Reduction of Eosinophilic Inflammation in the Airways of Patients With Asthma Using Montelukast*

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Objective: Leukotrienes (LTs) are involved in airway eosinophilic inflammation in patients with asthma. We examined the effects of a cysteinyl LT 1-receptor antagonist, montelukast, on sputum eosinophil levels, and the correlation between sputum eosinophils and bronchodilatation in patients with asthma.

Design: Double-blind, randomized, crossover study.

Setting: University hospital and private hospital.

Patients: Twenty-nine patients with mild-to-moderate asthma.

Interventions: Montelukast, 10 mg, and placebo tablet, once daily, each for 4 weeks.

Measurements: Sputum eosinophils analyzed using hypertonic saline solution-induced sputum and airway hyperresponsiveness to histamine were evaluated before and after treatment. In addition, morning and evening peak expiratory flow (PEF), asthma symptoms, and peripheral blood eosinophil levels were assessed.

Results: The percentage of eosinophils in sputum decreased from 24.6 ± 12.3% at baseline to 15.1 ± 11.8% after montelukast treatment, for a change of −9.5 ± 12.7% (n = 20). During placebo administration, the percentage of eosinophils fell from 21.3 ± 12.1% to 21.0 ± 11.5%, resulting in a decrease of −0.3 ± 10.8% (n = 20). There was a statistically significant difference in the change in sputum eosinophil levels between these two periods (p < 0.005). The number of peripheral blood eosinophils also significantly decreased after montelukast treatment (314.1 ± 237.6/mL) compared with placebo (413.1 ± 232.1/mL; p < 0.005, n = 21). Although morning and evening PEF values were significantly improved from baseline after montelukast treatment (p < 0.01, n = 20), asthma symptoms and airway responsiveness to histamine were not significantly altered. Furthermore, there was no significant correlation between the decrease in sputum eosinophils and the increase in PEF.

Conclusion: These results suggest that montelukast has anti-inflammatory effects on the airway in patients with asthma, and that its bronchodilatory effect is not solely dependent on a decrease in airway eosinophilia. (CHEST 2002; 121:732–738)

Key words: cysteinyl leukotriene 1-receptor antagonist; eosinophil; montelukast; sputum

Abbreviations: CysLT = cysteinyl leukotriene; LT = leukotriene; NS = not significant; PC20 = provocative concentration of histamine causing a 20% fall in FEV1; PEF = peak expiratory flow

Studies have demonstrated that asthma is associated with chronic airway inflammation with recruitment of a number of inflammatory cells, including T cells and eosinophils.1 Although several chemical mediators are released during chronic airway inflammation, evidence strongly suggests that the cysteinyl leukotrienes (CysLTs) C4, D4, and E4 play key roles in asthma.2,3 Leukotrienes (LTs) are not stored in cells but are newly generated from arachidonic acid after cellular activation and are produced by eosinophils, mast cells, alveolar macrophages, and neutrophils.2 LTs are at least 1,000

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times more potent as bronchoconstrictors than histamine or methacholine.4,5

The pharmacologic actions of CysLTs include not only bronchoconstriction but also chemotraction of eosinophils and increases in microvascular leak and mucus secretion.2 Because LTs mediate many of the pathophysiologic features of asthma, they may play an important role in asthma. In fact, LTs are released in BAL fluid and urine during acute exacerbation of asthma and after allergen, cold-air, exercise, or aspirin challenge.6–9 Furthermore, several anti-LT modifiers, including CysLT1-receptor antagonists and 5-lipoxygenase inhibitors, significantly inhibited the bronchoconstriction in response to these challenges.10–14 The efficacy of CysLT1-receptor antagonists in the chronic management of asthma has been reported.3 Treatment of patients with asthma with CysLT1-receptor antagonists results in an improvement of respiratory function and a decrease in asthma symptoms. Furthermore, CysLT1-receptor antagonists have steroid-sparing effects and significantly decrease the frequency of episodes of acute exacerbation of asthma.15–17

Montelukast is a potent and specific CysLT1-receptor antagonist.18 In 12-week, multicenter, randomized, double-blind studies in adult patients with persistent asthma, treatment with montelukast resulted in significant improvements in respiratory function, asthma symptoms, as-needed β2-agonist use, peripheral eosinophil counts, and health-related quality of life.19–22 Furthermore, Pizzichini et al23 demonstrated that 4 weeks of treatment with montelukast resulted in decreases in both sputum and peripheral blood eosinophils, suggesting that this drug may exert anti-inflammatory actions in patients with asthma.

In this study, we investigated the effects of montelukast on airway inflammation and airway hyperresponsiveness in patients with mild-to-moderate asthma in a randomized, double-blind, placebo-controlled, two-period crossover study. Furthermore, the correlation between the decrease in sputum eosinophils and the increase in peak expiratory flow (PEF) was also evaluated.

Materials and Methods

Patients

Twenty-nine adults with mild-to-moderate bronchial asthma, who were capable of producing induced sputum before the study and had >10% sputum eosinophils, were enrolled from among outpatients of the First Department of Internal Medicine, Showa University; the Department of Respiratory, Kihara Hospital; and the Department of Allergy and Respiratory Medicine, Doai Memorial Hospital, Tokyo, Japan. The severity of bronchial asthma in patients participating in this study was considered mild to moderate based on the Japanese Guideline for the Treatment of Asthma.24 All of the patients had demonstrated an improvement of ≥15% in FEV1 after inhalation of 200 μg of salbutamol sulfate within 1 year before entering the study. All of the patients were nonsmokers for at least 6 months before starting this study. None of the patients received any type of steroids within 4 weeks before enrollment or anti-allergy agents, including antihistamines, within 2 weeks before enrollment. Patients receiving Chinese medicines, immunotherapy, or nonspecific therapy were also excluded from this study. None of the patients had respiratory infections within 4 weeks before enrollment, or chronic bronchitis, pulmonary emphysema, bronchiectasis, or other lung diseases that could interfere with the evaluation of the efficacy of montelukast. Patients with liver disorders, renal disorders, heart disorders, or other serious disorders were excluded. None of the female patients were pregnant or lactating. The study was approved by the institutional review board of each hospital, and all patients gave written informed consent.

Study Design

This study was conducted according to a randomized, double-blind, placebo-controlled, two-period crossover design (Fig 1). The study comprised two periods, each consisting of a 2-week washout period and a 4-week treatment phase. In period 1, after a 2-week run-in phase, the patients received the first test drug (montelukast, 10-mg tablet or matched placebo tablet) once daily at the beginning of period 1 and at the end of both test periods. Asthma symptoms were recorded in a daily diary, and PEF was monitored throughout the study periods.

Asthma Symptoms and PEF Measurement

Each patient kept a diary card during the study, detailing the intensity and frequency of symptoms, as well as the use of concomitant drugs and therapies. The symptom scores were graded according to the rating standards established by the Japanese Society of Allergology as follows: breathlessness and wheezing, on a scale of 0 to 9 (0 = no symptoms; 1 = breathlessness or wheezing; 3 = mild asthma attack; 6 = moderate asthma attack;...
Sputum Induction and Eosinophil Counts

Sputum induction was performed as described previously. Medications were stopped for at least 12 h, after which sputum was induced by inhalation of increasing concentrations of hypertonic saline solution (0.9%, 1.8%, 3%, 4%, and 5%) until an adequate volume of sputum was collected. Patients were encouraged to cough deeply after each inhalation. Cell plugs in sputum were separated from saliva and collected. Sputum cells were then spread on the slides and treated with Wright-Giemsa so that the inflammatory cells could be counted. We reported the reproducibility of the proportion of sputum eosinophils by this method. The percentage of total eosinophils was determined by counting 900 inflammatory cells under a light microscope. All slides were independently counted by two readers blinded to treatment, study site, and visit. The average values of the percentage of sputum eosinophils were expressed. The sputum induction test was performed after the histamine challenge test.

Measurement of Airway Responsiveness to Histamine

Bronchial responsiveness to histamine was measured by a standard technique as described previously.26,27 Patients inhaled doubling concentrations of histamine via nebulizer (model 646; DeVilbiss; Somerset, PA) for 2 min by tidal breathing. Increasing concentrations of histamine were administered until FEV1 decreased by > 20% of the baseline value. Results were expressed as the provocative concentration of histamine that caused a 20% decrease in FEV1 (PC20) from post-saline solution baseline. Bronchodilators were withheld for at least 12 h before the histamine challenge test, which was performed between 9 AM and 10 AM; all patients were tested on the same equipment and by the same investigator.

Peripheral Blood Measurement

Complete leukocyte counts, including differential cell counts, were evaluated on the days specified in the protocol.

Data Analysis

Because this study used a crossover design, the effects of carry-over and period were evaluated. The homogeneity of background factors between the two groups was examined by χ² test (significance level, two-tailed, 15%). The changes from baseline in eosinophil percentages in sputum and peripheral blood were analyzed using an analysis of variance model including the factors of carry-over, period and treatment effects (significance levels: carry-over effect, two-tailed, 10%; period effect, two-tailed, 5%; and treatment effect, one-tailed, 2.5%). Sputum eosinophil percentage, airway responsiveness to histamine (PC20), pulmonary function, and asthma symptoms were analyzed using the same model. All data except PC20 are expressed as mean ± SD. PC20 is expressed as the geometric mean. For safety information, the incidence of total adverse events was compared between periods using Fisher’s Exact Test (significance level, two-tailed, 5%).

Results

Twenty-six patients completed the study, and 3 patients dropped out. The baseline characteristics of the 26 patients are presented in Table 1. One patient was not included in the study because he had received steroid therapy within 4 weeks before enrollment, and two patients were excluded because their percentage of sputum eosinophils at the beginning of period 1 was < 10%. Changes in asthma symptoms and peripheral blood eosinophils were analyzed in 21 eligible patients; 5 patients were excluded because of a lack of data (n = 3), exacerbation of asthma caused by respiratory infection (n = 1), and lack of washout between periods 1 and 2 (n = 1). Induced sputum, PEF, and airway responsiveness to histamine were analyzed in 20 eligible patients; 5 patients were excluded by the reasons described above, and 1 more patient was excluded because of a lack of data.

The symptom score decreased from baseline after 2 weeks and 4 weeks of treatment with montelukast, but these decreases did not achieve statistical significance (8.2 ± 12.1 at baseline, 6.8 ± 10.2 at 2 weeks, 6.6 ± 10.3 at 4 weeks). Placebo administration was not associated with a decrease in asthma symptoms (6.4 ± 7.4 at baseline, 6.5 ± 8.4 at 2 weeks, 6.4 ± 9.0 at 4 weeks).

Sputum Eosinophils

Montelukast decreased the mean percentage of sputum eosinophils from 24.6 ± 12.3% at baseline to

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*Data are presented as mean ± SD unless otherwise indicated.
15.1 ± 11.8% at the end of the treatment period, resulting in a change of −9.5 ± 12.7% (p < 0.005). In contrast, placebo treatment decreased the mean percentage of sputum eosinophils from 21.3 ± 12.1% at baseline to 21.0 ± 11.5% at the end of the treatment period, resulting in a change of −0.3 ± 10.8%. The reduction in the percentage of sputum eosinophils was significantly greater in the montelukast period than in the placebo period (p < 0.005, Fig 2). Because this parameter showed a significant period effect, analysis was also performed using the data in period 1 only. The reduction in the percentage of sputum eosinophils was again significantly greater in the montelukast period than in the placebo period (p < 0.005, data not shown).

**Peripheral Blood Eosinophils**

Because baseline values were not measured in period 2, the posttreatment values in each period were compared directly. The mean baseline number of peripheral blood eosinophils was 439.7 ± 223.5/mL. The value after montelukast treatment (314.1 ± 237.6/mL) was significantly different from the value after placebo administration (413.1 ± 232.1/mL; p < 0.005; Fig 3). These results demonstrate that treatment with montelukast resulted in a significantly lower number of peripheral blood eosinophils.

**PEF**

Morning PEF increased significantly with montelukast treatment from 427.7 ± 98.3 L/min at baseline to 444.4 ± 97.9 L/min (p < 0.005) at 2 weeks of treatment, and to 448.6 ± 97.9 L/min (p < 0.0005) at 4 weeks of treatment, resulting in mean increases of 16.7 ± 19.7 L/min and 20.9 ± 19.6 L/min, respectively. No significant
change in morning PEF was observed during the placebo period. Morning PEF changed from 426.9 ± 95.0 L/min at baseline to 429.4 ± 90.6 L/min (not significant [NS] vs baseline) at 2 weeks, and to 437.5 ± 103.6 L/min (NS vs baseline) at 4 weeks, resulting in mean increases of 2.5 ± 18.7 L/min and 10.6 ± 31.9 L/min, respectively. There was no statistically significant difference in the extent of change from baseline in morning PEF between montelukast and placebo at 4 weeks (Fig 4).

Evening PEF increased significantly with montelukast treatment from 438.1 ± 102.5 L/min to 455.4 ± 100.6 L/min (p < 0.005) at 2 weeks and to 464.3 ± 99.0 L/min (p < 0.0005) at 4 weeks, resulting in mean increases of 17.3 ± 20.8 L/min and 26.2 ± 21.2 L/min, respectively. During the placebo period, PEF changed from 442.1 ± 102.2 L/min at baseline to 441.4 ± 96.1 L/min (NS vs baseline) at 2 weeks, and to 444.3 ± 105.6 L/min (NS vs baseline) at 4 weeks, resulting in mean changes of −0.7 ± 21.9 L/min and 2.3 ± 30.7 L/min, respectively. There was a statistically significant difference in evening PEF between the montelukast and placebo periods at the end of each period (p < 0.005; Fig 4).

**Correlation Between the Decrease in Sputum Eosinophils and the Increase in PEF**

The relationship between the decrease in sputum eosinophils and the increase in PEF was investigated in 18 patients in whom both sputum eosinophils and PEF could be monitored. Increases in either morning or evening PEF were not significantly correlated with the decrease in sputum eosinophils, suggesting that the bronchodilatory effects of 4 weeks of treatment with montelukast were not dependent solely on the improvement in airway eosinophilia (Fig 5 and data not shown).

**Airway Responsiveness to Histamine**

In the montelukast treatment period, PC_{20} increased from 277.6 ± 861.3 μg/mL at baseline to 305.4 ± 1762.1 μg/mL at 4 weeks (NS vs baseline), resulting in a geometric mean change of 1.1 ± 1.6-fold. In the placebo period, PC_{20} increased from a baseline value of 263.5 ± 1412.3 μg/mL to 333.7 ± 1690.8 μg/mL (NS vs baseline) at 4 weeks, resulting in a mean change of 1.3 ± 3.1-fold. There was no statistically significant difference between the two periods in the degree of change in this parameter.

**Tolerability**

There were no significant differences between montelukast and placebo in the frequency of clinical and laboratory adverse effects. While 27 patients were receiving montelukast, 13 patients reported a total of 26 clinical adverse effects; in comparison, 15 of 28 patients reported a total of 30 clinical adverse effects while receiving placebo. Respiratory events, especially cold syndrome, were most commonly observed. Concerning laboratory test findings, 4 of 27 patients receiving montelukast experienced a total of five laboratory adverse effects compared with 5 of 28 patients who had a total of six laboratory adverse effects while receiving placebo. Three patients, including one who received only montelukast, were
withdrawn from the study because of worsening of asthma before starting period 2.

**Discussion**

In the present randomized, placebo-controlled crossover study, we investigated the potential anti-inflammatory effects of 4 weeks of treatment with montelukast on sputum and peripheral blood eosinophils, and on airway responsiveness to histamine in patients with mild-to-moderate asthma. In addition, the clinical efficacy of montelukast was evaluated. Although treatment with montelukast but not placebo resulted in significant decreases in sputum and peripheral blood eosinophils, neither montelukast nor placebo significantly changed airway responsiveness to histamine. Furthermore, only montelukast significantly increased both morning and evening PEF compared with baseline values. There was no significant relationship between the increase in PEF and the decrease in sputum eosinophils after treatment with montelukast, suggesting that improvement of PEF was not solely dependent on the improvement of airway eosinophilia. Because this study involved a relatively small sample size and did not have adequate power, asthma symptoms did not show significant improvement with montelukast treatment.

The effects of montelukast on airway eosinophilic inflammation have been reported previously. Although a significant decrease in sputum eosinophils was demonstrated in the previous study, baseline percentages of sputum eosinophils were significantly higher in the placebo group than in the montelukast group. In the present study, none of the baseline parameters, including the percentage of sputum eosinophils, was significantly different between the montelukast and placebo periods and investigated by a crossover design.

Noninvasive assessment of airway inflammation in asthma has been the focus of increased interest in the past 10 years. Although several techniques have been developed, analysis of induced sputum is particularly useful because it is repeatable and can be performed in outpatient clinics. Therefore, analyzing induced sputum is useful for evaluating the response to anti-inflammatory therapy. Although our method for the analysis of sputum cells was different from the one that was reported by Pizzichini et al., sputum eosinophils were analyzed using the same method in the placebo group and montelukast group, and no significant difference in the percentage of sputum eosinophils was observed in the placebo group, suggesting that our method was reliable and reproducible. In the present study, we used this technique to investigate the potential effects of montelukast on airway inflammation and found that sputum eosinophils significantly decreased with montelukast treatment. It has been reported that inhalation of \( \text{LTE}_4 \) resulted in the recruitment of eosinophils into the airway. In addition, \( \text{LTD}_4 \) possessed chemotactic activity for eosinophils in vitro. More recently, an autocrine CysLTs pathway that supports eosinophil survival by inhibiting apoptosis has been revealed. These findings and the results of the present study suggest that CysLTs are released within the airway of asthmatic patients and that montelukast inhibits the chemotactic activity of CysLTs for eosinophils and promotes apoptosis, resulting in a decrease in sputum eosinophils.

Prompt improvement of bronchoconstriction by CysLT\(_1\)-receptor antagonists has been reported in asthma. In fact, bronchodilatory effect was observed several hours after treatment with CysLT\(_1\)-receptor antagonists. In the present study, we found that treatment with montelukast resulted in significant increases in both morning and evening PEF compared with the baseline values. Because the number of patients evaluated in this study was small, significant difference in evening PEF was only observed between montelukast and placebo treatment at 4 weeks of treatment.

In this study, we also investigated the relationship between the increase in PEF and the decrease in sputum eosinophils before and after administration of montelukast and placebo. However, we failed to demonstrate a significant relationship, suggesting that the increase in PEF may be caused not only by the decrease in eosinophils in the airway. Therefore, we speculate that improvement of respiratory function may be induced by the wide variety of inhibitory actions of CysLTs-receptor antagonist such as bronchodilation. In addition, because the present study was neither designed nor powered to determine the correlation between clinical parameters and airway inflammation, further clinical trials will be needed to reach a definitive conclusion.

Airway hyperresponsiveness is a characteristic feature of asthma. Although several factors are involved in the pathophysiologic features of airway hyperresponsiveness, ongoing airway inflammation may play an important role in inducing, maintaining, and enhancing airway hyperresponsiveness. In our study, treatment with montelukast significantly decreased eosinophils in induced sputum but did not alter airway responsiveness to histamine. In addition, a previous report also failed to show a significant improvement in airway responsiveness to methacholine after 12 weeks of treatment with montelukast. These results suggest that although CysLT\(_1\) receptor antagonists have both bronchodilatory and anti-
inflammatory actions, airway responsiveness may be regulated mainly by factors other than CysLTS.

In conclusion, our study confirms that the CysLT<sub>1</sub>-receptor antagonist, montelukast, exerts both bronchodilatory and anti-inflammatory effects in patients with asthma.

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Clinical Investigations