fig. 3). Knorr et al. (24) observed the same effect in asthmatic children aged 6–14 years. In a randomized, double-blind, placebo-controlled study, children who received montelukast as a 5-mg chewable tablet exhibited a steady decrease in peripheral blood eosinophils compared with patients who received placebo ( $P = 0.02$  over the 8-week treatment period).

#### Montelukast decreases asthma-related exhaled nitric oxide

The concentration of nitric oxide in exhaled air ( $F_{\text{ENO}}$ ) is increased in both adults and children with asthma and correlates with severity of disease (25). Thus, increased  $F_{\text{ENO}}$  appears to be another hallmark of asthmatic inflammation, although little is known about the mechanism that links NO with airway inflammation. We recently examined the effects of montelukast on  $F_{\text{ENO}}$  in asthmatic children 6–15 years of age (26). In this double-blind, placebo-controlled, cross-over study,  $F_{\text{ENO}}$  was significantly ( $P = 0.02$ ) decreased by once-daily treatment with montelukast 5 mg (Fig. 4). The effect of montelukast was rapid:  $F_{\text{ENO}}$  decreased by

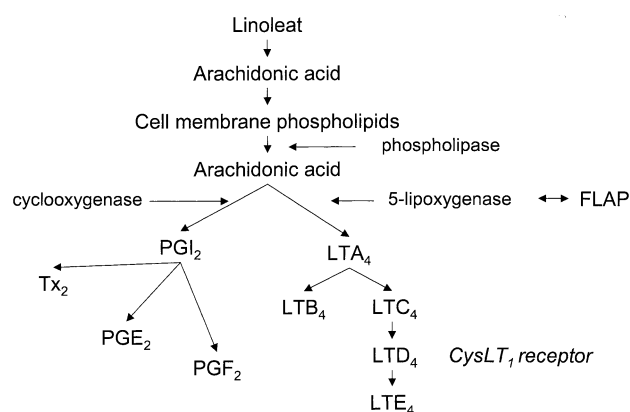


Figure 1. Schematic showing pathway of eicosanoid biosynthesis.  $\text{CysLT}_1$  receptor, cysteinyl leukotriene Type 1 receptor; FLAP, 5-lipoxygenase activating protein; LT, leukotriene; PG, prostaglandin; Tx, thromboxane.

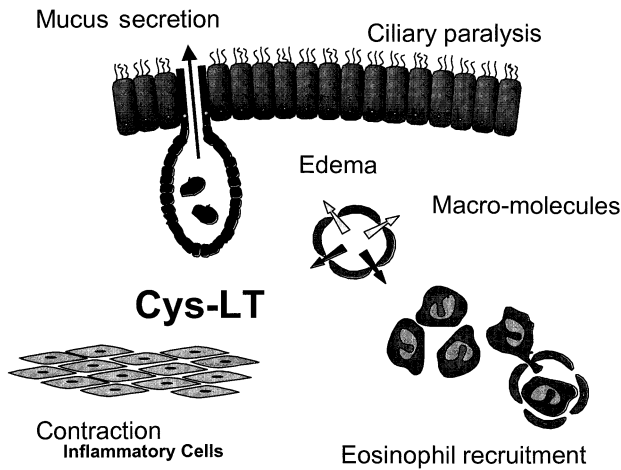


Figure 2. Roles of the cysteinyl leukotrienes in asthma pathophysiology.

15% after just 2 days of treatment and by 20% after 14 days of treatment. The effect of montelukast was not attributable to bronchodilation, since  $F_{ENO}$  was significantly increased after inhalation of the bronchodilator terbutaline. Inhaled budesonide, given to all patients during a 2-week run-out period, decreased  $F_{ENO}$  by 43% but also did not normalize levels.

#### Corticosteroids do not affect leukotriene release

Inhaled corticosteroids, a mainstay of therapy for asthma, modify allergen-induced bronchoconstriction. Although it has been conjectured that effects on the 5-lipoxygenase pathway might in part mediate this effect, results from a number of recent *in vivo* studies provide strong evidence that corticosteroids do not affect cysteinyl leukotriene synthesis or release. In a double-blind, cross-over study of 10 patients with mild atopic asthma, O'Shaughnessy et al. (27) measured the effects of the potent inhaled corticosteroid fluticasone propionate 1 mg per day on urinary  $LTE_4$  levels.

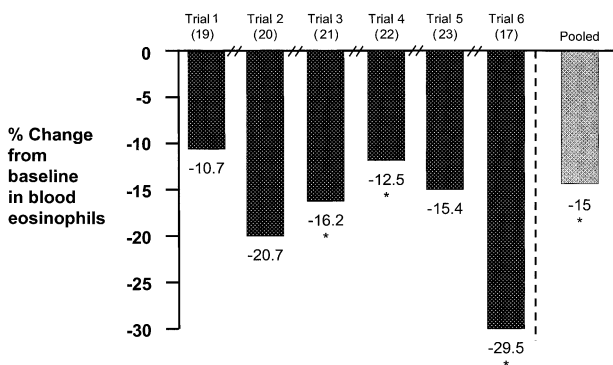


Figure 3. Montelukast consistently reduced mean numbers of peripheral blood eosinophils in asthmatic adults enrolled in six clinical trials (17, 19–23) (calculation of treatment effect based on placebo-adjusted change from baseline in least square means) \* $P \leq 0.05$  compared with placebo.

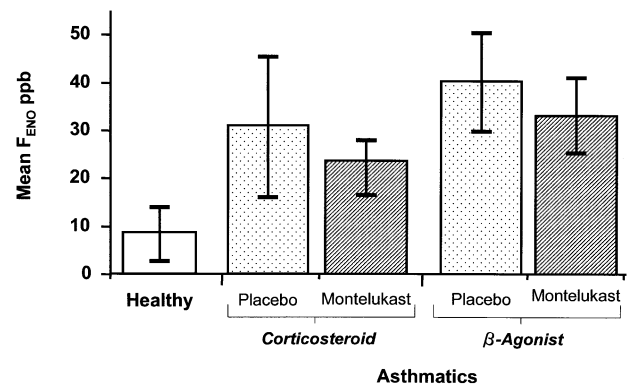


Figure 4. Exhaled nitric oxide ( $F_{ENO}$ ) was significantly reduced after treatment with montelukast as compared with placebo in children aged 6–15 years receiving concurrent treatment with inhaled corticosteroid or as-needed inhaled  $\beta$  agonist (26). Vertical lines represent 95% confidence limits.

Measurement of urinary  $LTE_4$ , the most stable of the cysteinyl leukotrienes, is used as a whole-body index of  $LTC_4$ ,  $LTD_4$ , and  $LTE_4$  production. Despite significantly ( $P < 0.02$ ) decreasing allergen-induced bronchoconstriction, fluticasone had no significant effect on urinary  $LTE_4$  excretion after allergen challenge. A similar study performed by Dworski and colleagues (28) led to the same conclusion. These investigators found that treatment with oral prednisone, while reducing asthma symptoms, had no effect on  $LTE_4$  concentration in bronchoalveolar lavage fluid in 14 atopic asthmatics, corroborating earlier results in normal subjects, in which administration of oral prednisone had no effect on urinary  $LTE_4$  levels (29).

These important observations suggest that new therapeutic gains for asthma, above that which corticosteroids can provide, can be achieved by specifically blocking of cysteinyl leukotriene activity. Moreover, because corticosteroids appear to act through a mechanism that does not involve the leukotrienes, concomitant leukotriene blockade should provide additive benefits to corticosteroid therapy. In fact, this was shown to be the

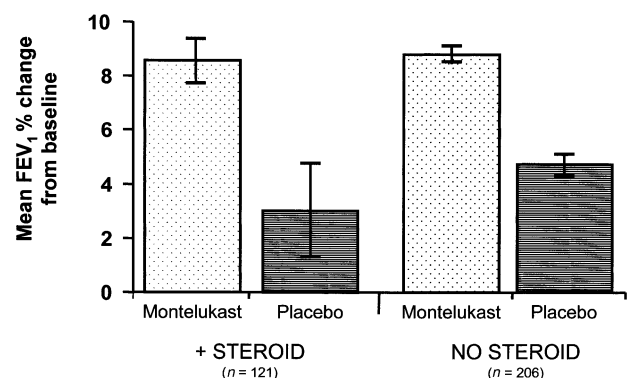


Figure 5. Montelukast provides additional clinical benefit in pediatric patients receiving inhaled corticosteroid, with mean improvement in  $FEV_1$  similar to that for children not receiving concomitant inhaled corticosteroid. Vertical lines represent SE.

case in a recent large, controlled clinical trial: therapy with montelukast provided additional asthma control in patients benefiting from but incompletely controlled on inhaled beclomethasone (30). Patients receiving concomitant once-daily montelukast experienced significantly improved daytime symptom scores, fewer nocturnal awakenings, and a lower percentage of days with asthma exacerbations relative to patients receiving beclomethasone alone ( $P < 0.05$  for all comparisons).

The effect of montelukast in reducing  $F_{ENO}$  in asthmatic children seemed independent of concurrent corticosteroid treatment (Fig. 4) (26). Similarly, in previous clinical trials, montelukast therapy improved asthma control, in both adults and children, irrespective of the use of concomitant inhaled corticosteroids (22, 24, 31, 32). *Post-hoc* subgroup analyses (33) of the large pediatric trial reported by Knorr et al. (24) provide further support for the additive effects of montelukast with corticosteroids. In this trial, the two subgroups of montelukast-treated children who were and who were not receiving concomitant inhaled corticosteroids showed similar numerical improvements in quality of life, parental global evaluations and asthma exacerbations. Improvements in  $FEV_1$  were also similar in the two subgroups (Fig. 5).

As well as providing additional clinical benefit to patients receiving concomitant inhaled corticosteroids, the use of montelukast can reduce the need for inhaled corticosteroids. In another large clinical trial, montelukast allowed a mean reduction of 47% in the inhaled corticosteroid dose among patients requiring moderate to high doses of corticosteroid to maintain asthma control (34).

## Conclusions

The effects of the cysteinyl leukotrienes mimic most of the essential features of asthma pathophysiology. In particular,  $LTC_4$ ,  $LTD_4$ , and  $LTE_4$  have potent bronchoconstrictor activity and induce eosinophilia in the submucosa of the airways. Substantial evidence shows that the LTRA can attenuate airway inflammation in both children and adults with asthma. Most notably, eosinophilia in the airways and peripheral blood is significantly reduced by treatment with an LTRA such as montelukast. Moreover, exhaled NO concentrations, another correlate of airway inflammation, are decreased during montelukast treatment in children.

Corticosteroid therapy does not block the release of cysteinyl leukotrienes in asthmatic patients but rather appears to work through a leukotriene-independent mechanism. This important observation suggests that the LTRA represent a novel therapeutic approach, one that may provide benefits that are additive to corticosteroid therapy. This supposition is supported by clinical observations that treatment with LTRA significantly improves asthma control when added to inhaled corticosteroid therapy. Moreover, the bronchodilator properties of the LTRA are additive with those of  $\beta$  agonists. These data provide strong support for the use of LTRA as complementary treatment for asthma.

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