Effects of Cysteinyl-Leukotriene Receptor Antagonist, Thromboxane A2 Receptor Antagonist, and Thromboxane A2 Synthetase Inhibitor on Antigen-Induced Bronchoconstriction in Patients With Asthma*

Yasushi Obase, MD; Terufumi Shimoda, MD; Nobuko Matsuo, MD; Hiroto Matsuse, MD; Sadahiro Asai, MD; and Shigeru Kohno, MD, FCCP

Background: Leukotriene (LT) and thromboxane A2 (TXA2) receptor antagonists have been used in the treatment of asthma.

Objectives: We examined the effects of an LT receptor antagonist, TXA2 receptor antagonist, and TXA2 synthetase inhibitor on bronchoprovocation test (BPT) in patients with mild-to-moderate atopic asthma.

Methods: BPT was performed four times in each of six asthmatics. Development of the immediate asthmatic reaction (IAR) and late asthmatic reaction (LAR) was confirmed on the first BPT (BPT1). After a 7-day washout period, an LT receptor antagonist (pranlukast, 450 mg/d), TXA2 receptor antagonist (seratrodast, 80 mg/d), or TXA2 synthetase inhibitor (ozagrel, 800 mg/d) was administered orally over 7 days at random using a cross-over method (BPT2-4). Blood levels of LTβ4, LTC4, LTD4, 11-dehydrothromboxane B2, eosinophil cationic protein, and histamine were measured at reaction phases of pre-BPT, IAR, and LAR.

Results: Administration of pranlukast suppressed IAR by 80.5% (p < 0.0001) and LAR by 54.6% (p = 0.0391). Ozagrel significantly suppressed IAR by 39.5% (p = 0.0413), but the fall in FEV1 was >20% (21.56 ± 4.173%). Seratrodast did not suppress IAR or LAR. Blood levels of chemical mediators did not correlate with the suppressive effects of the tested drugs.

Conclusions: The LT receptor antagonist was considered to be the most effective. LT might play a more important role in the pathogenesis of asthma than TXA2. Our data showed that measurement of blood levels of chemical mediators is not useful in identifying the pathogenic mechanisms of asthma.

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Key words: antigen-induced bronchoconstriction; chemical mediators; cysteinyl-leukotriene receptor antagonist; thromboxane A2 receptor antagonist; thromboxane A2 synthetase inhibitor

Abbreviations: BDP = beclomethasone dipropionate; BPT = bronchoprovocation test; ECP = eosinophil cationic protein; HD = house dust; IAR = immediate asthmatic reaction; LAR = late asthmatic reaction; LT = leukotriene; LT-C4,-D4,-E4 = leukotriene -C4,-D4,-E4; RIA = radioimmunoassay; TX = thromboxane; TXA2 = thromboxane A2; 11-dhTXB2 = 11-dehydrothromboxane B2

Among antiallergic compounds, only antihistamines, such as ketotifen, are recognized in the report of “Global Strategy for Asthma Management and Prevention” issued by the National Heart, Lung, and Blood Institute and the World Health Organization (1995) as antiasthmatic agents. In addition to histamine, recent studies have emphasized the role of arachidonic acid metabolites, eg, leukotriene C4 (LTC4), leukotriene D4 (LTD4), leukotriene E4 (LTE4), and thromboxane A2 (TXA2), in the pathogenesis of asthma.2-9 LTC4, LTD4, and LTE4 are produced by mast cells, neutrophils, and eosinophils. These leukotrienes (LTs) induce a strong contraction of smooth muscles, and enhance vascular permeability and mucous secretion in the respiratory
tract. TXA₂ has also attracted attention recently due to its strong physiologic activity.⁵ TXA₂, a product of cyclooxygenase in arachidonic acid metabolism, was first described as a constrictive substance of arteries in the rabbit and then named by Hamberg et al.⁶ TXA₂, mainly produced by platelets, induces strong contraction of respiratory and vascular smooth muscles and has platelet agglutination activity.⁷ TXA₂ is thought to play an important role in the pathogenesis of asthma as well as that of circulatory disturbances.⁸,⁹ In recent years, anti-inflammatory drugs with antagonistic activities to LT and TXA₂ have been developed and proved to be useful to some extent against asthma. Several placebo-controlled clinical trial studies have shown the effectiveness of LT or TXA₂ receptor antagonists on antigen-induced bronchoconstriction.¹⁰,¹¹ However, to our knowledge, the effects of these drugs have not been compared in a single experimental protocol.

To assess the role of LT and TXA₂ in the pathogenesis of asthma, we compared the suppressive effects of pranlukast (LT receptor antagonist), seratrodast (TXA₂ receptor antagonist), and ozagrel (TXA₂ synthetase inhibitor) on immediate asthma reaction (IAR) and late asthmatic reaction (LAR) during bronchoprovocation test (BPT). Blood levels of LTB₄, LTC₄, LTD₄, thromboxane B₂ (TXB₂), eosinophil cationic protein (ECP), and histamine were also measured to allow assessment of the effects of these agents.

**Materials and Methods**

**Subjects**

After obtaining informed consent, six inpatients (four male and two female) with atopic bronchial asthma were enrolled in the study. The study was approved by the Ethics Review Committee of Nagasaki University Hospital. The diagnosis, severity, and disease type in these subjects were determined according to the “diagnosis and classification” criteria of the Global Initiative for Asthma (global strategy for asthma management and prevention; NHLB/WHO workshop report). All subjects were nonsmokers ranging between 19 and 43 years of age (mean, 31 years) with disease severity ranging from mild-persistent to moderate-persistent. All subjects had a positive response to methacholine challenge test. Control of asthma was achieved with inhaled beclomethasone dipropionate (BDP) at 400 µg/d in two patients and at 500 µg/d in four patients. In all patients, inhaled β₂-agonists were used when necessary (Table 1). All patients had no episodes of airway infection 4 weeks before the present study, and had a positive radioallergosorbent test and a positive skin test for house dust (HD) antigen extract (Tori; Tokyo, Japan).

**Bronchoprovocation Test**

Allergen inhalation challenge was performed using the standard method advocated by the International Society of Allergy using a commercially available HD allergen extract.¹² In this test, the threshold for positive skin reaction was first determined by skin sensitization tests using 10-fold serial dilutions of the HD antigen extract. Oral administration of antihistaminic, antiallergic drugs, or steroids was avoided for 24 h before the test. Inhalation of β-stimulants, anticholinergic drugs, or steroids and oral administration of theophylline were also prohibited for 12 h before the test. The baseline FEV₁ was measured with a spirometer (Superspiro, DISCOM-21FX; Chest M.I. Co.; Tokyo, Japan) and confirmed to be >70% of the predicted value. Clinical examination confirmed a lack of dyspnea or rales even on forced expirations.

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**Table 1—Characteristics of Patients Participating in the Study**

<table>
<thead>
<tr>
<th>Subject/Age, yr/Sex</th>
<th>Severity*</th>
<th>IgE, IU/mL</th>
<th>RAST Score to HDM†</th>
<th>Therapy† BDP, µg/d</th>
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<td>544</td>
<td>6</td>
<td>400</td>
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<td>756</td>
<td>6</td>
<td>800</td>
</tr>
<tr>
<td>3/23/M</td>
<td>Mod</td>
<td>490</td>
<td>6</td>
<td>800</td>
</tr>
<tr>
<td>4/28/F</td>
<td>Mild</td>
<td>2,490</td>
<td>6</td>
<td>400</td>
</tr>
<tr>
<td>5/38/M</td>
<td>Mod</td>
<td>524</td>
<td>4</td>
<td>800</td>
</tr>
<tr>
<td>6/19/F</td>
<td>Mod</td>
<td>278</td>
<td>5</td>
<td>500</td>
</tr>
</tbody>
</table>

*Severity of asthma was determined according to the Global Initiative for Asthma (global strategy for asthma management and prevention; NHLB/WHO workshop report).†RAST = radioallergosorbent test; HDM = house dust mite.†Asthma was controlled with inhaled BDP at 400 µg/d in two patients and at 800 µg/d in four patients. In all patients, inhaled β₂-agonists were used when necessary.

**Protocol**

Each of six patients with atopic asthma completed a total of four BPTs. BPT 1 commenced at 9 AM without premedication; IAR and LAR were confirmed to be positive for HD antigen. The following drugs were used: 450 mg/d of pranlukast (Ono Pharmaceutical Co.; Osaka, Japan), 80 mg/d of seratrodast (Takeda Chemical Industries; Osaka, Japan), and 500 mg/d of ozagrel (Kissei Pharmaceutical Co.; Matsumoto, Japan). The protocol consisted of performing BPT 1 followed by a 7-day washout period, then administration of the first medication for 7 days. At the end of this treatment, another BPT (BPT 2) was performed using the same dose of HD allergen. The same regimen was repeated for the second and third drugs, as illustrated in Figure.
1. Each drug was also taken 2 h before the test. Based on blood levels and half-lives of the tested drugs, 1-week intervals were considered to be necessary and adequate for washout. In each of the four BPTs, blood samples were obtained 15 min before the test, IAR and LAR, for measurement of chemical mediators. The time of withdrawal of blood sample on BPT 2 to 4 was similar to that used in BPT 1.

Measurement of Leukotrienes

For measurement of LTB4, LTC4, and LTD4, serum samples were immediately fourfold diluted with an LT extract (ethyl acetate: methanol solution at 2:1) and cryopreserved at −80°C. The concentrations of LTB4, LTC4, and LTD4 were determined by radioimmunoassay (RIA) with a low limit of detection of 20 pg/mL (LTB4 [3H] assay system, TRK 940 Amersham, Little Chalfont, UK; LTC4 [3H] assay system, TRK 905 Amersham; peptidyl-leukotriene [3H] RIA kit, NED-043 DuPont NEN, Boston, MA).

Measurement of 11-Dehydrothromboxane B2

Blood samples for measurement of 11-dehydrothromboxane B2 (11-dhTXB2) were collected in specially designed tubes (Vacutainer, Brand Blood Collection Tube; Becton Dickinson; Franklin Lakes, NJ), left at room temperature for 1 h and centrifuged at 1,300g for 10 min. The supernatant was cryopreserved at −80°C. We determined the concentration of 11-dhTXB2 in the serum by RIA (thromboxane B2 [125I] RIA Kit; DuPont NEN) with a low detection limit of 35 pg/mL.

Measurement of ECP

Blood samples for measurement of ECP were collected in a specially designed tubes (Vacutainer, Brand Blood Collection Tube; Becton Dickinson; Franklin Lakes, NJ), left at room temperature for 1 h and centrifuged at 1,300g for 10 min. The supernatant was cryopreserved at −80°C. The concentration of ECP in the serum was determined by RIA (Pharmacia ECP RIA; Pharmacia; Uppsala, Sweden) with a low detection limit of 15.7 μg/L.

Measurement of Histamine

The concentration of histamine in the serum was determined by RIA (Histamine RIA Kit; Eiken, Tokyo, Japan), with a low detection limit of 1 nM.

Data Analysis

Data were expressed as mean ± SEM. Changes in FEV1 on BPT 1 and BPT 2 to 4 were compared using one-way analysis of variance for repeated measures. Changes in the concentration of chemical mediators were analyzed using a two-tailed Student’s t test with the level of significance (p value) set at 0.05.

RESULTS

No adverse effects were noted and no abnormal changes in vital signs or ECG were detected in any patient throughout the tests. Test drugs were allocated at random. Baseline FEV1 was similar in all BPTs irrespective of the type of test medication used (data not shown).

On BPT 1 (no treatment), a biphasic change in FEV1 was noticed. FEV1 improved significantly by pranlukast and IAR-related fall in FEV1 by 80.5% (−6.95 ± 5.35%, p < 0.0001) and LAR-related fall in FEV1 by 54.6% (−11.47 ± 4.98%, p = 0.0391), compared with the falls in FEV1 on BPT 1. Administration of ozagrel suppressed IAR-related fall in FEV1 by 39.5% (−21.56 ± 4.17%, p = 0.0413), but failed to change LAR-related fall in FEV1 compared with the fall in FEV1 on BPT 1.Administration
of seratrodast did not significantly change IAR- or LAR-related falls in FEV\textsubscript{1} (Fig 2).

LTB\textsubscript{4}, LTE\textsubscript{4}, LTD\textsubscript{4}, 11-dhTXB\textsubscript{2}, ECP, and histamine levels did not change significantly after the administration of any of the test drugs. There was no correlation between changes in blood or serum levels of chemical mediators and changes in FEV\textsubscript{1} (data not shown). The concentration of 11-dhTXB\textsubscript{2} in blood samples obtained 15 min before the test tended to be lower following the administration of ozagrel (115.81 ± 266.60 to 5.87 ± 2.66 pg/mL), but the difference was not statistically significant.

**Discussion**

The major finding of the present study was that pranlukast (LT receptor antagonist) suppressed IAR- and LAR-related falls in FEV\textsubscript{1} induced by HD antigen inhalation more effectively than ozagrel (TXA\textsubscript{2} synthetase inhibitor) and seratrodast (TXA\textsubscript{2} receptor antagonist) in patients with mild-to-moderate atopic asthma. These results suggest that LT plays a more important role than TXA\textsubscript{2} in the pathogenesis of asthma. Our results also indicated that measurement of chemical mediators in the blood is not useful for investigating the pathogenesis of asthma.

Several studies that examined the efficacy of antiasthmatic drugs showed that the suppression of falls in FEV\textsubscript{1} during BPT was significantly greater after administration of the test drug than after placebo.\textsuperscript{13-18} Thus, the results shown in Figure 2 are not new, but are supportive of previous data examining the role of leukotrienes in asthma.

Based on clinical use, however, it is more important to reduce the fall in FEV\textsubscript{1} within 20% and prevent asthmatic attacks. Therefore, even if significant differences were found between test drugs and placebo, evaluation of the drug should be performed carefully in cases in which falls in FEV\textsubscript{1} were ≥ 20% (associated with asthmatic attacks).

O’Byrne\textsuperscript{16} speculated that LAR was caused by LT and other products released from inflammatory cells in the airway. The present findings are in agreement with these early results, demonstrating that the LT receptor antagonist suppressed IAR-related fall in FEV\textsubscript{1} by 80.5% and LAR by 54.6%. A number of studies have reported the effectiveness of LT receptor antagonists on antigen provocation test in humans. Taylor et al\textsuperscript{11} found that ICI 204.219, an LTD\textsubscript{4} receptor antagonist, markedly improved IAR-related fall in FEV\textsubscript{1} from 32.0 to 6.3% and while LAR-related falls in FEV\textsubscript{1} improved from 27.9 to 12.7% in 11 asthmatics; falls in FEV\textsubscript{1} were suppressed within 20% both for IAR and LAR. However, Findlay and coworkers\textsuperscript{17} noted that a single oral administration of ICI 204.219 at a dose of 40 mg caused significant increases in the concentration of antigen required to produce a 20% fall in FEV\textsubscript{1} on BPT in 13 atopic asthmatics. According to a study of IAR on BPT by Taniguchi et al,\textsuperscript{10} a 7-day course of 300 mg/d of oral ONO-1078 (pranlukast) effectively suppressed the fall in FEV\textsubscript{1} within 20% during the period from 20 to 60 min postinhalation in 10 patients with atopic asthma.

To our knowledge, there is only one study that has examined the effectiveness of thromboxane (TX) inhibitors on BPT in humans. Manning and coworkers\textsuperscript{18} reported that oral administration of CGS 13080, a TX synthetase inhibitor, for 2 days significantly suppressed IAR-related fall in FEV\textsubscript{1} from 25 to 20%. However, the drug did not suppress LAR-related fall in FEV\textsubscript{1}. Our study confirmed these results and allowed the comparison between IAR- and LAR-related effects of these drugs. To our knowledge, there are no studies that have investigated the effectiveness of TX receptor antagonists on IAR and LAR following antigen provocation in humans. Matsumoto et al\textsuperscript{19} measured airway resistance during a biphasic asthmatic response following allergen challenge in a guinea pig model. They found that a TXA\textsubscript{2} receptor antagonist (AA-2414) suppressed both IAR and LAR dose dependently. They also reported that TXA\textsubscript{2} synthetase inhibitors (CV-4151 and OKY-046) suppressed both IAR and LAR, although no changes were found in cell components in BAL fluid. They concluded that although TXA\textsubscript{2} played an important role in both IAR and LAR, it did not influence inflammatory cells in their animal model of asthma.

In this study, the effect of ozagrel (TX synthetase inhibitor) on IAR was more than seratrodast (TX receptor antagonist). However, the fall in FEV\textsubscript{1} during treatment with ozagrel was still > 20% indicating that the drug is not effective clinically. These results suggest that anti-TX agents may not be very effective antiasthmatic drugs.

Although the use of test drugs was selected at random in the present study, it is possible that our results were still influenced by the experimental design (for example, no BPT 2 or BPT 4 tests were performed with ozagrel). However, we do not think that this had a major impact on our results since the actual period between the use of any drug and the next BPT was 2 weeks, including 7 days of the washout period after the first drug and 7 days of using the next test drug (Fig 1).

Our results also showed that determination of the level of chemical mediators in the serum or blood was not beneficial for investigating the mechanisms of asthma. Interestingly, our results showed that the
level of 11-dhTXB2 in the blood tended to decrease following the use of TX synthetase inhibitor. However, the high mean baseline concentration of this mediator was associated with a high variability, and statistical analysis showed no significant difference between the paired data. Combined together, our results showed no relationship between the level of chemical mediators and fall of FEV1 during IAR and LAR following BPT.

Endogenous levels of LTB4 are difficult to measure due to the low basal level and the possible effect of stimulating cells during collection. Furthermore, LTC4 and LTD4 are rapidly metabolized to LTE4, which represents the circulating leukotriene, and the blood level of the latter is also very low.20 Validation of the measured concentrations of LT in the circulation may be performed by determination of LTE4 concentration in the urine. However, our results showed that blood concentrations of TX and LT did not correlate with changes in FEV1. It is possible that determination of other parameters (eg, proportion of eosinophils, ECP in induced sputum, exhaled nitric oxide, etc) may be helpful to our understanding of the pathogenesis of asthma.

The purpose of this study was to investigate the role of LT and TXA2 in asthma by measuring changes in airway resistance during IAR and LAR following the administration of three drugs with different pharmacologic mechanisms in the same subject under the same conditions. Our results suggested that LT plays a more important role than TXA2 in asthma. Future studies should clarify the network of chemical mediators, cytokines, and chemokines involved in the pathogenic process of asthma.

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