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OBSERVATIONAL STUDY OF THE ADDITIVE EFFECTS OF PRANLUKAST ON INFLAMMATORY MARKERS OF CLINICALLY STABLE ASTHMA WITH INHALED CORTICOSTEROIDS AND LONG ACTING β2-AGONISTS

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Running head: Additional anti-inflammatory effects of LTRA to ICS and LABA

Abstract

Background: Little is understood about the additive effects of leukotriene receptor antagonists (LTRA) on asthmatics currently medicated with inhaled corticosteroids (ICS) and long acting β2-agonists (LABA).

Objectives: The present study examines the anti-inflammatory effects of the LTRA pranlukast in addition to ICS and LABA, among asthmatic patients with normal pulmonary function and unremarkable clinical symptoms.

Methods: Fifteen adult asthmatics participated in a 2-month, open-label, not controlled, prospective, multi-center, observational trial. Patients stabilized (predicted forced expiratory volume in 1.0 second > 80%) by medication with ICS and LABA were also given pranlukast (450 mg daily). Asthma-related symptoms, pulmonary function, blood eosinophil counts and several inflammatory markers in sputum were monitored at week 0, as well as at 4 and 8 weeks after starting pranlukast.

Results: Adding pranlukast did not further improve blood eosinophil counts, pulmonary function and symptoms, but significantly attenuated sputum cysteinyl leukotrienes, tumor necrosis factor (TNF)-α and interleukin (IL)-5 concentrations.

Conclusions: Although the clinical relevance remains obscure, adding an LTRA attenuates allergic airway inflammation in some asthmatics undergoing treatment with
ICS and LABA.

**Keywords:** Asthma

Leukotriene receptor antagonist

Long acting β2-agonist

Airway inflammation

Inhaled corticosteroid

Additive therapy
Introduction

Asthma is a chronic inflammatory disease of the airways that is characterized by reversible airway obstruction and bronchial hyperresponsiveness [1]. Although inhaled corticosteroids (ICS) comprise the foundation of treatment for asthmatics of all ages [2], asthma persists in a considerable number of patients undergoing this treatment. Current international guidelines recommend increasing the dose of ICS or adding inhaled long acting β2 agonists (LABA), leukotriene receptor antagonists (LTRA) or sustained released theophylline to ICS when patients are not fully controlled [2].

The role of cysteinyl leukotrienes (cysLTs) as mediators of asthma has been extensively studied and is widely accepted. In addition to their potent bronchoconstrictive properties [3], cysLTs induce other inflammatory responses characteristic of asthma, including tissue edema, mucous secretion and increased airway responsiveness to histamine [4]. Pranlukast is an LTRA that apparently improves clinical symptoms, pulmonary function and airway inflammation when taken in addition to ICS [5-8]. We previously reported that pranlukast significantly attenuates tumor necrosis factor (TNF)-α production from human peripheral monocytes, interleukin (IL)-5 production from human lung parenchyma and cysLT production in lung tissues of a murine model of allergic asthma [9-11]. The clinical efficacy of LTRA has been compared with that of LABA, another class of anti-asthmatic agents [12]. When ICS and LABA are applied in a single device to adult patients, 40% remain
symptomatic, and symptoms remain difficult to control in 5% to 10% of them [13]. Thus, the additional effects of LTRA on ICS and LABA should be thoroughly understood. One report has indicated that administering LTRA to patients suboptimally controlled by ICS and LABA significantly improves clinical symptoms and pulmonary function [14]. However, whether adding LTRA further improves airway inflammation among patients with normal pulmonary function and stable symptoms who are already on ICS and LABA therapy is uncertain.

The present study examines whether pranlukast confers anti-inflammatory effects on clinically stable patients with normal pulmonary function under ICS and LABA management.
Subjects and Methods

Subjects

Fifteen adult asthmatics participated in a 2-month, open-label, not controlled, prospective, multi-center observational trial between July 2005 and March 2006 at 4 institutions in Nagasaki prefecture (Japan). Local ethical committees approved the study protocol and written informed consent was obtained from all participants. Eligible individuals comprised adults with asthma diagnosed by a physician, and who had received a daily fixed dose of ICS and LABA for at least 1 year preceding the study. All participants had recorded peak expiratory flow (PEF) and kept an asthma diary. At entry, predicted forced expiratory volume in 1.0 second (FEV1) > 80% was acceptable. Exclusion criteria included pregnancy and/or lactation, history of life-threatening asthma, hospitalization for asthma within 6 months, and oral or parenteral corticosteroids and LTRA administered 4 weeks before entry.

Study design

Following the confirmation of clinical stability including a physical examination, questionnaire and pulmonary function measurements, all patients were orally treated with pranlukast (ONON®, ONO pharmaceutical Co. Ltd., Osaka, Japan; 225 mg b.i.d) for 8 weeks. During this period, patients were asked to grade symptoms of breathlessness, wheeze, chest tightness, sputum production, limitation of daytime
activity, and sleep disturbance on a scale from 0 - 3 (no symptoms, 0; mild, 1; moderate, 2; severe, 3). They also recorded the number of puffs of short-acting β2 agonists (SABA), as well as morning and evening PEF. All participants were required to be taking a stable dose of ICS and LABA throughout the treatment period. Other asthma medications such as xanthines and inhaled anticholinergics were prohibited during the study period. Peripheral blood was collected, sputum was induced and pulmonary function was measured at week 0, as well as at 4 and 8 weeks after starting pranlukast. Adverse effects associated with pranlukast were also monitored throughout the study period.

Sputum induction and measurement

Sputum was induced at each institute. All participants gargled with tap water before sputum collection to reduce possible contamination. Those who could not produce sputum received nebulized saline via an ultrasonic nebulizer (NE-U12; OMRON Co, Tokyo) to induce production. Sputum samples were collected from each participant after coughing deeply at 3 to 5 minute intervals, snap-frozen and sent to the Second Department of Internal Medicine, Nagasaki University School of Medicine for processing as described in our previous reports [15]. In brief, samples were diluted with 2 ml of Hank’s balanced salt solution containing 1% dithiothreitol (Sigma Chemicals, Poole, UK), and gently vortex mixed at room temperature. After centrifugation at 400 ×
g for 15 minutes at 4°C, the supernatant was collected and stored at -80°C for eosinophil cationic protein (ECP), cysLT and cytokine assays.

**Measurement of ECP, cysLT and cytokines**

Sputum levels of ECP, cysLTs, IL-5, TNF-α, eotaxin and interferon (IFN)-γ were determined by radioimmunoassay (Pharmacia ECP RIA, Pharmacia, Uppsala, Sweden), enzyme immunoassay (EIA, Cayman Chemical company, Ann Arbor, MI) and enzyme-linked immunosorbent assays (ELISA, R&D systems, Inc. McKinley Place, NE), respectively. The lower detection limits of ECP, cysLTs, IL-5, TNF-α, IFN-γ and eotaxin were 2.0 μg/ml, and 13, 3.0, 4.4, 27.5, and 10.0 pg/mL, respectively.

**Measurements of pulmonary function**

Clinical examinations confirmed the absence of dyspnea, wheezing, or rhonchi even on forced expiration on the day of the test. The FEV1 and forced vital capacity (FVC) were measured using a spirometer (Superspiro, DISCOM-21 FX; Chest MI Co, Tokyo, Japan).

**Statistical analysis**

Results are expressed as means ± standard error of the mean (SEM). The statistical significance of differences within groups was examined using the Wilcoxon rank-sum
test. Statistical significance was established at $P < 0.05$. 
Results

Clinical responses and pulmonary functions

Of the 19 initially enrolled patients, 3 dropped out due to failure to produce sputum samples at the next clinic visit. The remaining 15 patients participated in the analysis (6 from 1 institution, 5 from 1 and 2 each from 2 institutions). Table 1 shows the features of the patients. None of them smoked and all of them used the LABA, salmeterol (Serevent®, Glaxo Smith Kline K.K., Tokyo, Japan). None of the patients experienced asthma attacks, night symptoms or increased their SABA use during the study period. No-one needed additional rescue therapy with any type of drugs including oral corticosteroids. Furthermore, none of the patients were withdrawn because of adverse effects related to pranlukast. Even though the FEV1 of the participants at entry was normal, they had persistent, slight asthma-related symptoms (Table 2). Adding LTRA to the ICS and LABA did not significantly reduce symptom scores or SABA use and did not significantly increase FEV1, FVC and PEF (Table 2).

Inflammatory markers

Adding pranlukast did not significantly reduce peripheral blood eosinophil counts (weeks 0, 4 and 8: 123.1 ± 37.9, 99.5 ± 68.4 and 188.5 ± 139.2/μl, respectively; p > 0.1). Concentrations of ECP in induced sputum did not significantly change after adding pranlukast (weeks 0, 4 and 8: 54.8 ± 24.9, 67.4 ± 22.1 and 83.7 ± 30.0 μg/l,
respectively; $p > 0.1$). Neither eotaxin nor IFN-$\gamma$ was detected in induced sputum throughout the study period. Although patients remained clinically stable and pulmonary functions were essentially normal under ICS and LABA management before adding pranlukast, we detected TNF-$\alpha$, IL-5 and cysLT (Fig. 1). Adding pranlukast significantly reduced levels of these three inflammatory factors in induced sputum (Fig. 1).
Discussion

Inhaled corticosteroids comprise the central therapeutic regimen of current international guidelines for asthma management [2]. When asthmatic symptoms are inadequately controlled by ICS alone, the addition of LABA, LTRA or theophylline should be considered. Because of serious adverse effects and decreasing bronchodilative potency [2, 15, 16], theophylline is gradually being replaced with either LABA or LTRA. Studies of LABA and LTRA in addition to ICS have suggested that they have superior bronchodilative and anti-inflammatory effects, respectively [12, 18-20]. The addition of LTRA improves persistent airway inflammation but not pulmonary function even when symptoms are controlled well by ICS alone [21]. Nonetheless, few studies have evaluated the additive effects of LTRA on asthmatics already undergoing treatment with ICS and LABA. Many adult asthmatics remain symptomatic despite ICS and LABA therapy and cysLT levels are relatively resistant to ICS [22]. We therefore postulated that airway inflammation persists in some asthmatics despite ICS and LABA administration, and that adding LTRA could further attenuate allergic airway inflammation.

Some reports indicate that adding LTRA does not significantly improve either symptoms or pulmonary function in asthmatics already undergoing treatment with ICS and LABA [20, 23, 24]. The results of the present study agreed with these findings, but showed that simply monitoring symptoms and respiratory function can overlook the
potential benefits of LTRA in patients receiving ICS and LABA. The observed anti-inflammatory effects of LTRA on sputum could also perhaps be due to other intervention in this open study (e.g. improved compliance, optimization of the inhalation technique, or avoidance of exposure to allergens). Thus, a randomized, controlled study is required to confirm the present results.

One of the studies alluded to above found that adding montelukast to ICS and LABA significantly improved inflammatory biomarkers (exhaled NO and eosinophils), and airway hyperresponsiveness, even though PEF and symptoms were not altered [20]. In some patients with stable asthma treated with ICS (and in approximately half of patients receiving LABA), inflammation is not completely suppressed and adding LTRA can provide the complementary effect of inflammatory control, with significant improvement in the quality of life [21]. In accordance with these reports, pranlukast in the present study significantly inhibited inflammatory markers (cysLTs, TNF-α, and IL-5) in induced sputum. This in turn indicates that the potential benefits of LTRA on airway inflammatory components are dissociated from lung function in patients receiving LABA who are likely to be maximally bronchodilated [25]. When monitoring the effects of LTRA in conjunction with ICS and LABA, various end points including airway inflammation must be assessed before drawing conclusions. On the contrary, other inflammatory markers including eotaxin and IFN-γ remained below the limits of detection and pranlukast did not attenuate ECP concentrations in the present study.
Corticosteroids and salmeterol inhibit eotaxin production [26, 27]. These indicate that eosinophilic airway inflammation and IFN-γ production are steroid- and/or LABA-sensitive and thus cannot be further attenuated by adding pranlukast.

The clinical relevance of the finding that pranlukast somewhat improves airway inflammation without improving pulmonary function and clinical symptoms in asthmatics already receiving ICS and LABA is uncertain. The present study does not indicate simply that the anti-asthmatic effects of LTRA are superior to those of LABA. Previous well-controlled studies have shown the advantage of LABA over LTRA as add-on therapy to ICS in some outcomes including the time to first exacerbation and treatment failure [28, 29]. Leigh et al. also demonstrated that LTRA failed to show additive effects on ICS [30]. The persistence of airway inflammation might result in irreversible airway remodeling in the future even when bronchodilation is sufficient and asthma-related symptoms are optimally controlled by conventional therapy with ICS and LABA. Anti-remodeling effects of LTRA have been demonstrated in animal models, indicating that the addition of LTRA will improve pulmonary function as well as clinical symptoms in the future [31]. Presently, direct evaluation of airway remodeling in chronic asthma at clinics is not simple. For a better understanding of the role of LTRA in patients already receiving ICS and LABA, simple and non-invasive methods with which to evaluate airway remodeling and long-term studies using double-blind protocols are required.
References


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Figure legends

**Fig. 1. Serial changes among inflammatory parameters in sputum.**

Concentrations of cysLTs, IL-5, and TNF-α in induced sputum of asthmatics already treated with ICS and LABA at 0, 4, and 8 weeks after pranlukast initiation. Bars represent means ± SEM (n = 15). *p < 0.05 vs. 0 weeks, †p < 0.05 vs. 4 weeks.
Table 1. Patients’ characteristics.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (number)</td>
<td>15</td>
</tr>
<tr>
<td>Age (y)</td>
<td>48.9 ± 5.5</td>
</tr>
<tr>
<td>Male/female</td>
<td>7/8</td>
</tr>
<tr>
<td>Atopy/non atopy</td>
<td>10/5</td>
</tr>
<tr>
<td>Smoking</td>
<td>None</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>155.6 ± 13.7</td>
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<tr>
<td>Weight (kg)</td>
<td>52.4 ± 2.9</td>
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<tr>
<td>BMI*</td>
<td>21.5 ± 0.8</td>
</tr>
<tr>
<td>Symptom score /week</td>
<td>2.0 ± 1.2</td>
</tr>
<tr>
<td>Use of SABA** /week</td>
<td>3.4 ± 3.7</td>
</tr>
<tr>
<td>FEV1, %predicted</td>
<td>81.0 ± 5.6</td>
</tr>
<tr>
<td>Inhaled corticosteroids (µg/d)</td>
<td>780.0 ± 48.3</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SEM or as numbers. *BMI, Body Mass Index;
**SABA, short acting β2 agonist.
Table 2. Serial changes in symptoms and pulmonary functions.

<table>
<thead>
<tr>
<th></th>
<th>Week 0</th>
<th>Week 4</th>
<th>Week 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Symptom score (/week)</td>
<td>2.0 ± 1.2</td>
<td>1.8±1.2</td>
<td>1.8±1.2</td>
</tr>
<tr>
<td>Morning PEF (L/min)</td>
<td>326.5 ± 91.3</td>
<td>332.4 ± 77.2</td>
<td>329.5 ± 101.1</td>
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<tr>
<td>Evening PEF (L/min)</td>
<td>333.6 ± 56.6</td>
<td>340.2 ± 88.1</td>
<td>344.3 ± 87.6</td>
</tr>
<tr>
<td>FEV1 (%predicted)</td>
<td>81.0 ± 11.2</td>
<td>82.8 ± 14.0</td>
<td>82.5 ± 10.7</td>
</tr>
<tr>
<td>FVC (L)</td>
<td>2.67 ± 0.37</td>
<td>2.71 ± 0.41</td>
<td>2.69 ± 0.35</td>
</tr>
<tr>
<td>FEV1/FVC (%)</td>
<td>79.3 ± 3.9</td>
<td>80.1 ± 3.7</td>
<td>78.6 ± 4.1</td>
</tr>
<tr>
<td>Use of SABA* (/week)</td>
<td>3.4 ± 3.7</td>
<td>2.4 ± 4.2</td>
<td>2.3 ± 4.4</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SEM. *SABA, short acting β2 agonist.
Fig. 1. Serial changes among inflammatory parameters in sputum.