Pranlukast, a Cysteinyl Leukotriene Antagonist, Reduces Serum Eosinophil Cationic Protein Levels in Patients with Asthma

Shinichi ISHIOKA, Soichiro HOZAWA, Yoshinori HARUTA, Keiko HIYAMA, Akihiro MAEDA and Michio YAMAKIDO
Second Department of Internal Medicine, Hiroshima University School of Medicine, Hiroshima, Japan.

ABSTRACT

Cysteinyl leukotrienes (cysLTs) are considered to be important mediators involved in bronchial asthma and the ensuing eosinophilic inflammation. We evaluated the effects of pranlukast, a potent and selective cysLT receptor antagonist, on the clinical course and serum eosinophil cationic protein (ECP) levels of 10 asthmatic patients. A four-week administration of pranlukast increased the morning peak expiratory flow (PEF) ($p=0.007$) and decreased as-needed $\beta_2$-agonist use ($p=0.021$). Changes in the morning PEF inversely correlated with those in the serum ECP levels ($r=-0.80, p=0.0057$). These results suggest that cysLTs are important mediators involved in eosinophilic inflammation, a major pathophysiologic feature of bronchial asthma in humans.

Key words: Bronchial asthma, Cysteinyl leukotriene receptor antagonist, Pranlukast, Serum eosinophil cationic protein

Regardless of the type or severity, the underlying pathologic basis of bronchial asthma has been most recently characterized by chronic airway inflammation$^{20,24}$. Airway inflammation arises from many factors, including chemical mediators generated by resident airway cells and recruited leukocytes. In particular, cysteinyl leukotrienes (cysLTs) including LTC4, LTD4, and LTE4, metabolites of arachidonic acid via the 5-lipoxygenase pathway, make up the material known as slow-reactive substance of anaphylaxis (SRS-A). This was previously considered to play an important role in the pathophysiology of bronchial asthma$^{20}$. Moreover, cysLTs are potent mediators that induce bronchoconstriction$^{1,4}$, airway microvascular leakage, edema$^{20}$, mucus secretion$^{20}$ and eosinophil recruitment to the airway$^{4,10}$. Since pathophysiologic features of bronchial asthma can be explained by the action of cysLTs, cysLTs are considered to be the most important mediators involved in bronchial asthma. Therefore, the antiasthmatic drugs that alter the action or production of cysLTs are expected to show therapeutic potential.

Pranlukast is a novel, potent and selective cysLT receptor antagonist which is orally active$^{24}$. It has been shown to inhibit LTC4-and LTD4-induced bronchial contractions in guinea pigs$^{24}$ and humans$^{23,45}$, and also inhibit antigen-induced bronchoconstriction in guinea pigs sensitized with ovalbumin$^{24}$, and to attenuate allergen-induced early and late phase bronchoconstrictions in asthmatic patients$^{14,31}$. Furthermore, pranlukast has been shown to antagonize LTD4-induced microvascular leakage and eosinophil influx into the airways in guinea pigs$^{20}$. Moreover, the antiasthmatic effects of pranlukast have been confirmed clinically$^{5,13}$. Eosinophilic infiltration of the asthmatic airway is the most characteristic finding of bronchial asthma that differentiates asthma from other inflammatory airway diseases. Eosinophils are considered, therefore, to be the primary effector cells causing airway inflammation in bronchial asthma$^{10}$. Activated eosinophils are the major cells that produces cysLTs$^{17,20}$ and which release granule proteins such as major basic protein (MBP) and eosinophil cationic protein (ECP), thereby damaging the airway epithelium$^{12,20}$. Eosinophilic inflammation is considered to be a major component of airway inflammation in bronchial asthma. In recent years, serum ECP levels have been reported to reflect the presence of primed eosinophils and to serve as a clinical parameter for predicting the degree of eosinophil activation$^{30}$. They are reported also to be a good marker for evaluating eosinophilic inflammation in bronchial asthma$^{21}$. To date, few reports...
Table 1. Clinical characteristics of subjects

<table>
<thead>
<tr>
<th>No.</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Type</th>
<th>Severity</th>
<th>Eos (%</th>
<th>RIST (IU/ml)</th>
<th>RAST</th>
<th>Complication</th>
<th>Treatment*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>59</td>
<td>F</td>
<td>Ext</td>
<td>Moderate</td>
<td>5.0</td>
<td>645.8</td>
<td>None</td>
<td>AR</td>
<td>300  800</td>
</tr>
<tr>
<td>2</td>
<td>74</td>
<td>M</td>
<td>Ext</td>
<td>Moderate</td>
<td>11.2</td>
<td>621.7</td>
<td>JC</td>
<td>AR</td>
<td>300  800</td>
</tr>
<tr>
<td>3</td>
<td>48</td>
<td>F</td>
<td>Ext</td>
<td>Moderate</td>
<td>4.7</td>
<td>150.0</td>
<td>JC</td>
<td>AR</td>
<td>200  200</td>
</tr>
<tr>
<td>4</td>
<td>27</td>
<td>M</td>
<td>Ext</td>
<td>Mild</td>
<td>4.8</td>
<td>100.2</td>
<td>JC</td>
<td>AR</td>
<td>400  400</td>
</tr>
<tr>
<td>5</td>
<td>21</td>
<td>M</td>
<td>Ext</td>
<td>Mild</td>
<td>13.3</td>
<td>941.9</td>
<td>HD, mite, JC</td>
<td>AR</td>
<td>400  400</td>
</tr>
<tr>
<td>6</td>
<td>16</td>
<td>F</td>
<td>Ext</td>
<td>Mild</td>
<td>4.5</td>
<td>110.7</td>
<td>HD, mite, JC</td>
<td>AR</td>
<td>400  400</td>
</tr>
<tr>
<td>7</td>
<td>65</td>
<td>F</td>
<td>Ext</td>
<td>Moderate</td>
<td>12.0</td>
<td>11.2</td>
<td>HD, mite, JC</td>
<td>AR</td>
<td>400  400</td>
</tr>
<tr>
<td>8</td>
<td>54</td>
<td>F</td>
<td>Ext</td>
<td>Mild</td>
<td>4.0</td>
<td>213.0</td>
<td>JC</td>
<td>None</td>
<td>400  400</td>
</tr>
<tr>
<td>9</td>
<td>53</td>
<td>F</td>
<td>Ext</td>
<td>Moderate</td>
<td>1.9</td>
<td>83.0</td>
<td>HD, mite, HD, mite</td>
<td>None</td>
<td>400  400</td>
</tr>
<tr>
<td>10</td>
<td>54</td>
<td>F</td>
<td>Int</td>
<td>Moderate</td>
<td>1.1</td>
<td>30.4</td>
<td>None</td>
<td>None</td>
<td>400  200</td>
</tr>
</tbody>
</table>

*Ext=extrinsic, Int=intrinsic, Eos=peripheral eosinophil, RIST=radioimmunosorbent test, RAST=radioallergosorbent test, JC=Japanese cedar, HD=house dust, AR=allergic rhinitis, BDP=beclomethasone dipropionate, Theo=theophylline, PSL=prednisolone. In addition, all patients were treated with on-demand β-agonist inhalation.

The subjects comprised 10 asthmatic patients (4 with mild and 6 with moderate asthma) aged 16 to 74 years (average 47.1 years). Three men and 6 women had extrinsic asthma and one woman had intrinsic asthma (Table 1). The forced expiratory volume in one second (FEV₁,₅₀) of each patient improved by more than 15% after inhalation of 300 μg salbutamol sulfate. All the patients were non-smokers who developed mild wheezing and chest tightness rather than asthma attacks. These patients had been treated with as-needed β₂-agonist inhalation in addition to the following agents, without changes, for 6 months before starting this study: beclomethasone dipropionate (BDP, 200–800 μg/day) by inhalation in 6 patients, orally administered slow-release theophylline (200–800 mg/day) in 8, and orally administered prednisolone (2.5 mg/day) in one. Except in one case, corticosteroids had not been administered systemically to these patients for at least 6 months before starting the study. No patient suffered a viral infection during the 2 months prior to the start of the study. Concomitant medication was continued without modification throughout. The purpose of this study was explained carefully, after which informed consent was obtained from each patient. Furthermore, the study was approved by the ethics committee of our hospital.

Study Design

The total duration of this study was 6 weeks. Patients took 225 mg of pranlukast orally twice daily for 4 weeks after a 2-week trial period. All patients visited the outpatient clinic every two weeks and kept a booklet in which all medication was recorded daily during the course of the study. Morning and evening peak expiratory flow (PEF) values (the best of 3 attempts before taking medication) and the amount of as-needed inhaled β₂-agonist were recorded daily. The ASSESS™ peak flow meter (Health Scan Products Inc., Cedar Grove, NJ, USA) was used to measure PEF.

Measurement of Serum ECP Levels

Serum ECP levels were measured at the end of the trial and treatment periods, that is, before and after pranlukast administration. Blood samples were collected by venepuncture using an SST tube (Becton Dickinson, Tokyo, Japan), allowed to clot at room temperature for 60 min, centrifuged at 1,350 × g for 10 min; serum was collected and stored at −20°C. The ECP levels of these serum samples were measured collectively using a radioimmunoassay (RIA) kit (Pharmacia Upjohn, Tokyo, Japan).

Statistical Methods

The mean morning and evening PEF values and number of puffs of as-needed β₂-agonist were calculated every 2 weeks. The mean values of these parameters during the trial period were used as reference values. These variables, during trial and treatment periods were compared using the Wilcoxon signed rank test. The serum ECP level obtained at the end of the trial period, before starting treatment, was used as the reference value and compared with the value after treatment using the Wilcoxon signed rank test. All the
values are expressed as means ± standard errors of the mean (SEM). Differences at p values of less than 0.05 were considered to be statistically significant. In this study, treatment with pranlukast was considered to be clinically effective when the morning PEF values increased by more than 5% and the amount of β2-agonist inhaled decreased during the 3rd and 4th weeks of the treatment period compared with the values of the parameters obtained during the trial period.

RESULTS
The Effects of Pranlukast on the PEF and As-needed Inhaled β2-Agonist
The mean morning PEF was 356.9 ± 31.9 liters/min during the 2-week trial period, increasing significantly to 383.8 ± 28.6 liters/min during the first 2 weeks (weeks 1–2, p=0.015) and 385.6 ± 26.8 liters/min during the next 2 weeks (weeks 3–4, p=0.007) of pranlukast treatment (Fig. 1). The mean evening PEF was 400.0 ± 34.8 liters/min during the 2-week trial period and did not change significantly after pranlukast administration (415.4 ± 35.9 liters/min during weeks 1–2 and 417.7 ± 37.3 liters/min during weeks 3–4 of pranlukast treatment). As-needed use of inhaled β2-agonist during the 2-week trial period was 15.7 ± 6.3 puffs/week and it decreased significantly to 9.1 ± 3.7 puffs/week during weeks 1–2 (p=0.028) and 9.6 ± 4.5 puffs/week (p=0.021) during weeks 3–4 of pranlukast treatment (Fig. 2).

Based on the above changes in the variables, increase of the morning PEF by more than 5% and decrease of as-needed use of inhaled β2-agonist during weeks 3–4 of pranlukast treatment were observed in 7 of the 10 subjects. This suggested the clinical effectiveness of pranlukast.

Effects of Pranlukast on Serum ECP Levels
The mean serum ECP level before pranlukast treatment of all 10 subjects was 14.0 ± 2.3 µg/liter and did not change significantly after treatment (11.2 ± 3.0 µg/liter). However, the mean serum ECP level of the 7 patients in whom pranlukast administration was clinically effective decreased significantly from 15.5 ± 2.8 to 8.3 ± 1.8 µg/liter after pranlukast treatment (p=0.018), while that of the 3 patients in whom pranlukast administration was not clinically effective did not change significantly after pranlukast treatment (Fig. 3).

Fig. 1. Morning peak expiratory flow (PEF) before (Trial period) and after pranlukast treatment (Weeks 1–2 and Weeks 3–4). The mean morning PEF value during each 2-week period was calculated for 10 asthmatics and expressed as means ± SEM.

Fig. 2. The amount of inhaled β2-agonist needed before (Trial period) and after pranlukast treatment (Weeks 1–2 and Weeks 3–4). The number of puffs of β2-agonist needed during each 2-week period was calculated for 10 asthmatics and expressed as means ± SEM.

Fig. 3. Serum eosinophil cationic protein (ECP) levels before and after pranlukast treatment for 4 weeks in clinically effective patients (A, n=7) and ineffective patients (B, n=3). Pranlukast treatment was considered to be clinically effective when the morning peak expiratory flow (PEF) increased by more than 5% and the amount of inhaled β2-agonist decreased during the 3rd and 4th weeks of the treatment period compared with the corresponding values during the trial period.
Relationship between Morning PEF and Serum ECP Levels

The changes in the morning PEF (ΔPEF=morning PEF after treatment-morning PEF before treatment) and the changes in the serum ECP levels (ΔECP=serum ECP level after treatment-serum ECP level before treatment) were related to reduced serum ECP levels. After pranlukast treatment, changes in the morning PEF inversely correlated with changes in the serum ECP levels.

Based on the results of previous animal and in vitro experiments, it is suggested that cysLTs are important mediators involved in bronchial asthma. Clinically, cysLTs have been detected in plasma, urine, and bronchoalveolar lavage fluid (BALF) obtained from patients with asthma attack and after antigen challenge. Moreover, inhalation of cysLTs evoked bronchoconstriction in both normal and asthmatic subjects, and increased bronchial hyperresponsiveness. In recent years, based on the theory that cysLTs are important mediators involved in bronchial asthma, selective cysLT receptor antagonists such as pranlukast, zafirlukast, and montelukast, have been developed to control the action of endogenous cysLTs. These selective cysLT receptor antagonists inhibited allergen-induced early and late asthmatic responses (EARs and LARs) and showed antiasthmatic effects in clinical trials.

Chronic airway inflammation in bronchial asthma is mainly due to eosinophilic inflammation. It occurs even in patients with stable asthma, and activated eosinophils are the major cells that produce cysLTs. In view of the importance of cysLTs in bronchial asthma, attention has been focused on the relation between cysLTs and eosinophilic inflammation. Pranlukast attenuated LTD4-induced eosinophil influx into the airways of guinea pigs and biopsy analysis showed that inhalation of LTE4 by asthmatic subjects resulted in marked eosinophil infiltration of the airways. Therefore, cysLTs are considered to induce eosinophil recruitment into the airway.

Serum ECP was reported to be composed mainly of ECP released by eosinophils during the clotting process after blood collection and it reflects the releasability of peripheral primed eosinophils. In asthmatic patients, it was reported that serum ECP level correlates with the ECP concentration in BALF. Clinically, the serum ECP level is considered to be a good marker for evaluating eosinophil activation and ensuing eosinophilic inflammation in bronchial asthma. Tamaoki et al. reported that adding pranlukast prevented asthma deterioration and a rise in the serum ECP level provoked, in well-controlled asthmatic patients receiving high-dose inhaled corticosteroid, by a 6-week reduction of the inhaled dose by half. This was a finding compatible with our results.

In this study, pranlukast, a potent and selective cysLT receptor antagonist, exerted clinical effects in patients with asthma: pranlukast increased morning PEF and reduced the as-needed use of inhaled β2-agonist. These clinical effects of pranlukast were related to reduced serum ECP levels. After pranlukast treatment, changes in the morning PEF inversely correlated with changes in the serum ECP levels.

FIG. 4. Relationship between the changes in the morning peak expiratory flow (APEF) and serum eosinophil cationic protein level (ΔECP) following pranlukast treatment (n=10). An inverse correlation was observed (r=−0.80, p=0.0057). APEF=morning PEF after treatment-morning PEF before treatment. ΔECP=(serum ECP level after treatment-serum ECP level before treatment) × serum ECP level before treatment × 100.
the 7 patients in whom pranlukast administration was clinically effective did decrease significantly. Furthermore, analysis of the whole group demonstrated a significant inverse correlation between the changes in the morning PEF and those in the serum ECP levels after pranlukast treatment. Therefore, in asthmatic patients in whom pranlukast administration is effective for controlling the action of endogenous cysLTs, cysLTs are considered to play an important role in the major pathophysiologic features of bronchial asthma, such as eosinophil activation and the ensuing eosinophilic inflammation. By contrast, in asthmatic patients in whom pranlukast administration is ineffective for controlling the action of endogenous cysLTs, mediators other than cysLTs may make a greater contribution to the pathophysiology of bronchial asthma. In future, the cloning of cysLT receptors may elucidate the detailed mechanisms responsible for the action of cysLTs on eosinophils.

CONCLUSIONS

Pranlukast, a potent and selective cysLT receptor antagonist, was administered to patients with bronchial asthma. The clinical effects were related to serum ECP level reduction, a clinical parameter for evaluating eosinophilic inflammation. The results of our study, together with previous findings, suggest that cysLTs, which exert proinflammatory effects in humans, are important mediators contributing to the main pathophysiologic features of bronchial asthma, such as eosinophil activation and the ensuing eosinophilic inflammation.

ACKNOWLEDGEMENTS

Part of this study was supported by a research grant from the Japanese Ministry of Health and Welfare.

(Received August 11, 1999)
(Accepted November 1, 1999)


