Effect of a Thromboxane A₂ Antagonist on Sputum Production and Its Physicochemical Properties in Patients With Mild to Moderate Asthma*

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Study objective: To determine the effects of a specific thromboxane A₂ (TxA₂) receptor antagonist, seratrodast, on asthma control and airway secretions.

Design: Multicenter, double-blind, randomized, placebo-controlled study.

Patients: Forty-five patients with mild to moderate asthma who had been continuously expectorating sputum of > 20 g/d. Patients with a current pulmonary infection or taking oral corticosteroids, antibiotics, or mucolytic agents were excluded from the trial.

Interventions: Following a 2-week run-in period, while pulmonary function, sputum production, and mucociliary function were assessed, patients were assigned to receive seratrodast, 40 mg/d, or placebo for 6 weeks.

Measurements and results: During the treatment period, the changes in FEV₁ and peak expiratory flow (PEF) were not different between the two patient groups, but there were significant reductions in diurnal variation of PEF (p = 0.034), frequency of daytime asthma symptoms (p = 0.030), and daytime supplemental use of β₂-agonist (p = 0.032) in the seratrodast group. For sputum analysis, seratrodast treatment decreased the amount of sputum (p = 0.005), dynamic viscosity (p = 0.007), and albumin concentration (p = 0.028), whereas it had no effect on elastic modulus or fucose concentration. Nasal clearance time of a saccharin particle was shortened in the seratrodast group at week 4 (p = 0.031) and week 6 (p = 0.025), compared with the placebo group.

Conclusion: Blockade of TxA₂ receptor has minimal effects on pulmonary function, but may cause an improvement in mucociliary clearance by decreasing the viscosity of airway secretions.

Key words: airway secretion; elasticity; mucociliary clearance; thromboxane; vascular leakage; viscosity

Abbreviations: G' = elastic modulus; η' = dynamic viscosity; NCT = nasal clearance time; PEF = peak expiratory flow; PGD₂ = prostaglandin D₂; TxA₂ = thromboxane A₂

Impairment of mucociliary clearance in the respiratory tract is one of the characteristic features of asthma, which may be an important determinant of exacerbations, current severity, and prognosis of the disease.¹,² Furthermore, chronic airway hypersecretion can frequently be seen in patients with asthma, and the presence of a viscid exudate, composed of mucus and inflammatory cells, in the bronchial lumen likely worsens airway obstruction.³,⁴

Cyclooxygenase metabolites of arachidonic acid have been implicated in the inflammatory cascade that occurs in asthmatic airways.⁵ The metabolites include thromboxane A₂ (TxA₂) and prostaglandin D₂ (PGD₂), both of which are capable of producing potent bronchoconstriction, airway microvascular leakage, and bronchial hyperresponsiveness through activation of a TxA₂ receptor.⁶ There is increasing evidence that various TxA₂ synthase inhibitors and TxA₂ receptor antagonists attenuate the increased bronchoconstrictor responses to methacholine in asthmatic patients.⁷–⁹ This would indicate an involvement of TxA₂ and/or PGD₂ in the pathogenesis of...
bronchial hyperresponsiveness. However, the role of these cyclooxygenase products in airway hypersecretion and impairment of mucociliary clearance in asthma remains unknown. Therefore, in the present randomized trial, we examined the effect of treatment with seratrodast, (±)-7-(3,5,6-trimethyl-1,4-benzoquinon-2-yl)-7-phenylheptanoic acid, which is the first approved specific TxA2 receptor antagonist10 (now widely used for the treatment of asthma in Japan); specifically, we looked at effects on pulmonary function, airway mucus secretion, sputum physicochemical properties, and mucociliary transport function in mild to moderate asthma.

**Materials and Methods**

**Patients**

Four centers participated. Nonsmoking subjects (age range, 29 to 72 years) with mild to moderate asthma who had been expectorating sputum of > 20 g/d for at least 2 weeks prior to the study were recruited from the outpatient department. All patients conformed to the National Asthma Education and Prevention Program definition of the disease.11 Entry criteria included FEV1 > 60% of predicted normal, morning peak expiratory flow (PEF) > 60% of predicted normal, no course of oral corticosteroids during the previous 8 weeks, no course of antibiotics or mucolytic agents during the previous 4 weeks, no evidence of pulmonary infection on chest radiograph and sputum bacteriology (bacteria > 105/mL), and no evidence of apparent sinusitis or rhinitis. Written informed consent was obtained from each patient, and the study was approved by the Tokyo Women’s Medical University Medical Board and the local ethics committees.

**Study Design**

The study was performed as a multicenter, double-blind, randomized, placebo-controlled, parallel-group trial. After an initial 2-week run-in (baseline) period, patients were divided into two groups and given either seratrodast (Takeda Chemical Industries; Osaka, Japan), 40 mg/d, or placebo orally in the morning (between 7:00 AM and 8:00 AM) during the next 6-week double-blind treatment period. Of the 58 patients who participated in the trial, 51 were enrolled in the treatment period and randomly assigned study treatment. Randomization into two treatment groups was made separately for each center in blocks of four at the end of the baseline period. Patients were allowed to take β2-agonist, procaterol (Otsuka Pharmaceutical; Tokyo, Japan), from a metered-dose inhaler (10 µg/dose) if supplemental medication was needed, and all other treatment remained unchanged.

**Clinical Assessments**

All patients visited an outpatient clinic once a week during the baseline and treatment periods. At the first visit, demographic details were recorded. Each patient recorded daily in a booklet all medication taken throughout the study. Symptoms of asthma (breathlessness, wheezing, and cough), sleep disturbance, morning and evening PEF (best of three attempts before taking medication), and the use of supplemental β2-agonist inhalation were recorded daily. At each visit, the physician recorded changes in medication, intercurrent illness, adverse events, and asthma exacerbations, and calculated diurnal variation in PEF (highest evening PEF minus lowest morning PEF as a percentage of the highest value). At the end of the baseline period and at the end of the treatment period, FEV1 was measured in the morning (between 10:00 AM and 12:00 noon).

**Sputum Analysis**

To analyze sputum, patients were given preweighed, covered plastic cups, and asked to collect and weigh all sputum expectorated during a 24-h period in the baseline and treatment periods. A scale (model HF-200; Kensei-Kogyo; Tokyo, Japan) for the measurement of sputum weight at home was supplied, and each patient was carefully instructed in the use of the scale. The weight of the sputum was recorded in the booklet daily. Patients were also asked to swallow saliva immediately before expectoration of sputum to minimize salivary contamination. On the day of the beginning, and after 2 weeks and 6 weeks of the trial, the samples of sputum collected in the morning (8:00 AM to 11:00 AM) were transported to the laboratory, and parameters of sputum viscoelasticity (ie, elastic modulus [G’] and dynamic viscosity [η]) were measured by a microrheometric method of Lutz and associates.12 To do so, a magnetically oscillated steel microsphere suspended in a drop of mucus was used as a mechanical probe, and the oscillation amplitude of a 100- to 200-mm iron sphere driven by sinusoidal magnetic forces was recorded. The measurements were made at a frequency of 10 Hz, because this frequency approximates to human airway ciliary beat frequency.13 Whenever possible, three specimens from each sputum sample were tested, and the results were expressed as the mean.

For chemical analysis of the sputum, the samples of sputum were homogenized in a glass homogenizer and centrifuged (13,000g for 20 min). The supernatant was taken, and the concentrations of fucose and albumin were measured in duplicate by a sulfuric acid and thioglycolic acid assay and by an enzyme-linked immunosorbent assay employing a mouse monoclonal anti-human albumin antibody (Sigma Chemical; St. Louis, MO) and a rabbit polyclonal antibody directed against albumin (Sigma Chemical), respectively.

**Mucociliary Clearance**

In evaluating mucociliary clearance, measurement of mucus velocity using a bronchofiberscope and scanning of inhaled technetium-99m-labeled particles are so invasive and time consuming, respectively,14 that these techniques are difficult to apply to many patients. We thus adopted the saccharin method and measured nasal clearance time (NCT) as an alternative noninvasive method.15,16 At the beginning and the end of the baseline period, and every 2 weeks during the treatment period, NCT was determined in all patients. A 1-mm diameter particle of saccharin (Nakalai Tesque; Kyoto, Japan) was placed 1 cm from the anterior end of the inferior nasal turbinate of a nostril verified by inspection not to be obstructed. Patients were asked not to eat, drink, cough, or sneeze during the test. The time from the placing of the particle to the first perception of a sweet taste was recorded. Control values for NCT were also measured in 40 age-matched normal volunteers.

**Statistical Analysis**

The variables recorded in the diaries (number of asthma symptoms, PEF, diurnal variation in PEF, the number of puffs of supplemental β2-agonist, and the amount of sputum) were
Table 1—Baseline Patient Characteristics*

<table>
<thead>
<tr>
<th>Variables</th>
<th>Seratrodast Group (n = 21)</th>
<th>Placebo Group (n = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>48 ± 4</td>
<td>49 ± 4</td>
</tr>
<tr>
<td>Range</td>
<td>33–72</td>
<td>29–70</td>
</tr>
<tr>
<td>Sex, No.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Female</td>
<td>12</td>
<td>15</td>
</tr>
<tr>
<td>Duration of asthma, yr</td>
<td>4.6 ± 1.5</td>
<td>5.3 ± 2.0</td>
</tr>
<tr>
<td>Daily sputum production, g</td>
<td>39 ± 5</td>
<td>45 ± 6</td>
</tr>
<tr>
<td>FEV1, L</td>
<td>2.07 ± 0.24</td>
<td>1.87 ± 0.26</td>
</tr>
<tr>
<td>PEF, L/min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morning PEF</td>
<td>388 ± 20</td>
<td>370 ± 26</td>
</tr>
<tr>
<td>Evening PEF</td>
<td>423 ± 22</td>
<td>411 ± 18</td>
</tr>
<tr>
<td>Other medication, No.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inhaled corticosteroid</td>
<td>16</td>
<td>17</td>
</tr>
<tr>
<td>Inhaled anticholinergic agent</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Oral β2-agonist</td>
<td>21</td>
<td>24</td>
</tr>
<tr>
<td>Oral theophylline</td>
<td>15</td>
<td>18</td>
</tr>
</tbody>
</table>

*Data are presented as mean ± SEM unless otherwise indicated.

Results

Patients

Of the 51 patients who started the treatment phase of the study, 45 patients completed the 6-week treatment (21 patients received seratrodast, and 24 patients received placebo). During the treatment period, four patients were withdrawn from the seratrodast group, two because of a missed visit to the clinic, one because of addition of antibiotics and oral corticosteroids to the treatment due to bacterial infection and acute exacerbation of asthma, and one because of pregnancy. Two patients were also withdrawn from the placebo group, one because of no visit to the clinic, and one because of failure to collect sputum. Pharmacologically predictable adverse events (dyspepsia, headache) were reported by two patients in the seratrodast group within 1 week of treatment, but they were spontaneously relieved during the next few days in spite of continuing medication. None of the patients dropped out because of adverse effects.

Baseline patient characteristics are shown in Table 1. Patients had sputum production between 23 g/d and 69 g/d during the baseline period. There were no significant differences in the distribution of age, gender, sputum production, pulmonary function, and other medications between the two treatment groups.

Clinical Assessments

The effects of seratrodast on pulmonary function, asthma symptoms, and supplemental use of inhaled β2-agonist are shown in Table 2. Although mean values for FEV1, morning PEF, and evening PEF increased from the baseline values after a 6-week treatment period, the changes in these variables were not significantly different between the seratrodast group and the placebo group. Mean diurnal variation of PEF decreased in the seratrodast group from the baseline value of 14.6 ± 2.2% to 8.1 ± 2.2% at week 6, whereas it remained unchanged in the placebo group. There was a significant difference between the two groups with respect to the change in diurnal variation of PEF (p = 0.034). The number of daytime asthma symptoms and the number of daytime use of β2-agonist decreased during the seratrodast treatment, and the changes of these variables were significantly different between two treatment groups at week 6 (p = 0.030 and p = 0.032, respectively). The mean values for nighttime asthma symptoms and β2-agonist use were not significantly different between the groups.

Table 2—Changes in Pulmonary Function, Asthma Symptoms, and Use of Rescue β2-Agonist After 6-Wk Treatment*

<table>
<thead>
<tr>
<th>Variables</th>
<th>Seratrodast Group</th>
<th>Placebo Group</th>
<th>Seratrodast vs Placebo p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV1, L</td>
<td>+0.14 ± 0.07</td>
<td>+0.07 ± 0.05</td>
<td>0.066</td>
</tr>
<tr>
<td>Morning PEF, L/min</td>
<td>+12 ± 7</td>
<td>+9 ± 5</td>
<td>0.184</td>
</tr>
<tr>
<td>Evening PEF, L/min</td>
<td>+5 ± 4</td>
<td>+3 ± 6</td>
<td>0.421</td>
</tr>
<tr>
<td>Daytime asthma symptoms, episodes/wk</td>
<td>−5.5 ± 1.1</td>
<td>+0.2 ± 1.6</td>
<td>0.030</td>
</tr>
<tr>
<td>Nighttime asthma symptoms, episodes/wk</td>
<td>−2.0 ± 1.6</td>
<td>+1.4 ± 0.5</td>
<td>0.064</td>
</tr>
<tr>
<td>Daytime use of β2-agonist, puffs/wk</td>
<td>−6.4 ± 1.7</td>
<td>−1.0 ± 1.9</td>
<td>0.032</td>
</tr>
<tr>
<td>Nighttime use of β2-agonist, puffs/wk</td>
<td>−2.8 ± 1.6</td>
<td>+1.3 ± 1.6</td>
<td>0.102</td>
</tr>
</tbody>
</table>

*Values are presented as mean ± SEM.
Sputum Analysis

As shown in Figure 1, in both the seratrodast group and the placebo group, the values for sputum production immediately before the treatment period did not differ from those recorded during the previous 2-week run-in period. During the 6-week treatment period, the amount of daily sputum production in the placebo group did not change. In contrast, seratrodast treatment gradually decreased sputum production, and there was a significant difference between the two groups with respect to the change in mean sputum production at week 3 ($p = 0.008$), week 4 ($p = 0.022$), week 5 ($p = 0.039$), and week 6 ($p = 0.005$).

For the assessment of rheologic properties of the sputum, mean values for $G'$ and $\eta'$ in the placebo group did not differ significantly from those in the seratrodast group before the treatment. Administration of placebo did not alter $G'$ or $\eta'$ during the treatment period (Fig 2). On the other hand, in the seratrodast group, $G'$ remained unchanged, but $\eta'$ decreased from the baseline value of $48 \pm 3$ poise to $33 \pm 4$ poise at week 6 ($p = 0.007$). There was a significant difference between the two treatment groups with respect to the change in $\eta'$ from the baseline ($p = 0.016$). For chemical analysis of the sputum, during the 6-week treatment period, the concentration of fucose in the sputum did not change from the baseline value in the placebo group and the seratrodast or placebo group, the values for sputum production immediately before the treatment period did not differ from those recorded during the previous 2-week run-in period. During the 6-week treatment period, the amount of daily sputum production in the placebo group did not change. In contrast, seratrodast treatment gradually decreased sputum production, and there was a significant difference between the two groups with respect to the change in mean sputum production at week 3 ($p = 0.008$), week 4 ($p = 0.022$), week 5 ($p = 0.039$), and week 6 ($p = 0.005$).

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trodast group (Fig 3). The concentration of albumin remained unchanged in the placebo group, but it decreased from the baseline value of 814 ± 50 µg/g to 529 ± 58 µg/g (p = 0.028) at week 6 in the seratrodast group. There was a significant difference between the two groups with respect to the change in albumin concentration from baseline (p = 0.044).

Nasal Mucociliary Clearance

To determine the effect of seratrodast on mucociliary transport function, NCT was measured by a saccharin method. The NCT measured at the beginning of the treatment period was 24 ± 4 min in the placebo group and 28 ± 5 min in the seratrodast group (Fig 4). These values did not significantly differ from each other, and were greater than the values for normal volunteers (7 ± 4 min). During the treatment period, placebo did not alter NCT, while seratrodast significantly shortened NCT at week 4 (p = 0.031) and week 6 (p = 0.025). There was a significant difference between the two groups with respect to the change from the baseline values of NCT.

Discussion

Our double-blind, placebo-controlled study demonstrates that 6-week treatment with seratrodast, a specific TxA2 receptor antagonist,10 elicits a decrease in the amount of daily production of sputum and improves breathlessness, wheezing, and cough in patients with mild to moderate asthma with minimal side effects. The decreases in sputum production were accompanied by beneficial alterations in sputum physicochemical properties, including reductions in η' and albumin concentration. Furthermore, seratrodast shortened NCT, indicating an improvement in upper airway mucociliary clearance.

It is well known that the cyclooxygenase product TxA2 induces potent bronchoconstriction and airway hyperresponsiveness, a key feature of asthma. One study suggests that TxA2 also possesses an immunomodulating action, because the blockade of TxA2 synthesis or antagonism of TxA2 receptor inhibits antigen-induced accumulation of eosinophils into the airways.17 However, the role of TxA2 in the pathogenesis of asthma seems controversial. In spite of the fact that the levels of TxA2 metabolites in BAL fluid and urine are elevated after antigen inhalation
in asthmatic subjects, a number of clinical trials on TxA2 synthase inhibitors or TxA2 receptor antagonists have failed to demonstrate improvements in obstructive impairment in pulmonary function. Fujimura and coworkers have shown that treatment of mild asthma with seratrodast for 4 days does not change baseline pulmonary function. In the present study, the mean values for FEV1 and PEF tended to increase after a 6-week administration of seratrodast, but these changes did not reach significant levels compared with the placebo group. Thus, involvement of TxA2 in the baseline airflow limitation seems unlikely in stable asthma.

On the other hand, in the seratrodast group, diurnal variation of PEF was significantly decreased after a 6-week administration of seratrodast, but these changes did not reach significant levels compared with the placebo group. Thus, involvement of TxA2 in the baseline airflow limitation seems unlikely in stable asthma.

In addition to increasing the viscosity of airway secretions, albumin can agglutinate individual cilia and destroy coordinated ciliary motion, which may lead to impairment of mucociliary clearance. In the present trial, instead of studying bronchial mucus velocity or inhalation scanning of radiolabeled aerosols, an alternative noninvasive method was used to measure nasal clearance of a saccharin particle. We found that nasal clearance was prolonged in asthma patients compared with normal subjects, and that the disturbed clearance significantly improved after the treatment with seratrodast. However, further studies are required to determine whether this improvement is a result of the decreased mucus hypersecretion or the reduced airway microvascular leakage.

It has been recognized that mucociliary transport function is generally disturbed in asthmatic airways. However, the importance of airway secretion and mucociliary dysfunction in asthma severity is not clear. It is postulated that the change in airway geometry induced by the accumulation of intrabronchial secretions and mucus plugging increases resistance to airflow, but mucus hypersecretion may not necessarily correlate with impaired mucociliary clearance. Ahmed and associates have reported that the impaired mucus transport in asthma is attributed, at least in part, to cysteinyl leukotrienes liberated during airway anaphylaxis. In contrast, our study showed that seratrodast caused a marked decrease in the amount of sputum production along with changes in physicochemical properties of the sputum (i.e., decreases in fucoidan and albumin concentration). To date, little is known of the effect of TxA2 on glycoprotein secretion from submucosal glands and goblet cells, and it is thus uncertain whether seratrodast reduced sputum by acting on mucus-producing cells. On the other hand, TxA2 has been shown to induce airway microvascular leakage. Lotvall et al. showed that U-46619, a TxA2 mimetic, caused plasma exudation in guinea pig airways. They also found that the TxA2 synthase inhibitor OKY-046 and the TxA2 receptor antagonist ICI-192605 each inhibited albumin leakage induced by platelet-activating factor. In fact, we measured concentrations of fucoidan and albumin in the sputum, the former being derived from mucous secretions and the latter being viscous and regarded as a marker of serum-derived constituents. The seratrodast group, fucoidan and albumin concentration were decreased after the treatment, while fucose contents remained unchanged. Taken together, the observed effects of seratrodast may be associated with attenuation of airway microvascular permeability and the concomitant reduction of albumin leakage into the airway mucosa.

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is associated with the action by seratrodast on nasal microvascular permeability.

In conclusion, addition of a TxA₂ receptor antagonist to conventional antiasthma medications may be considered in the management of patients with mild to moderate asthma having increased airway secretions. Blockade of TxA₂ receptor has minimal effects on pulmonary function, but decreases the amount of sputum along with alterations in its physicochemical properties, and causes an improvement in nasal mucociliary clearance.

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