Impact of add-on pranlukast in stable asthma; the additive effect on peripheral airway inflammation

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KEYWORDS
Controlled asthma; Distal airway inflammation; Fraction of exhaled nitric oxide (FeNO); Leukotriene receptor antagonist (LTRA); Peripheral airway/alveolar NO concentration (CANO); Pranlukast

Summary
Background: The importance of airway inflammation has been highlighted in the pathophysiology of asthma. Even in controlled asthmatics treated with inhaled corticosteroid (ICS), residual airway inflammation is reported. Systemic therapy with oral leukotriene receptor antagonist, pranlukast, may have additive effects to improve asthma control.

Methods: Twenty-five controlled asthmatics treated with ICS or ICS plus long-acting β2-agonist (LABA) were enrolled for a randomized crossover trial evaluating the effect of additional oral pranlukast. The patients were assigned to two groups receiving ICS (+LABA) or ICS (+LABA) + pranlukast for 8 weeks. After washout period, two groups were switched over for another 8 weeks. Fraction of exhaled nitric oxide (FeNO), lung function tests, peak expiratory flow (PEF) and asthma control test (ACT) were evaluated at the beginning and end of each period. Central airway NO flux (J'awNO) and peripheral airway/alveolar NO concentration (CANO) were measured and adjusted for axial NO back-diffusion.

Results: FEV1, % predicted, forced expired flow (FEF)25 e75, % predicted, morning PEF and ACT were significantly increased after the addition of pranlukast. Oral pranlukast administration significantly decreased both CANO and corrected CANO.

Abbreviations: ICS, inhaled corticosteroid; LABA, long-acting β2-agonist; NO, nitric oxide; FeNO, fraction of exhaled nitric oxide; J'awNO, central airway nitric oxide flux; CANO, peripheral airway/alveolar nitric oxide concentration; LTRA, leukotriene receptor antagonist; COPD, chronic obstructive pulmonary disease; PEF, peak expiratory flow; ACT, asthma control test; SFC, salmeterol/fluticasone propionate combination; FEF, forced expired flow.

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Introduction

Asthma is an airway inflammatory disease of uncertain etiology, involving the large and small airways and alveoli resulting in varying airway hyper-responsiveness and bronchoconstriction. Current guidelines for asthma management concur that inhaled corticosteroids (ICS) are the mainstay of anti-inflammatory therapy in asthma. Long-acting \( \beta_2 \)-agonist (LABA) is recommended as add-on therapy to ICS in patients with moderate persistent asthma that is not well-controlled by regular ICS administration. Even though asthma symptoms are stable with ICS or ICS plus LABA therapy, residual airway inflammation often occurs in both central and peripheral airways in the lung, which may lead to airway remodeling.

Recent studies have redirected attention to the role of airway inflammation in both the central and the small airways in asthma. Several studies using lung tissues from asthmatic patients revealed inflammatory and structural changes in the small airways and alveoli, which were comparable to those within the central airways. Moreover, the severity of peripheral airway changes has been correlated with disease instability, resistance to therapy and excessive airway narrowing. Therefore, not only the central airways but also the distal airways should be important therapeutic targets in optimization of asthma control and prevention of airway remodeling. However, routine approaches, such as lung function tests, are limited by their test-retest variability and lack of specificity in reflecting small airways inflammation.

Measurement of the fraction of exhaled nitric oxide (FeNO) is a relatively simple, non-invasive test for monitoring endogenous inflammatory signals and responses to therapy in asthma. Tsoukias and George have developed a two-compartment model that has been used to quantify the amounts of FeNO arising from the large central airways and the peripheral small airway/alveolar site separately. Several studies have shown increased levels of both central airway NO flux (\( J_{awNO} \); nl/s) and peripheral airway/alveolar NO concentration (\( C_{ANO} \); ppb) in asthma. In particular, \( C_{ANO} \) is correlated with eosinophil cationic protein levels and eosinophil count in bronchoalveolar lavage fluid. \( C_{ANO} \) is a reproducible parameter reflecting peripheral airway inflammation, which does not vary diurnally and can be measured in asthmatic by simple breathing maneuvers. It has been reported that elevated FeNO at 50 ml/s in asthmatics was reduced by ICS therapy, whereas systemic therapy, such as oral corticosteroid administration, was needed to reduce increased \( C_{ANO} \).

Leukotriene receptor antagonists (LTRAs) are recommended as an optimal alternative for add-on therapy to ICS or ICS plus LABA in guidelines. Pranlukast, a systemic delivered LTRA, has been shown to have clinical efficacy in moderate to severe asthma when added to ICS therapy. While the improvements in FEV\(_1\) are generally modest, asthma symptoms and quality of life appear to be improved to greater degree with LTRA administration than FEV\(_1\), which could be due to systemic therapeutic effects with better adherence for LTRA as an orally administered agent. However, the therapeutic benefit of add-on LTRA to controlled asthma has not been fully established. The aim of this prospective crossover study was to investigate the impact of add-on pranlukast administration to stable asthma treated with ICS alone or ICS plus LABA therapy. In particular, we evaluated both large and small airway/alveolar sites in terms of inflammation using NO measurement.

Materials and methods

Study subjects

We recruited patients with asthma whose disease was classified as “controlled” according to the GINA criteria of asthma control. Briefly, the patients were free from asthma symptoms, limitations of activities, exacerbation and more than three rescue treatments a week even when required. Each patient had received ICS or ICS plus LABA for 1 year or more, with no change in dose within the previous 8 weeks. Patients who met the following criteria were excluded from this study: 1) treatment with LTRA in the previous 3 months, 2) treatment with oral or intravenous corticosteroids in the previous 4 weeks, 3) chronic obstructive pulmonary disease (COPD) using high-resolution computed tomography or lung function tests, and exhibited no or weak responsiveness against an inhaled short-acting bronchodilator.

Ethics and study design

This randomized crossover trial was conducted at Hamamatsu University School of Medicine. Our Institutional Review Board approved the study protocol and each patient gave written informed consent. The study was registered in the UMIN Clinical Trials Registry (UMIN-CTR) system (http://www.umin.ac.jp/, NO. UMIN 00003781).

The patients were randomly divided into two groups receiving add-on oral pranlukast (450 mg daily) and no-add-on treatment for 8 weeks (phase 1). After a washout period (4–8 weeks), the two groups were switched over to the opposite group for 8 weeks (phase 2) so that the trial was performed in a crossover manner (Fig. 1). FeNO at 50, 100, 150, and 200 ml/s, lung function tests, peak expiratory flow (PEF) in the morning and evening, and asthma control test (ACT) were evaluated at the beginning and the end of each phase.
period. $J'_{\text{awNO}}$ and $C_{\text{ANO}}$ were calculated using the two-compartment model\(^{12}\) and corrected by axial NO back-diffusion.\(^{21}\) If the patients had already used therapeutic drugs other than LTRA, they were allowed to use these medications with no change in the dosage or regimen. Patients who had asthma exacerbation that required hospitalization were removed from the study. Rescue inhalation of a short-acting $\beta_2$-agonist was used on demand to control symptoms throughout the study.

Measurement of FeNO

All subjects abstained from food and coffee for 2 hours prior to the studies. FeNO was measured prior to spirometry at 4 separate constant expiratory flow rates: 50, 100, 150, and 200 ml/s, in triplicate and the mean of 3 values obtained within 10% of each other was reported as determined using a Sievers NOA280i chemiluminescence analyzer (GE Analytical Instruments, Boulder, Colorado, USA). We used a restricted exhaled breath technique to exclude nasal NO. While sitting, subjects inhaled NO-free air to their total lung capacity and then exhaled into the analyzer mouthpiece attached to a one-way valve. Any exhalation that did not meet the American Thoracic Society requirements was not accepted by the REB system (GE Infrastructure Water & Process Technologies). The technique of Tsoukias and George was used to calculate $J'_{\text{awNO}}$ (y intercept) and $C_{\text{ANO}}$ (slope) using a linear regression line with data from at least 3 separate expiratory flow rates required for each subject.\(^{12}\) Correction was made for potential underestimation of $J'_{\text{awNO}}$ due to axial back-diffusion of NO using the method of Condorelli et al.\(^{21}\) by multiplying $J'_{\text{awNO}}$ by a factor of 1.7. Furthermore, to adjust for possible spurious overestimation of values for $C_{\text{ANO}}$, initial uncorrected $J'_{\text{awNO}}$ was divided by a correction factor of 0.53 and subtracted from initial uncorrected $C_{\text{ANO}}$.

Statistical analysis

The efficacy of pranlukast administration was assessed by comparing the changes in lung function tests, morning and evening PEF, asthma symptoms and FeNO levels. All values were analyzed using StatView version 5 (SAS Institute Inc. North Carolina, USA). Data for those with asthma were initially analyzed to determine normality of distribution using Kolmogorov–Smirnov test. Normally distributed variables are described as means and standard deviations, while non-normally distributed variables (FeNO data) were expressed as medians with interquartile ranges (medians, interquartile ranges). The comparison of lung function tests, PEF and ACT, was carried out using Student’s t-test for paired samples. The Wilcoxon matched-pair signed-rank test was used for FeNO. Values of $p < 0.05$ were considered significant.

Results

Characteristics of the patients

A total of 25 patients were enrolled in this study. All subjects completed both add-on oral pranlukast treatment and no-add-on treatment and were included in the analyses without any adverse effects. The characteristics of the patients at enrollment are shown in Table 1. Briefly, the median age was 66 years, 16 patients were women (64%) and the median duration of asthma was 132 months. Seventeen patients (68%) were atopic as judged by clinical manifestation and their serum IgE level. Among the group, 17 patients had never smoked and 8 patients were former smokers. All subjects used ICS or ICS plus LABA, and the median dose of ICS was 400 $\mu$g (fluticasone propionate conversion).

Figure 1  Study design. The patients were randomly divided into two groups receiving add-on oral pranlukast (450 mg daily) and no-add-on treatment for 8 weeks (phase 1). After a washout period (4–8 weeks), the two groups were switched over to the opposite group for 8 weeks (phase 2) so that the trial was performed in a crossover manner. Measurement of lung function tests, FeNO and ACT, were performed at the beginning and end of phase 1 and 2. FeNO, fraction expiratory nitric oxide; ACT, asthma control test; ICS, inhaled corticosteroid; LABA, long-acting $\beta_2$-agonist.
Lung function and asthma control

There were no significant differences in the lung function tests, morning PEF and ACT at the beginning of each period. During the no-add-on treatment period, no significant changes were found in lung function tests, PEF and ACT between the beginning and the end of the period (Table 2). FEV₁, % predicted, forced expired flow (FEF) 25–75, % predicted, morning PEF and ACT were significantly increased at the end of add-on oral pranlukast period compared with those at the beginning of the period.

NO Gas exchange

The values of FeNO at 50 ml/s at the beginning of add-on oral pranlukast treatment period and no-add-on treatment period were almost identical. No significant change was found in the FeNO at 50 ml/s between the beginning and the end of each period (Fig. 2A). The value of J'awNO was equal between the beginning and the end of the add-on oral pranlukast treatment period (1.35, 0.55–2.15 and 1.10, 0.40–2.50 nl/s, respectively, p = 0.44) as well as no-add-on treatment period (1.50, 0.80–3.30 and 1.50, 0.50–2.70 nl/s, respectively, p = 0.70) (Fig. 2B). Interestingly, there was a statistically significant reduction in CANO level at the end of the oral pranlukast treatment period (4.10, 2.30–6.60 ppb) compared with that at the beginning (6.45, 4.23–10.70 ppb) (p = 0.04) (Fig. 2C). As shown in Fig. 3, the results of J'awNO and CANO after correction for NO axial back-diffusion indicated a similar tendency, and corrected CANO was significantly lower at the end of oral pranlukast treatment period compared with that at the beginning of the period (p = 0.04).

Discussion

In the present study, we have shown that additional administration of oral pranlukast, a leukotriene receptor antagonist, to stable asthmatic patients under ICS or ICS plus LABA therapy have clinical benefit to improve asthma symptoms and physiology of the lung, FEV₁, % predicted, FEF 25–75, % predicted and morning PEF. In addition, add-on oral pranlukast significantly reduced the CANO level, a parameter of small airway inflammation, without any changes of FeNO at 50 ml/s and J'awNO. These results indicate that add-on oral administration of pranlukast has additive therapeutic benefit in asthma under ICS or ICS plus LABA therapy together with decreasing small airway inflammation.

Several clinical studies have already shown the therapeutic effects of pranlukast in asthma. Previous reports have demonstrated that pranlukast had steroid-sparing effects when it was administered to moderate to severe asthmatics combined with ICS. However, it has remained to be determined whether add-on oral pranlukast for controlled asthmatics treated with ICS or ICS plus LABA has additional benefit. In the present study, we have shown that add-on pranlukast administration has additive therapeutic effects, such as increasing ACT, FEV₁, % predicted, FEF 25–75, % predicted and morning PEF, even in controlled asthmatics under ICS or ICS plus LABA therapy. Recently, Ohbayashi et al. reported that add-on pranlukast with salmeterol/fluticasone propionate combination (SFC) did not change any pulmonary function index in well-controlled moderate asthma. All the patients in their study were treated with ICS plus LABA as SFC, whereas 9 of 25 patients in the present study were treated with ICS only (without LABA), which may have caused different effects of pranlukast administration in each study, especially in terms of bronchodilation. Further investigation will be needed to determine the different effects of add-on pranlukast to the patients treated with ICS only and ICS plus LABA.

The importance of distal airway inflammation has been highlighted in the pathophysiology of asthma. Increased mucus plugging and thickening of the smooth muscle, submucosa and adventitia in the peripheral airways have been demonstrated in the lung of patients with asthma. Activated eosinophils and expression of interleukin-5 mRNA were also increased in the distal lung of patients with mild-to-moderate asthma. In a clinical context, small airway inflammation is associated with increased risk of future exacerbation and increased bronchial hyper-responsiveness, which cause severe asthma, however, ICS is not sufficient to reduce CANO in asthmatics. Previous studies demonstrated that a major site of inflammation in distal airway is outer wall, which indicated that inhaled therapy may be insufficient to control distal airway inflammation and systemic therapy may be more preferable. The effects of oral LTRA administration to reduce peripheral airway inflammation in asthma have been reported. Zeidler et al. have found that montelukast, another LTRA, resulted in significantly less regional air-trapping on high-resolution computed tomography images and improvement in quality of life scores. In the present study, add-on oral pranlukast administration to ICS or ICS plus LABA resulted in significant reduction of CANO without any changes of FeNO at 50 ml/s and J'awNO, and improved asthma control. The action of pranlukast in the peripheral airway might lead to the improvement of asthma control.

In this study, we used a two-compartment model of NO exchange dynamics to assess central and distal airway

<table>
<thead>
<tr>
<th>Table 1 Patient characteristics.</th>
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<tbody>
<tr>
<td><strong>Number of patients</strong></td>
</tr>
<tr>
<td>Female sex, n (%)</td>
</tr>
<tr>
<td>Age (year)</td>
</tr>
<tr>
<td>Duration of asthma (month)</td>
</tr>
<tr>
<td>No. with atopy (%)</td>
</tr>
<tr>
<td>No. of former smokers (%)</td>
</tr>
<tr>
<td>Inhaled corticosteroid dose (μg)</td>
</tr>
<tr>
<td>No. with treatment</td>
</tr>
<tr>
<td>ICS only</td>
</tr>
<tr>
<td>SFC</td>
</tr>
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<td>ICS plus LABA</td>
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ICS, inhaled corticosteroid; SFC, salmeterol/fluticasone propionate combination; LABA, long-acting β₂-agonist.

* Data are expressed as median (range).

* Fluticasone equivalent.
inflammation separately. Recent studies have also demonstrated, both experimentally and theoretically, axial back-diffusion of NO from proximal to peripheral airway/alveoli against the direction of exhalation, which causes underestimation of $J_{awNO}$ and overestimation of $C_{ANO}$ levels. In the present study, we evaluated $J_{awNO}$ and $C_{ANO}$ using correction for NO axial back-diffusion according to the previous reports. The present data demonstrated that both $C_{ANO}$ level and corrected $C_{ANO}$ for axial back-diffusion were significantly decreased by add-on pranlukast to ICS or ICS plus LABA (Figs. 2C and 3B), which constitutes an advantage for the treatment of distal airway inflammation.

Some previous studies described that no significant change of $C_{ANO}$ was found after the addition of oral LTRA treatment to ICS therapy in asthmatics. In each study, the researchers used various kinds of LTRA (e.g., montelukast, zileuton) which may be one of the reasons for the difference in results. The differences of background in the subjects and study design also may cause different

<table>
<thead>
<tr>
<th>Add-on pranlukast</th>
<th>No add-on pranlukast</th>
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<tbody>
<tr>
<td></td>
<td>Beginning</td>
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<tr>
<td>FVC (L)</td>
<td>2.68 ± 0.97</td>
</tr>
<tr>
<td>FVC, % predicted (%)</td>
<td>94.1 ± 24.6</td>
</tr>
<tr>
<td>FEV$_1$ (L)</td>
<td>1.93 ± 0.91</td>
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<tr>
<td>FEV$_1$, % predicted (%)</td>
<td>80.8 ± 26.1</td>
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<tr>
<td>FEV$_1$/FVC (%)</td>
<td>70.0 ± 13.1</td>
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<tr>
<td>FEV$_{25–75}$, % predicted (%)</td>
<td>48.4 ± 35.3</td>
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<tr>
<td>FEF$_{50}$, % predicted (%)</td>
<td>55.6 ± 35.3</td>
</tr>
<tr>
<td>FEF$_{75}$, % predicted (%)</td>
<td>45.0 ± 32.9</td>
</tr>
<tr>
<td>morning PEF (L/min)</td>
<td>326.4 ± 116.4</td>
</tr>
<tr>
<td>ACT</td>
<td>21.4 ± 2.9</td>
</tr>
</tbody>
</table>

FEF, forced expired flow; PEF, peak expiratory flow; ACT, asthma control test. Data are expressed as mean ± SD.

**Figure 2** Results of FeNO at 50 ml/s, $J_{awNO}$ and $C_{ANO}$ at the beginning and end of the add-on pranlukast period and the no-add-on pranlukast period. The value of FeNO at 50 ml/s and $J_{awNO}$ were not significantly different between the beginning and the end of each period (A, B). Add-on pranlukast administration significantly reduced $C_{ANO}$ level (C). FeNO, fraction of exhaled nitric oxide; $J_{awNO}$, central airway nitric oxide flux; $C_{ANO}$, peripheral airway/alveolar nitric oxide concentration. Figures data include the median and interquartile ranges. The median is represented as a horizontal bar.
results in each study. In the present study, the subjects were controlled asthmatics under the treatment of ICS or ICS plus LABA according to the guideline. Add-on pranlukast period was 8 weeks in this study, which was longer than the other reports. Although the reduction of $C_{ANO}$ was modest and not statistically significant, Fritscher and coworker reported that the addition of oral montelukast to inhaled fluticasone might decrease small airway inflammation in mild asthma. Some limitations to these studies including ours should be noted. They were opened-label, non-controlled pilot studies on a relatively small number of patients. This study is important in terms of being the first randomized crossover trial observing the change of $C_{ANO}$ after additional oral LTRA treatment in stable asthmatics, which is an advantage of the present study over the others.

In summary, the present study has demonstrated that add-on pranlukast treatment to ICS or ICS plus LABA improved asthma control together with a significant reduction of $C_{ANO}$. Although asthmatic patients treated with ICS or ICS plus LABA are clinically stable, there is a possibility that small airway inflammation is not regulated completely and add-on oral pranlukast decreases residual small airway inflammation, which can improve asthma control. A long-term study involving assessment of various airway inflammation markers and pulmonary functions is required to confirm the benefit of add-on pranlukast administration in stable asthma.

**Author contribution**

Hideki Yasui: Conception and design, Data collection, Data analysis and interpretation, Manuscript writing; Tomoyuki Fujisawa: Conception and design, Data analysis and interpretation, Manuscript writing. Final approval of manuscript; Naoki Inui: Conception and design, Data collection; Masato Kato: Data collection; Dai Hashimoto: Data collection; Noriyuki Enomoto: Data collection; Yutaro Nakamura: Data collection; Toshihiro Shirai: Data collection; Takafumi Suda: Data analysis; Hirotoshi Nakamura: Data analysis and interpretation; Kingo Chida: Conception and design, Administrative support, Data analysis and interpretation.

**Conflict of interest**

All authors in this manuscript have no conflict of interest.

**References**


