

## Original Article

# Effect of a leukotriene receptor antagonist, pranlukast hydrate, on airway inflammation and airway hyperresponsiveness in patients with moderate to severe asthma

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### ABSTRACT

Asthma is characterized by chronic airway inflammation and the recruitment of inflammatory cells, typically eosinophils and lymphocytes, into the airway. Although several chemical mediators are released during the inflammatory process of asthma, evidence strongly suggests that the cysteinyl leukotrienes (LT), LTC<sub>4</sub>, LTD<sub>4</sub>, and LTE<sub>4</sub>, play key roles in asthma. The short-term clinical efficacy of an LT receptor antagonist, pranlukast hydrate, in symptomatic patients with asthma who had already been treated with moderate to high doses of inhaled corticosteroids was therefore investigated. Treatment with pranlukast hydrate for 4 weeks significantly improved respiratory function and decreased asthma symptoms, the rescue use of inhaled β<sub>2</sub>-agonists, the number of peripheral blood eosinophils and serum levels of eosinophil cationic protein. Furthermore, airway inflammation, as evaluated by the percentage of eosinophils in induced sputum and airway responsiveness to histamine, decreased significantly after treatment. There were no significant changes in these parameters in control patients with asthma whose treatment was not changed over 4 weeks. These preliminary results

suggest that pranlukast hydrate, an LT receptor antagonist, is an effective agent in the management of asthma in combination with moderate to high doses of inhaled corticosteroids.

**Key words:** airway hyperresponsiveness, airway inflammation, asthma, eosinophils, leukotriene, leukotriene receptor antagonist.

### INTRODUCTION

Asthma is caused by chronic airway inflammation involving numerous inflammatory cells, including eosinophils and lymphocytes.<sup>1</sup> Airway inflammation, together with epithelial desquamation, edema, hypertrophy of airway smooth muscle and mucus glands and mucus plugging, induces airway obstruction and enhances airway responsiveness in asthma.<sup>2</sup> Although several chemical mediators are released during chronic airway inflammation, evidence strongly suggests that the cysteinyl leukotrienes (LT) C<sub>4</sub>, D<sub>4</sub>, and E<sub>4</sub> play key roles in asthma.<sup>3</sup>

In 1938, Feldberg and Kellaway first reported that chemical activity in lung perfusate causes slow-onset, sustained contraction of airway smooth muscle; these mediators were later called slow-reacting substances of anaphylaxis (SRS-A).<sup>4</sup> In 1983, the component molecules of SRS-A were identified as LTC<sub>4</sub>, D<sub>4</sub>, and E<sub>4</sub> by Samuelsson.<sup>5</sup> Although LT are produced by several types of inflammatory cell, most cysteinyl LT are produced by eosinophils and mast cells.<sup>6</sup> Leukotrienes are not stored in cells, but are newly generated from arachidonic acid after cellular activation.

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Pharmacologic actions of cysteinyl LT include potent bronchoconstriction, increased microvascular leakage and mucus secretion, chemoattraction of eosinophils and proliferation of human airway smooth muscle.<sup>7</sup> Because of their many actions, cysteinyl LT likely play important roles in asthma. In fact, LT are elevated in bronchoalveolar lavage fluid and urine during acute exacerbations of asthma.<sup>8–10</sup> Furthermore, challenges with allergen, exercise, cold air inhalation and aspirin result in the release of cysteinyl LT in asthma and antagonists of LT significantly inhibit the bronchoconstriction in response to these challenges.<sup>11–14</sup> Therefore, treatment with LT modifiers would be beneficial in the management of asthma.

In the present study, we investigated the short-term effects of an LT receptor antagonist, pranlukast hydrate, on asthma symptoms and respiratory function. Furthermore, changes in airway inflammation, as evaluated by hypertonic saline-induced sputum and airway responsiveness to histamine, were examined before and after treatment in patients with asthma who had already been treated with moderate to high doses of inhaled corticosteroids.

## METHODS

### Subjects

Eighty patients with asthma were enrolled in this study. No subjects had used oral steroids or had suffered from respiratory infections in the 4 weeks before enrolment or during the study period. The baseline forced expiratory volume in 1 s (FEV<sub>1</sub>) was more than 50% of the predicted values and improved more than 15% with inhalation of a  $\beta_2$  agonist. All patients had been treated with an inhaled corticosteroid (beclomethasone dipropionate (BDP), 800–1200  $\mu$ g/day) and bronchodilators (slow-release theophylline or an inhaled  $\beta_2$  agonist or both) for more than 3 months, but asthma symptoms persisted. Oral medications and inhaled corticosteroids were withheld for at least 12 and 24 h, respectively, before each examination visit. Plasma concentrations of theophylline in patients receiving this drug were measured before every study and were confirmed to be less than 5  $\mu$ g/mL. Informed consent was obtained before the study.

### Study design

After a 2-week run-in period, all patients underwent blood tests and tests of respiratory function and were randomly separated into two groups by an enveloped

method. One group of patients was additionally treated with pranlukast hydrate (225 mg twice daily, ONON, ONO Pharmaceutical Co. Ltd, Osaka, Japan), a specific LT receptor antagonist, and the other group of patients was treated without changes in all medications for 4 weeks as a control. Asthma symptoms, morning peak expiratory flow (PEF) and rescue use of  $\beta_2$ -agonists were monitored. Sputum induction with hypertonic saline inhalation and airway responsiveness to histamine were evaluated at enrolment and at the end of the study period.

### Clinical measurements

A daily record was kept of asthma symptoms, including breathlessness and wheezing on a scale of 0–9 (0 = no symptoms, 1 = breathlessness or wheezing, 3 = mild asthma, 6 = moderate asthma, 9 = severe asthma); cough on a scale of 0.5–1 (0.5 = infrequent cough, 1 = frequent cough); and sleep disturbance on a scale of 0–12 (0 = slept well through the night, 4 = slept most of the night despite dyspnea, 8 = slept with difficulty because of asthma, 12 = kept awake most of the night by asthma). Asthma symptoms were compared on the basis of the mean symptom scores during the observation period and the last 2 weeks of the study period. Morning PEF was recorded each day as the best of three successive trials with a Mini-Wright peak flow meter (Clement Clark International Ltd, Harlow, UK).

### Sputum induction and analysis

Sputum induction was performed before and after 4 weeks of treatment, as described previously.<sup>15</sup> Briefly, medications were stopped for at least 12 h, after which sputum was induced by inhalation of increasing concentrations of hypertonic saline (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 treated with Wright-Giemsa stain so that inflammatory cells could be counted. The percentages of eosinophils were determined by counting 900 cells under a light microscope.

### Airway responsiveness to histamine

Airway responsiveness to histamine was measured with a method described previously.<sup>16</sup> Briefly, patients inhaled normal saline, then inhaled doubling concentrations of histamine by tidal breathing from a Devilbis nebulizer

(Somerset, PA, USA) for 2 min until FEV<sub>1</sub> had decreased by more than 20% of the baseline value. Results are expressed as the provocative concentration causing a 20% decrease in FEV<sub>1</sub> (PC<sub>20</sub>).

### Peripheral blood analysis

Eosinophils were counted and serum concentrations of eosinophil cationic protein (ECP) were measured with radioimmunoassay.<sup>17</sup> Total and Der f-specific IgE antibody titers were measured with CAP-radioallergosorbent test (RAST; Pharmacia Diagnostics, Uppsala, Sweden).<sup>18</sup>

### Statistical analysis

The Wilcoxon test and the Mann–Whitney *U*-test were used for statistical analysis. Data are expressed as the mean ± SEM and a probability less than 0.05 was considered to indicate significance.

## RESULTS

Thirty-seven patients completed the study and three patients dropped out because of exacerbations of asthma that required oral or intravenous steroids in pranlukast treatment group. In the control group, 33 patients completed the study and seven patients dropped out due to asthma exacerbation. Characteristics of patients who completed the study are summarized in Table 1. Sputum was successfully collected from 28 and 27 patients and airway responsiveness to histamine was evaluated in 34 and 30 patients in pranlukast treatment group and control groups, respectively, before and at the end of the study period. There were no significant differences between the pranlukast treatment group and the control group in age ( $P = 0.233$ ), duration of asthma ( $P = 0.381$ ), symptom score ( $P = 0.426$ ), rescue use of inhaled  $\beta_2$ -agonist ( $P = 0.50$ ), pulmonary function including forced vital capacity (FVC) ( $P = 0.36$ ), %FVC ( $P = 0.503$ ), FEV<sub>1</sub> ( $P = 0.394$ ), FEV<sub>1%</sub> ( $P = 0.480$ ), maximum expiratory

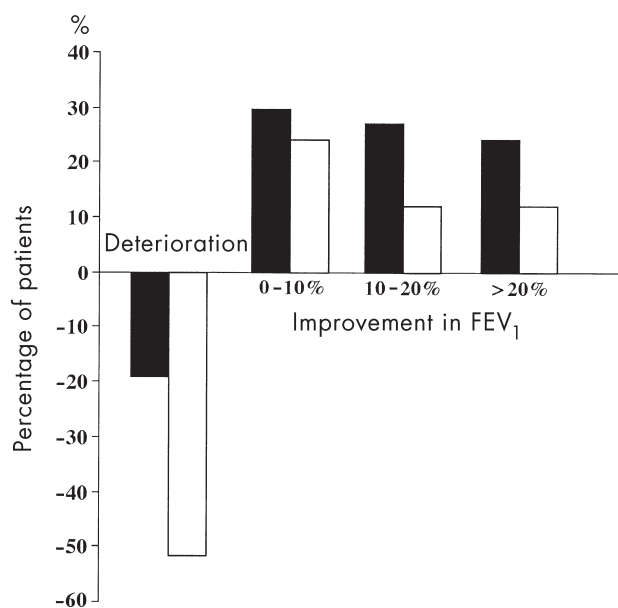
flow at 25% of the FVC divided by height ( $\dot{V}_{25/HT}$ ;  $P = 0.724$ ), morning PEF ( $P = 0.432$ ), number of peripheral blood eosinophils ( $P = 0.107$ ) and serum ECP levels ( $P = 0.868$ ) at baseline.

Outcome measures are summarized in Table 2. Although asthma symptoms continued during the observation period, treatment with pranlukast hydrate for 4 weeks significantly decreased the severity of asthma symptoms ( $4.7 \pm 1.2$  to  $1.8 \pm 0.4$ ;  $P < 0.03$ ). Significant improvements in respiratory function, including increases in morning PEF ( $366 \pm 17$  to  $389 \pm 17$  L/min;  $P < 0.01$ ) and FEV<sub>1</sub> ( $1.83 \pm 0.11$  to  $1.95 \pm 0.11$  L;  $P < 0.01$ ), were observed. Of the pranlukast group patients, 51.4% had an increase of more than 10% in FEV<sub>1</sub> after treatment with pranlukast hydrate compared with 24.2% of patients in the control group (Fig. 1). Deterioration in FEV<sub>1</sub> was observed for 18.9% of patients in the pranlukast group and 52.1% of patients in the control group (Fig. 1). A marker of peripheral airway dilatation,  $\dot{V}_{25/HT}$ , increased slightly but not significantly after treatment with pranlukast hydrate ( $0.46 \pm 0.05$  to  $0.48 \pm 0.04$  L/min per m;  $P = 0.057$ ). The rescue use of inhaled  $\beta_2$ -agonists was also significantly decreased after treatment with pranlukast hydrate ( $1.2 \pm 0.4$  to  $0.3 \pm 0.2$  puffs/week;  $P < 0.01$ ). There were no significant changes in asthma symptoms and respiratory function in control patients with asthma.

**Table 1** Patient characteristics

	Pranlukast group	Control group
Number	37	33
Male/Female	22/15	17/16
Age (years)	50.8 ± 2.4	48.4 ± 2.2
Duration (years)	8.8 ± 1.6	10.9 ± 1.9
Atopy/Non-atopy	20/17	17/16
BDP dose (µg/day)	1048.6 ± 39.1	1127.3 ± 53.5

BDP, beclomethasone dipropionate.



**Fig. 1** Percentage of patients with an increase or decrease in forced expiratory volume in 1 s (FEV<sub>1</sub>) at the end of the study period in the pranlukast (■) and control (□) groups.

Because asthma is caused by airway inflammation, we next investigated the effects of pranlukast hydrate on the number of peripheral blood eosinophils, serum ECP levels and percentages of sputum eosinophils. Treatment with pranlukast hydrate significantly decreased the number of peripheral blood eosinophils ( $437.1 \pm 55.0$  to  $266.1 \pm 30.9/\mu\text{L}$ ;  $P < 0.01$ ), serum ECP levels ( $27.9 \pm 5.8$  to  $18.1 \pm 2.1 \text{ ng/mL}$ ;  $P < 0.03$ ) and the percentages of sputum eosinophils ( $26.1 \pm 3.2$  to  $18.9 \pm 3.6\%$ ;  $P < 0.01$ ; Fig. 2). Furthermore, airway responsiveness to histamine was significantly decreased after treatment with pranlukast hydrate ( $715.3 \pm 228.3$  to  $1404.2 \pm 401.1 \mu\text{g/mL}$ ;  $P < 0.01$ ; Fig. 3). There was no significant change in the number of peripheral blood eosinophils, the serum ECP levels, the percentages of sputum eosinophils and airway responsiveness to histamine in control patients with asthma (Table 2, Figs 2,3).

## DISCUSSION

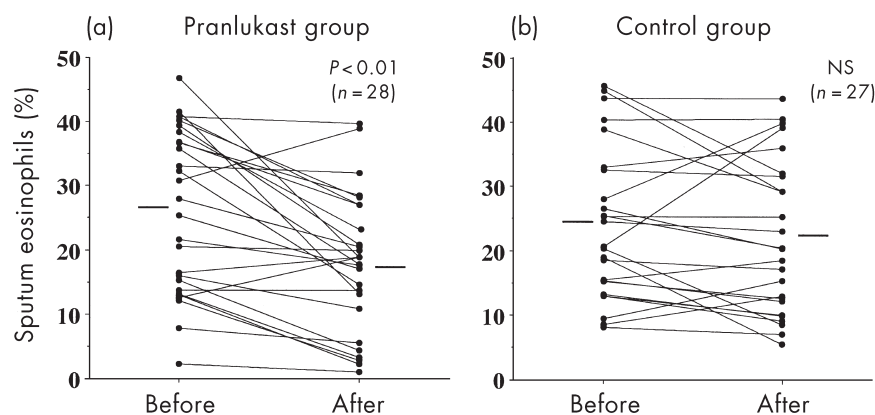
In this preliminary study, we have demonstrated that treatment with pranlukast hydrate, an LT receptor antagonist, significantly improves respiratory function and decreases asthma symptoms, airway inflammation and airway hyperresponsiveness in patients with asthma. Although our study was parallel and not a placebo-controlled design, it clearly shows that short-term treatment with this LT receptor antagonist significantly decreases airway inflammation and airway hyperresponsiveness. Furthermore, our results suggest that a LT receptor antagonist would be effective against asthma that was not completely controlled with moderate to high doses of inhaled corticosteroids.

Because the pathogenesis of asthma is chronic airway inflammation, guidelines for the treatment of asthma in

**Table 2** Outcome measures

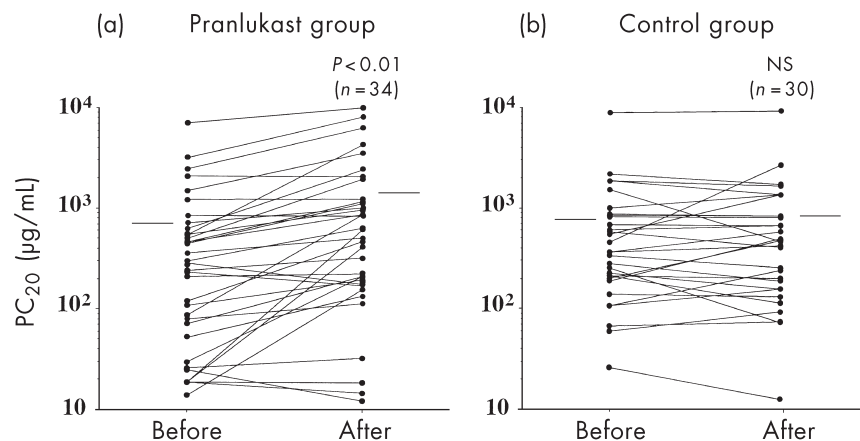
	Pranlukast group			Control group		
	Before	After	<i>P</i> value	Before	After	<i>P</i> value
Symptom score (score/week)	$4.7 \pm 1.2$	$1.8 \pm 0.4$	$<0.03$	$3.6 \pm 0.6$	$4.0 \pm 0.7$	NS
Rescue use of inhaled $\beta_2$ -agonist (puffs/week)	$1.2 \pm 0.37$	$0.3 \pm 0.2$	$<0.01$	$1.6 \pm 0.4$	$2.0 \pm 0.5$	NS
Pulmonary function						
FVC (L)	$2.41 \pm 0.13$	$2.57 \pm 0.15$	NS	$2.57 \pm 0.12$	$2.64 \pm 0.15$	NS
%FVC	$83.7 \pm 3.65$	$85.6 \pm 3.05$	NS	$86.5 \pm 2.29$	$88.7 \pm 2.68$	NS
FEV <sub>1</sub> (L)	$1.83 \pm 0.11$	$1.95 \pm 0.11$	$<0.01$	$1.96 \pm 0.10$	$1.92 \pm 0.10$	NS
FEV <sub>1\%</sub>	$74.3 \pm 1.64$	$74.7 \pm 2.11$	NS	$75.8 \pm 1.51$	$74.8 \pm 1.65$	NS
$\dot{V}_{25/HT}$ (L/s per m)	$0.46 \pm 0.05$	$0.48 \pm 0.04$	0.056	$0.48 \pm 0.04$	$0.46 \pm 0.04$	NS
Morning PEF (L/min)	$366 \pm 17$	$389 \pm 17$	$<0.01$	$386 \pm 18$	$394 \pm 17$	NS
Blood analysis						
Eosinophils(/ $\mu\text{L}$ )	$437.1 \pm 55.0$	$266.1 \pm 30.9$	$<0.01$	$318.2 \pm 43.2$	$321.2 \pm 34.2$	NS
Serum ECP(ng/mL)	$27.9 \pm 5.8$	$18.1 \pm 2.1$	$<0.03$	$27.0 \pm 2.1$	$23.9 \pm 2.4$	NS

FVC, forced vital capacity; FEV<sub>1</sub>, forced expiratory volume in 1 s;  $\dot{V}_{25/HT}$ , maximum expiratory flow at 25% of the FVC divided by height; PEF, peak expiratory flow; ECP, eosinophil cationic protein; NS, not significant.



**Fig. 2** Effects of pranlukast hydrate on the percentage of sputum eosinophils. The percentage of sputum eosinophils was measured during the observation period and at the end of the study period in pranlukast and control groups. NS, not significant.

**Fig. 3** Effects of pranlukast hydrate on airway responsiveness to histamine. Airway responsiveness to histamine was assessed during the observation period and at the end of the study period of the pranlukast and control groups. Results are expressed as the provocative concentration causing a 20% decrease in the forced expiratory volume in 1 s ( $PC_{20}$ ). NS, not significant.



Japan and other countries suggest that inhaled corticosteroids be used as the central agent in the treatment of mild to severe asthma.<sup>19–21</sup> Recently, the Expert Panel Report II of the National Heart, Lung and Blood Institute stated that treatment with LT modifiers, such as LT receptor antagonists and 5-lipoxygenase inhibitors, may be considered an alternative to low-dose inhaled corticosteroid therapy for patients with mild persistent asthma.<sup>20</sup> In contrast, Japanese guidelines suggest that LT receptor antagonists be used to treat not only mild persistent to moderate asthma but also severe asthma.<sup>19</sup>

Recent studies have shown that LT modifiers have inhibitory effects in clinical models of asthma, including allergen-induced, exercise-induced, cold air hyperventilation-induced and aspirin-induced asthma.<sup>11–14</sup> These results suggest that leukotriene modifiers may be useful for treating aspirin-induced asthma and exercise-induced asthma. Although LT modifiers have been reported to reduce symptoms, improve air flow, and reduce the rescue use of inhaled  $\beta_2$ -agonists in asthma, their effects on airway inflammation and airway hyperresponsiveness have not been fully investigated.

The finding that activated eosinophils and mast cells play important roles in asthma suggests that persistent generation of cysteinyl LT is a consequence of persistent airway inflammation. Several reports have confirmed that cysteinyl LT cause eosinophil chemotaxis *in vitro*.<sup>22,23</sup> In addition, inhalation of  $LTE_4$  increases the number of eosinophils in human bronchial biopsy specimens.<sup>24</sup> In the present study, we have demonstrated that short-term treatment of moderate to severe asthma with an LT receptor antagonist significantly decreases the percentage of eosinophils in induced sputum and serum ECP levels. In addition, we have found in a recent double-blind, placebo-controlled

trial that MK-476, another LT receptor antagonist, significantly decreases the number of sputum eosinophils in patients with mild to moderate asthma.<sup>25</sup> These results suggest that cysteinyl LT may be involved in airway eosinophilia in asthma.

Airway hyperresponsiveness is also a characteristic of asthma. Inhaled cysteinyl LT have been shown to induce airway hyperresponsiveness.<sup>26,27</sup> In addition, several clinical studies suggest that antagonists of cysteinyl LT can block airway hyperresponsiveness. One study has shown that treatment with pranlukast hydrate, an LT receptor antagonist, for 1 week decreased airway responsiveness to methacholine and another report has shown that treatment with a 5-lipoxygenase inhibitor, zileuton, for 13 weeks decreased airway responsiveness to cold air.<sup>28,29</sup> In the present study, we have demonstrated that treatment with pranlukast hydrate for 4 weeks decreased both airway inflammation and airway hyperresponsiveness in patients with moderate to severe asthma. Because baseline  $FEV_1$  was also increased after treatment with pranlukast hydrate, we cannot conclude that the resolution of airway inflammation was the only cause of the decreased airway hyperresponsiveness. In addition, although improvement of airway responsiveness to histamine was statistically significant, it remains unknown how this improvement might contribute to the clinical efficacy of this drug.

Several studies that have measured clinical outcome have shown that some patients respond to LT modifiers. If an increase in  $FEV_1$  of more than 10% after treatment is used as a cut-off point, 19 of the 37 patients (51.4%) in the present study were responders. Although the mean decrease in sputum eosinophils in responders ( $11.2 \pm 2.9\%$ ) was greater than that in non-responders

( $3.26 \pm 5.11\%$ ), it was not statistically significant ( $P = 0.056$ ). These results suggest that the LT receptor antagonist pranlukast hydrate has both bronchodilator and anti-inflammatory effects in responders.

In summary, our results provide biological proof that LT are important mediators in asthma and that an LT receptor antagonist, pranlukast hydrate, is a useful agent for patients with symptomatic asthma who have been treated with moderate to high doses of inhaled corticosteroids. Therefore, we conclude that the addition of a LT receptor antagonist to treatment with inhaled corticosteroids is beneficial in patients with moderate to severe asthma.

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