BAY u3405, a Thromboxane A₂ Antagonist, Reduces Bronchial Hyperresponsiveness in Asthmatics*

Hisamichi Aizawa, MD, PhD; Mutsumi Shigyo, MD; Hiroko Nogami, MD; Takahito Hirose, MD, PhD; and Nobuyuki Hara, MD, PhD

Objective: Thromboxane A₂ (TXA₂) is reported to induce bronchial hyperresponsiveness along with the well-documented bronchoconstrictor action on smooth muscles. We examined the effect of the TXA₂ antagonist, BAY u3405, on bronchial hyperresponsiveness to methacholine (MCh) in asthmatics.

Patients: Twelve adult asthmatics were studied in a randomized, double-blind, placebo-controlled, crossover fashion.

Design: Following a 2-week run-in period, the subjects were administered 75 mg of BAY u3405 or placebo orally, twice a day for 2 weeks each in a crossover design, interposing a 2-week washout period. Bronchial hyperresponsiveness was measured by the astograph method. Briefly, the respiratory resistance (Rrs) was measured by the forced oscillation method during continuous inhalation of MCh in stepwise incremental concentrations, until Rrs reached twice the baseline value. Bronchial hyperresponsiveness was evaluated as the minimum cumulative dose (Dmin) of MCh that induced an increase in Rrs. Dmin was calculated so that 1 U of Dmin equals to 1 min of inhalation of aerosol solution at 1.0 mg/mL during quiet breathing.

Results: Three subjects were withdrawn from the evaluation because they had asthmatic attacks or wheezing during the study. The Dmin value of 0.533 U (GSEM 1.675) after the BAY u3405 treatment was significantly greater than that of 0.135 U (GSEM 1.969) after the placebo treatment (p=0.0139). There were no safety concerns in either treatment group.

Conclusion: We conclude that BAY u3405 may be a useful drug for attenuating bronchial hyperresponsiveness in bronchial asthma.

(CHEST 1996; 109:338-42)

ANOVA-analysis of variance; Dmin-minimum cumulative dose; GSEM=geometric standard error of mean; MCh=methacholine; Rrs-respiratory resistance; TXA₂=thromboxane A₂

Key words: astograph; bronchial asthma; methacholine; thromboxane A₂

Bronchial asthma is a disease defined by reversible airway obstruction, bronchial hyperresponsiveness, and inflammation.¹ Bronchial hyperresponsiveness to a wide variety of stimuli is a characteristic feature of asthma, and the level of hyperresponsiveness usually correlates with the clinical severity of asthma and medication requirements.¹

Although the exact mechanism of bronchial hyperresponsiveness is not yet known, thromboxane A₂ (TXA₂) has been reported to be an important mediator involved in the development of bronchial hyperresponsiveness. Thus, TXA₂ is a potent bronchoconstrictor² and has been demonstrated to induce bronchial hyperresponsiveness in dogs³ and humans.⁴ In dogs, the TXA₂ synthetase inhibitor, OKY-046, inhibits bronchial hyperresponsiveness induced by ozone³ and allergen.⁵

BAY u3405 is an indole sulfonamide⁶ that is a potent and selective TXA₂ receptor antagonist on the airway smooth muscle⁷-⁹ and vascular smooth muscle.⁸ Therefore, BAY u3405 may be effective in the treatment of bronchial asthma by reducing bronchial hyperresponsiveness. In this study, we examined the effects of BAY u3405 on airway hyperresponsiveness in patients with bronchial asthma.

MATERIALS AND METHODS

Subjects

The study group comprised 12 subjects (6 men and 6 women) between 18 and 42 years old, with mild asthma who were attending the clinics at Kyushu University Hospital or National Minami Fukuoka Chest Hospital, Fukuoka, Japan. Informed consent was obtained from each subject in accordance with the Declaration of Helsinki. Table 1 summarizes the anthropometric data as obtained at the time of inclusion in the study. All had been diagnosed as having asthma based on a history consistent with episodic airway obstruction, diffuse expiratory wheezes heard on auscultation of the chest, and the demonstration of an increase of 15% or more in their
Table 1—Characteristics of Patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No.</th>
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<tr>
<td>No.</td>
<td>12</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>6/6</td>
</tr>
<tr>
<td>Age, yr</td>
<td>24.1±6.6* (18-42)</td>
</tr>
<tr>
<td>%FVC</td>
<td>94.8±12.3* (76-111)</td>
</tr>
<tr>
<td>%FEV₁</td>
<td>79.3±14.6* (59-111)</td>
</tr>
<tr>
<td>FEV₁/FVC (%)</td>
<td>81.7±9.9* (66-100)</td>
</tr>
<tr>
<td>Rs, cmH₂O/L/s</td>
<td>3.7±1.0* (2.4-5.9)</td>
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</table>

*Mean±SD (range).

FEV₁ following the inhalation of salbutamol. Increased bronchial responsiveness to methacholine (MCh) was observed in all patients. All subjects were maintained on a regimen of the inhaled B₂-stimulant as needed. The patients who had received inhalers or sodium cromoglycate therapy either currently or in the last 4 weeks before the study were excluded from the investigation.

Study Design

The patients were studied in a randomized, double-blind, placebo-controlled, crossover study. The study period was 8 weeks and four MCh inhalation tests were performed at bi-weekly intervals. The subjects were requested to take only aerosol salbutamol or procaterol on demand for a run-in period of 2 weeks at the start of the study. We defined a "run-in period" as the observation period before BAY u3405 or placebo. Treatment with all other medications for asthma was withheld. Two weeks later, the patients were administered 75 mg of BAY u3405 or placebo tablet orally twice a day for 2 weeks in a double-blind fashion. At the end of the first period of treatment, the drugs were crossed over, interposing a 2-week washout period.

Measurement of Bronchial Responsiveness to MCh

The treatments of asthma were withheld for at least 12 h before the measurement of bronchial responsiveness. Bronchial responsiveness was measured by the method of Takishima et al.¹⁰ by an astograph (TCK-6100H; Chest; Tokyo). Briefly, respiratory resistance (Rs) was measured by the forced oscillation method (3 Hz) during inhalation of MCh continuously in stepwise incremental concentrations, until Rs reached twice the baseline value. MCh chloride was diluted in physiologic saline solution with twofold increasing concentrations from 0.049 to 25 mg/mL. Each concentration of MCh solution was inhaled for a period of 1 min. All subjects were examined during quiet breathing in a sitting position. Bronchial hyperresponsiveness was evaluated as the minimum cumulative dose (Dmin) of MCh that induced an increase in Rs. Dmin was calculated so that 1 U of Dmin equals to 1 min of inhalation of aerosol solution at 1.0 mg/mL. Thus, a decrease in Dmin indicates an increase in bronchial hyperresponsiveness, and an increase in Dmin indicates decrease in bronchial hyperresponsiveness. Previous studies have reported a significant correlation between the bronchial hyperresponsiveness measured by astograph and that by conventional methods, and clinical usefulness of astograph.¹¹-¹⁴

Drugs

The drugs used in this study were MCh chloride (Sigma; St. Louis) and BAY u3405 (Bayer Yakuhin Ltd; Osaka, Japan).

Statistical Analysis

The values of Dmin are expressed as the geometric means and geometric standard errors of means (GSEM). Statistical analysis was performed by use of the analysis of variance (ANOVA) for crossover design after the logarithmic transformation. We considered the differences to be statistically significant when the p value was less than 0.05.

Results

Three subjects had asthmatic attacks or wheezing on the day of provocation test. They were withdrawn from evaluation because treatments or airway narrowing may affect the bronchial hyperresponsiveness. The Dmin values before the treatment were not significantly different between 0.242 U (GSEM 1.561) for BAY u3405 and 0.183 U (GSEM 1.789) for placebo. Figure 1 shows a typical tracing of MCh challenge in one subject. The dose-response curve of Rs shifted to the right after administration of BAY u3405. Table 2 and Figure 2 show the results of the analyses. The mean Dmin after 2 weeks of treatment with the placebo was 0.175 U (GSEM 2.326). Two weeks after the crossover to BAY u3405, there was an increase in Dmin to the mean of 0.352 U (GSEM 2.677).

![Figure 1. Typical astograph tracing of MCh test in patient 4, a 30-year-old man. Solid line indicates the dose response curve of Rs before BAY u3405 treatment, and dotted line indicates that after BAY u3405 treatment. Concentrations of MCh were as follows (mg/mL): (c) saline solution; (1) 0.049; (2) 0.098; (3) 0.195; (4) 0.391; (5) 0.781; (6) 1.56; (7) 3.13; (8) 6.25; (9) 12.5; (10) 25.0](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21727/ on 04/07/2017)
Table 2—Summary of Dmin Values*

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of Patients</th>
<th>BAY u3405</th>
<th>Placebo</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>BAY u3405 first</td>
<td>5</td>
<td>Dmin</td>
<td>0.415 (1.597)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dmin (post)/Dmin (pre)</td>
<td>1.789 (1.735)</td>
</tr>
<tr>
<td>Placebo first</td>
<td>4</td>
<td>Dmin</td>
<td>0.123 (2.102)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dmin (post)/Dmin (pre)</td>
<td>2.854 (1.508)</td>
</tr>
</tbody>
</table>

*Values are geometric mean (GSEM). Results of ANOVA for Dmin: direct treatment effect, p=0.0139 BAY u3405 > placebo; period effect, p=0.1747, NS; and treatment by period interaction, p=0.9097, NS. Results of ANOVA for Dmin (post)/Dmin (pre): direct treatment effect, p=0.0322 BAY u3405 > placebo; period effect, p=0.7294, NS; and treatment by period interaction, p=0.5388, NS.

Dmin after 2 weeks of treatment of BAY u3405 was 0.743 U (GSEM 1.751). Two weeks after the crossover to placebo, there was a decrease in Dmin to the mean of 0.110 U (GSEM 2.993). The direct treatment effects were statistically significant (p=0.0139 in Dmin, p=0.0322 in post/pre ratio of Dmin). The value of Dmin after the BAY u3405 treatment was fourfold higher than that after the placebo treatment (Fig 3, top). Figure 3, bottom, shows the ratio of Dmin (postmedication)/Dmin (premedication). Thus, when this ratio is larger than 1, the bronchial hyperresponsiveness is decreased. This ratio was 2.202 for the treatment with BAY u3405, which was significantly larger than that for the treatment of placebo. The baseline lung function before astograph is shown in

![Figure 2](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21727/ on 04/07/2017)

**Figure 2.** Changes in Dmin (geometric mean with GSEM) during crossover study in each group. Closed circles indicate BAY u3405 first group, and open triangles indicate placebo first group.

![Figure 3](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21727/ on 04/07/2017)

**Figure 3.** Posttreatment Dmin (top) and post/pre ratio in Dmin (bottom). Significant differences were observed between BAY u3405 treatment and placebo treatment. (n=9, p=0.0139 for Dmin; p=0.0322 for the post/pre ratio of Dmin.)

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Figure 4 (top: FEV₁; bottom: FVC). There are no differences before and after each treatment. There were no safety concerns in either treatment.

**Discussion**

In this study we demonstrated that a selective TXA₂ antagonist, BAY u3405, reduces the severity of bronchial hyperresponsiveness in the asthmatic subjects. The bronchial responsiveness to MCh decreased by the treatment with BAY u3405, but not by that with the placebo, in the present double-blind and crossover study. There are no changes in lung function before and after treatment with BAY u3405, suggesting that the suppression of bronchial hyperresponsiveness is not due to bronchodilatory effect.

Bronchial asthma is a disease characterized by bronchial hyperresponsiveness to many physical, chemical, and pharmacologic agents, including MCh.15 Bronchial hyperresponsiveness also appears to be important in the pathogenesis of asthma and it is closely linked to bronchial inflammation.16 In addition, the degree of bronchial hyperresponsiveness is related to the severity of the asthma symptoms,17 the number of previous hospital admissions,18 and treatment requirements.19 Bronchial hyperresponsiveness may be caused and perpetuated by agents that cause bronchial inflammation, indicating important therapeutic implications. Therapeutic interventions that reduce bronchial inflammation in bronchial asthma patients decrease the bronchial hyperresponsiveness and the severity of asthma. Treatment with anti-inflammatory agents may modify bronchial hyperresponsiveness, improve asthma symptoms, and reduce the need for frequent use of a bronchodilator. Inhaled steroids not only provide symptomatic benefit but also reduce the bronchial hyperresponsiveness.20 Cromolyn sodium and nedocromil are also known to reduce bronchial hyperresponsiveness in asthmatics.1 Although B-sympathomimetics and theophylline are potent bronchodilators, neither drug has been reported to improve bronchial hyperresponsiveness.1

TXA₂ is known to be a potent constrictor of the airway2 and vascular smooth muscle21 and to cause platelet aggregation.22 TXA₂ was originally described as being released from the platelets23 but it is now known to be released from other cells, including macrophages and neutrophils.24 TXA₂ is considered to play an important role for the development of airway hyperresponsiveness. Thus, a TXA₂ synthase inhibitor has been demonstrated to prevent the development of airway hyperresponsiveness induced by ozone,3 leukotriene B₄,25 platelet activating factor,26 and allergen.5 Furthermore, a TXA₂ mimetic, U-46619, has been reported to cause airway hyperresponsiveness in dogs.3 Jones et al4 also reported that U-46619 enhanced MCh-induced contractions in humans. Although the mechanism by which TXA₂ causes airway hyperresponsiveness is not yet known, prejunctional enhancement of acetylcholine release induced by TXA₂ has been reported.27,28 U-4661927 or TXA₂ released from the aggregated platelets28 caused an increase in the contractile response to vagal nerve stimulation, but no increase in the response to exogenous cholinergic agonists was observed.

The role played by TXA₂ in human airway diseases is not yet known, but several studies have suggested the importance of this agent. In human subjects, increased levels of TXB₂, the metabolite of TXA₂, have been demonstrated in the plasma following allergen challenge29 and in the BAL fluid following exposure to ozone.30 Kirby et al31 reported that indomethacin significantly inhibits the development of bronchial hyperresponsiveness after inhalation of allergens in allergy subjects. In an uncontrolled study, the TXA₂ synthase inhibitor, OKY-046, administered orally, reduced bronchial hyperresponsiveness to acetylcholine in patients with stable asthma.32 The present controlled study clearly showed that BAY u3405 is effective against bronchial hyperresponsiveness in asthmatics.

BAY u3405 is a selective and potent TXA₂ receptor antagonist.6-9 It is reported to inhibit U-46619-induced contractions in human, guinea pig, rat, and ferret air-
way smooth muscle.\textsuperscript{7-8} BAY u3405 given either IV, orally, or by aerosol has also been reported to attenuate U-46619-induced bronchoconstriction in guinea pigs.\textsuperscript{9} Furthermore, BAY u3405, administered orally, has been reported to suppress prostaglandin D2-induced bronchoconstriction in asthmatics.\textsuperscript{33} Johnson et al did not find acute inhibitory effect of BAY u3405 on histamine-induced bronchoconstriction.\textsuperscript{33} In the present study, as we attempt to attenuate bronchial hyperresponsiveness itself, which is presumably accompanied by chronic airway inflammation, we administered this drug for 2 weeks. Suppression of bronchial hyperresponsiveness in asthmatics by BAY u3405 indicates the importance of TXA\textsubscript{2} in the pathogenesis of asthma. BAY u3405 may be of value in the treatment of asthma by modifying bronchial hyperresponsiveness. However, further investigations are needed.

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