Effect of Montelukast on Exhaled Leukotrienes and Quality of Life in Asthmatic Patients*

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Study objectives: In some patients with asthma treated with inhaled corticosteroids, suppression of inflammation is incomplete. This may be because the effect of corticosteroids on cysteinyl-leukotriene (cys-LT) biosynthesis is limited. Montelukast is a cys-LT antagonist that significantly improves asthma control in corticosteroid-treated asthmatic patients. However, not all patients treated with cys-LT antagonists show a clinical improvement.

Design: We have studied the effect of treatment for 4 weeks with montelukast (10 mg/d) on exhaled cys-LTs and leukotriene B4 (LTB4), exhaled nitric oxide, asthma quality of life (AQL), and respiratory function in patients with stable asthma.

Setting: Asthma clinics in general practice.

Patients: We studied 50 patients (30 men; mean ± SEM age, 53 ± 2 years) who were treated with inhaled corticosteroids.

Measurements and results: We detected cys-LTs in exhaled breath condensate in 25 of 50 patients; however, in the normal nonasthmatic subjects, cys-LTs were below the limit of detection. After treatment with montelukast, there was a fall in cys-LT concentrations from 14.6 ± 3.3 to 8.5 ± 2.6 pg/mL after 2 weeks (p > 0.05) and to 3.9 ± 1.3 pg/mL after 4 weeks (p < 0.01). Exhaled LTB4 levels were also elevated. After treatment with montelukast, LTB4 levels fell from 33.0 ± 3.9 to 20.4 ± 2.5 pg/mL after 2 weeks of treatment (p < 0.05), and to 17.0 ± 2.2 pg/mL after 4 weeks of treatment (p < 0.01). These changes in exhaled cys-LT and LTB4 were associated with significant improvements in AQL scores.

Conclusions: It appears that in some patients with stable asthma treated with inhaled corticosteroids, the suppression of inflammation is incomplete. Adding a leukotriene receptor antagonist can provide a complementary effect of controlling inflammation, with a significant improvement in quality of life.

(Key words: asthma; cysteinyl-leukotrienes; exhaled breath condensate; leukotriene B4; leukotriene receptor antagonist)

Asthma is a chronic inflammatory disease of the airways characterized by an infiltration of the airways by inflammatory cells including eosinophils, mast cells, and T lymphocytes.1 Inhaled corticosteroids remain a first-line therapy for the control of inflammation in patients with asthma. However, it appears that suppression of inflammation may not be complete, and the effect of inhaled corticosteroids on cysteinyl-leukotriene (cys-LT) biosynthesis is limited.2 cys-LTs are important inflammatory mediators of asthma produced by the 5-lipoxygenase pathway predominantly in the mast cells and eosinophils.3 cys-LTs cause constriction of airway muscle, increase microvascular permeability, stimulate mucus secretion, repair ciliary activity, and induce smooth-muscle cell proliferation.4–6 Allergen-induced eosinophilia and bronchoconstriction are in part mediated by cys-LTs.4 Increased amounts of cys-LTs have been found in BAL in patients with asthma7 and in exhaled breath condensate (EBC) in adults and children with asthma.8–11
Leukotriene B4 (LTB4) is a potent chemoattractant and activator of neutrophils but has no significant effects on airway smooth muscle.12 Inhalation of LTB4 increases the number of neutrophils in the alveolar spaces.4 LTB4 has been found at sites of inflammation in which neutrophils are in close proximity. LTB4 has also been found to be a potent stimulator of T-cell migration.13

Montelukast is a cyst-LT antagonist that improves asthma control in children14 and in adults,15 and is effective in the prevention of exercise-induced bronchoconstriction.16 Leukotriene receptor antagonists improve pulmonary function, reduce asthma symptoms, and decrease the use of rescue bronchodilators.17,18 and may prevent exacerbations of asthma.19 Addition of a cyst-LT antagonist to inhaled corticosteroids in patients with uncontrolled asthma has been shown to improve the control of asthma.20 However, several studies15,21 have shown that not all patients treated with cyst-LT antagonists will have a significant clinical improvement, and no factors have been identified to reliably predict the clinical response to cyst-LT antagonists.

The aim of this study was to examine the effect of treatment with montelukast on exhaled markers of inflammation (cys-LTs and LTB4), asthma quality of life (AQL), exhaled nitric oxide (NO), and respiratory function in patients with asthma who were already receiving with inhaled corticosteroids. This study was conducted in a primary care setting.

**Materials and Methods**

All patients were recruited from asthma clinics in a primary care practice (Table 1). Patients had been receiving a stable dose of inhaled steroids for at least 2 months. All patients used short-acting β2-agonists as needed, and 26 of 50 patients were also receiving a long-acting β2-agonist. After an initial visit and baseline measurements, all patients received 4 weeks of treatment with montelukast (10-mg tablet daily). A control group included 13 age-matched, healthy subjects with no history of asthma or atopy, no respiratory infection for at least 6 weeks prior to testing, and no current medications. The study was approved by the ethics committees of the Royal Brompton Hospital and West Kent Health Authority, and written consent was obtained from all patients.

| Table 1—Patient Characteristics* |
|-----------------|----------------|----------------|
| Characteristics | No Asthma | Asthma |
| Patients, 13   | 50         |
| Age, yr 50 ± 5 | 53 ± 2     |
| Male/female gender | 5/8 | 30/20 |
| FEV1, % predicted | 101 ± 4.1 | 69 ± 3.4 |
| FVC, % predicted | 99 ± 2.1 | 80 ± 3.0 |
| Inhaled steroids, μg/d | 0 | 759 ± 56 |

*Data are expressed as mean ± SEM or No.

**Study Design**

The study consisted of four visits: an initial assessment, after 2 weeks and 4 weeks of active treatment with montelukast, and 2 weeks after discontinuation of treatment. At each visit, the patients underwent spirometry and completed an AQL questionnaire, and exhaled NO and EBC were measured.

**Measurements**

Spirometry was performed with a dry spirometer (Vitalograph; Buckingham, UK). Exhaled NO was measured using an NO analyzer (NIOX; Aerocrine; Stockholm, Sweden). EBC was collected by condenser (EcoScreen; Jaeger; Würzburg, Germany). Patients were asked to breath tidally for a period of 8 min. Condensate samples were transferred on dry ice and stored at -70°C until analyzed. Cys-LTs (leukotriene C4, leukotriene D4, and leukotriene E4) and LTB4 concentrations were measured with a specific enzyme immunoassay (Cayman Chemicals; Ann Arbor, MI). The lower limit of detection for these assays was 4.0 pg/mL for cys-LTs and 4.4 pg/mL for LTB4. An AQL was used to assess subjective improvement.22

**Statistical Analysis**

Data were expressed as means ± SEM. For comparison between groups, a nonparametric Mann-Whitney U test was used. Significance was defined as a value of p < 0.05. Correlations were evaluated by nonparametric Spearman test. Results were considered significant at a value of p < 0.05.

**Results**

**Exhaled Cys-LTs**

At the initial visit, Cys-LTs were detected in 25 of 50 patients (14.6 ± 3.3 pg/mL; Fig 1, left, a). However, in all our normal subjects, the levels were undetectable. After treatment with montelukast, cys-LT levels fell to 8.5 ± 2.6 pg/mL after 2 weeks (p < 0.05) and to 3.9 ± 1.3 pg/mL after 4 weeks (p < 0.01). When we included into analysis only patients with detectable cys-LTs at the initial visit, the reduction in cys-LT levels became more significant (Fig 1, right, b).

**Exhaled LTB4**

At the initial visit, LTB4 levels were significantly higher compared to normal subjects (33.0 ± 3.9 pg/mL and 9.0 ± 2.1 pg/mL, respectively; p < 0.001). After treatment with montelukast, LTB4 fell to 20.4 ± 2.5 pg/mL after 2 weeks (p < 0.05) and to 17.0 ± 2.2 pg/mL after 4 weeks (p < 0.01; Fig 2, left, a). Only 27 patients attended a follow-up visit after discontinuation of treatment. In those patients, there was a slight but statistically insignificant increase in LTB4 (Fig 2, right, b).

**AQL**

There was a significant improvement of pooled AQL scores after 2 weeks and 4 weeks of treatment.
with montelukast (Fig 3, top, a). Thus, the mean score improved from 145.5 ± 5.8 to 172.7 ± 5.1 after 2 weeks (p < 0.005) and remained stable at 172.3 ± 6.1 after 4 weeks of treatment. The most responsive domain was “activity.” However, in 27 patients who were followed up for 2 weeks after discontinuation of treatment, there was a significant fall of pooled AQL scores (Fig 3, bottom, b). The most responsive domain was “symptoms.” There were no significant correlations between the changes in exhaled cys-LTs and changes of AQL scores, and also between changes in exhaled LTB4 and changes of AQL scores.

**Lung Function and Exhaled NO**

After treatment with montelukast, no significant changes were found in FEV1, FVC, and exhaled NO. Initial FEV1 was 2.01 ± 0.11 L; after 2 weeks and 4 weeks of treatment with montelukast, FEV1 levels were 2.09 ± 0.11 L and 2.06 ± 0.11 L, respectively. Initial exhaled NO was 26.5 ± 4.6 ppm; after 2 weeks and after 4 weeks, exhaled NO levels were 24.2 ± 4.2 ppm and 24.5 ± 4.7 ppm, respectively.

**Discussion**

In this study, we have shown elevation of cys-LTs in EBC in a group of patients with stable asthma receiving inhaled corticosteroids. The increased levels of cys-LTs may indicate the presence of continuing inflammation in the airways despite treatment with inhaled corticosteroids. MacFarlane et al23 found a strong correlation between increased cys-LTs and eosinophilia in induced sputum. Elevated levels of cys-LT in EBC have been also found in adults and children with mild-to-moderate asthma treated with inhaled corticosteroids. However, in some studies,9,24 there were difficulties in detecting any cys-LTs in breath condensate. We were unable to detect cys-LTs in a significant number of patients. This may be because the values obtained by measuring cys-LTs with currently available assays are often close to the limit of detection; assays that are more sensitive in detecting cys-LTs may be available in the future.

Inflammation in airways of patients with asthma may persist despite treatment with inhaled steroids. Duncan et al25 and Louis et al26 have shown a wide range of sputum eosinophilia despite treatment with inhaled corticosteroids. The reason for that could be the inadequate dosage of inhaled corticosteroids, or that treatment with corticosteroids was unable to fully control the inflammatory process in asthma. Indeed in a study,27 in patients with severe asthma treated with inhaled corticosteroids, there was an elevation of leukotriene excretion in urine, which could indicate that the cys-LT pathway in asthmatic...
airway inflammation remains relatively unaffected by corticosteroids. Also, in children with moderate-to-severe asthma treated with inhaled corticosteroids, there was an elevation of \( \text{LTE}_4 \) in urine.\(^{28}\) Furthermore, in a study\(^{29}\) with BAL, there was elevation of cys-LTs despite treatment with high doses of corticosteroids. One explanation of these finding could be that inhaled corticosteroids have only a small effect on 5-lipoxygenase.\(^{21}\) This may imply that even in asthmatic patients with normal lung function and good symptomatic control of their disease, suppression of inflammation is incomplete.\(^{30}\) This suggests that the addition of a leukotriene antagonist may be beneficial in blocking the effects of residual cys-LTs.

Our study shows that treatment with 10 mg of montelukast significantly reduces concentrations of cys-LTs, and this is associated with the improvement in an AQL questionnaire. The benefits of adding a leukotriene antagonist to treatment with inhaled corticosteroids in patients with asthma have been shown in several previous studies. Treatment with a leukotriene receptor antagonist may reduce eosinophils in circulating blood\(^{15}\) and in the airways.\(^{31}\) In the study by Laviolette et al.,\(^{32}\) combined therapy with inhaled beclomethasone and montelukast resulted in almost total suppression in blood eosinophil numbers. In children with corticosteroid-dependent asthma, montelukast reduced eosinophil cationic protein in sputum.\(^{33}\)

Our study also showed an elevation of exhaled \( \text{LTB}_4 \) levels in our patients treated with inhaled corticosteroids, in agreement with previous studies\(^{8,10,11}\) in adults and children. Subsequently, levels of \( \text{LTB}_4 \) have fallen significantly after treatment with montelukast. However, after 2 weeks of discontinuation of treatment, there was a slight but not significant rise in exhaled \( \text{LTB}_4 \) levels that coincided with the fall of AQL scores. \( \text{LTB}_4 \) is a potent chemoattractant that activates neutrophils and T-lymphocytes. An \( \text{LTB}_4 \) antagonist reduces neutrophil influx into airways of asthmatic patients at 24 h after airway antigen challenge.\(^{34}\) The reduction in cys-LTs and \( \text{LTB}_4 \) after treatment with leukotriene antagonist is unexpected and difficult to explain. However, a recent study\(^{35}\) showed that montelukast inhibits 5-lipoxygenase, the rate-limiting enzyme in the biosynthesis of leukotrienes. This could explain the decrease of leukotrienes during treatment with montelukast in our study.

We measured inflammatory markers in EBC. EBC provides access to volatile and nonvolatile respiratory compounds, and the measurements are reproducible.\(^{36}\) EBC is noninvasive, safe, and easy to perform even in patients with severe airways obstruction. EBC can be measured regularly to monitor airway inflammation. All of our patients were recruited from general practice clinics, confirming the feasibility of performing such studies in a primary care setting.
Despite elevated levels of exhaled cys-LTs and LTB4, the levels of NO remained normal. This may indicate a limitation of NO as an accurate monitor of the control of inflammation in patients treated with inhaled corticosteroids. In our study, the addition of montelukast to inhaled corticosteroid had no additional bronchodilator effect. This result was not surprising to us since all our patients were receiving inhaled corticosteroids and were clinically stable. Similarly, Robinson et al38 and Currie et al39 found no benefit of cysteinyl-leukotriene antagonists on respiratory function. Monitoring respiratory function on its own can therefore miss the potentially beneficial effects of cys-LT antagonist in patients receiving inhaled corticosteroids.

In this study, considerable improvement was seen in all domains of the AQL questionnaire. This improvement was noticed after 2 weeks of treatment and was maintained after 4 weeks. A possible explanation of the improvements in AQL scores could be an additional antiinflammatory effect of montelukast. However, stopping treatment with montelukast caused deterioration in AQL scores. Similarly, Currie et al39 found benefit of treatment with montelukast on surrogate inflammatory markers in corticosteroid treated asthmatics, but without any changes in respiratory function tests. Also Strauch et al33 and Tamaoki et al19 have found improvement in AQL scores in patients treated with montelukast. In addition Tamaoki at al19 also found that montelukast prevented deterioration of asthma after reduction of inhaled corticosteroids. Price et al20 also reported benefit of treatment with montelukast on a specific quality of life questionnaire, but in this study there was also improvement in respiratory function tests. It seems therefore that symptoms-score grading is more sensitive that these respiratory function tests in the assessment of treatment and should be used as an adjunct to objective measurements of markers of inflammation. We have found no significant correlation between the changes in exhaled cys-LTs and AQL scores and also between the changes in exhaled LTB4 and AQL scores. One of the explanations for this could be that we have only studied a relatively small group of patients. However, in a recent analysis40 of the few much larger studies, only a very weak correlation was found between clinical outcomes and quality of life. The explanation for this finding was the possible “noise” of measurements, and that quality of life is a distinct component of asthma health status, which could be responsible for the lack of significant correlation in our study.

In conclusion, we have found elevated levels of exhaled cys-LTs and LTB4, the levels of NO remained normal. This may indicate a limitation of NO as an accurate monitor of the control of inflammation in patients treated with inhaled corticosteroids.37 It may also suggests that NO is more sensitive to the inhibitory effects of corticosteroids than are exhaled cys-LTs and LTB4.

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In conclusion, we have found elevated levels of exhaled cys-LTs and LTB4 in patients with stable asthma treated with inhaled corticosteroids. This suggests that inflammation may persist in airways despite treatment with corticosteroids. Adding an antileukotriene provides a complementary role in the control of asthma and improvement in AQL questionnaire scores. However, for a better understanding the role of treatment with the leukotriene receptor antagonist montelukast in patients receiving with inhaled corticosteroids, further studies using double-blind protocols are required.

REFERENCES

Figure 3. Top, a: mean AQL questionnaire score at the initial visit (white bars), at 2 weeks (gray bars), and at 4 weeks (black bars) of treatment with montelukast (**p < 0.01; ***p < 0.005). Bottom, b: mean AQL questionnaire score at the initial visit (white bars), at 2 weeks (gray bars), and at 4 weeks (black bars) of treatment with montelukast, and 2 weeks after discontinuation of treatment (dark gray bars) [only 27 patients who attended four visits were included; *p < 0.05; **p < 0.01].


