Inhibitory Effect of a Selective Thromboxane Synthetase Inhibitor, OKY-046, on Acetaldehyde-Induced Bronchoconstriction in Asthmatic Patients*

Shigeharu Myou, M.D.; Masaki Fujimura, M.D., F.C.C.P.; Kohichi Nishi, M.D.; Takio Ohka, M.D.; and Tamotsu Matsuda, M.D.

We recently reported that inhaled acetaldehyde causes bronchoconstriction indirectly via histamine release in patients with asthma. The purpose of this study was to investigate a role of thromboxane A2 in acetaldehyde-induced bronchoconstriction in asthmatic airways. We investigated the bronchial response to inhalation of ascending doses (5, 10, 20, and 40 mg/ml) of acetaldehyde in nine asthmatic subjects who were treated with placebo or OKY-046, a selective thromboxane A2 synthetase inhibitor, of 200 mg twice a day for 3 days, and 200 mg on the fourth day (test day) in a double-blind, randomized, placebo-controlled, crossover fashion. Percentage decreases in FEV1 caused by 20 and 40 mg/ml of acetaldehyde inhalation were significantly (p<0.05 and p<0.01, respectively) prevented by the pretreatment with OKY-046. Geometric mean value (geometric standard error of the mean) of acetaldehyde concentration producing a 20 percent fall in FEV1 (PC20-Ac-CHO) was significantly (p<0.01) greater with the OKY-046 pretreatment (72.2 [1.1] mg/ml) than with the placebo pretreatment (19.8 [1.2] mg/ml). We conclude that thromboxane A2 is one of contributors to acetaldehyde-induced bronchoconstriction in asthmatic subjects. It suggests that thromboxane A2 may play an important role in endogenous histamine-induced bronchoconstriction caused by acetaldehyde in asthmatic airways. We believe that this is a first report on the interaction between endogenous histamine and thromboxane A2 in asthmatic subjects.

**Key words:** acetaldehyde; asthma; OKY-046; thromboxane A2

Blood acetaldehyde and histamine concentrations in asthmatics with alcohol-induced bronchoconstriction increased after ingestion of ethanol. We9 recently reported that inhaled acetaldehyde produces bronchoconstriction indirectly via histamine release in asthmatic patients *but not in healthy nonasthmatic subjects.*

However, thromboxane A2, a cyclo-oxygenase product of arachidonic acid metabolism, has been implicated in acute bronchoconstriction after allergen inhalation in asthmatics10,11 and synthetic analogues of thromboxane A2 such as the endoperoxide U46619 have been demonstrated to be a potent bronchoconstrictor in asthmatics.12 Moreover, thromboxane synthetase inhibitors have been found to suppress bronchoconstriction caused by various bronchoconstrictive agents,13 and we previously demonstrated that OKY-046, a selective thromboxane synthetase inhibitor, reduced bronchial hyperresponsiveness to acetylcholine14 and methacholine15 in asthmatics. In this study, we investigated a role of thromboxane A2 in acetaldehyde-induced bronchoconstriction using OKY-046.

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*From the Division of Respiratory Medicine, Ishikawa Prefectural Central Hospital, (Drs. Myou, Nishi, and Ohka), and the Third Department of Internal Medicine, Kanazawa University School of Medicine (Drs. Fujimura and Matsuda), Kanazawa, Japan.

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Reprint requests: Dr. Myou, Third Dept. of Internal Medicine, Kanazawa University School of Medicine, 13-1 Takara-machi, Kanazawa 920, Japan

**ALDH**=aldehyde dehydrogenase; **FEV1**=forced expiratory volume in 1 s; **FVC**=forced vital capacity; **PC20-Ac-CHO**=acetaldehyde concentration producing a 20 percent fall in **FEV1**; **PC20-MCh**=the provocative concentration of methacholine producing a 20 percent fall in **FEV1**; **PGI2**=prostaglandin I2

**Key words:** acetaldehyde; asthma; OKY-046; thromboxane A2

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1. Alcohol is oxidized to acetaldehyde, and then acetaldehyde is oxidized to acetate mainly by aldehyde dehydrogenase (ALDH). ALDH consists of two main isozymes with low and high Km for aldehyde. About 50 percent of Japanese lack the low Km enzyme (ALDH 2) and show an elevation of serum acetaldehyde concentration. Sakurai et al reported that ALDH 2 activity was a major determining factor of asthmatic exacerbation after drinking pure ethanol in Japanese asthmatic patients, and that changes in specific airway conductance closely related to blood acetaldehyde levels. Watanabe demonstrated that blood acetaldehyde and histamine concentrations in asthmatics with alcohol-induced bronchoconstriction increased after ingestion of ethanol. We recently reported that inhaled acetaldehyde produces bronchoconstriction indirectly via histamine release in asthmatic patients *but not in healthy nonasthmatic subjects.*

2. However, thromboxane A2, a cyclo-oxygenase product of arachidonic acid metabolism, has been implicated in acute bronchoconstriction after allergen inhalation in asthmatics and synthetic analogues of thromboxane A2 such as the endoperoxide U46619 have been demonstrated to be a potent bronchoconstrictor in asthmatics. Moreover, thromboxane synthetase inhibitors have been found to suppress bronchoconstriction caused by various bronchoconstrictive agents, and we previously demonstrated that OKY-046, a selective thromboxane synthetase inhibitor, reduced bronchial hyperresponsiveness to acetylcholine and methacholine in asthmatics. In this study, we investigated a role of thromboxane A2 in acetaldehyde-induced bronchoconstriction using OKY-046.
Table 1—Subject Characteristics

<table>
<thead>
<tr>
<th>Subject No./Age, yr./Sex</th>
<th>Baseline Spirometry</th>
<th>Bronchial Reversibility Test*</th>
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<tbody>
<tr>
<td></td>
<td>FVC, %</td>
<td>FEV1, %</td>
</tr>
<tr>
<td>1/48/F</td>
<td>117.4</td>
<td>102.6</td>
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<td>2/30/F</td>
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<td>3/45/F</td>
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<td>4/60/F</td>
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<td>5/26/M</td>
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<td>8/23/F</td>
<td>106.0</td>
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<tr>
<td>9/52/M</td>
<td>90.3</td>
<td>67.9</td>
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<tr>
<td>Mean 39.2</td>
<td>110.6</td>
<td>97.7</td>
</tr>
<tr>
<td>SEM 4.3</td>
<td>4.1</td>
<td>4.7</td>
</tr>
</tbody>
</table>

*FEV1 was taken before and 30 min after inhalation of 0.4 ml of 0.5 percent salbutamol solution.  
†Sa=salbutamol via metered-dose inhaler; Th=oral administered theophylline.

Materials and Methods

Subjects

Nine asthmatic patients with a mean age of 39.2±4.3 years participated in this study (Table 1). Each patient with asthma satisfied the American Thoracic Society’s definition, with symptoms of episodic wheezing, cough, and shortness of breath responding to bronchodilators and reversible airflow obstruction (more than 15 percent of reversibility) documented on at least one pulmonary function study25 (Table 1). In bronchial reversibility test, spirometry was taken before and 30 min after inhalation of 2 mg of salbutamol. All subjects were nonsmokers, had not experienced any occupational exposure, and had not experienced any respiratory tract infection for at least 8 weeks prior to the study. Moreover, they had no history of excessive mucus expectoration, and there was no low attenuation area in thin-slice chest computed tomography among all the subjects.

This study was carried out when their symptoms were mild and stable and while they were receiving aerosol β2-stimulant (salbutamol) and/or orally administered theophylline. They had not received steroid therapy for at least 8 weeks. Informed consent was obtained from all subjects. This study was approved by the ethics committee of our hospital.

Acetaldehyde Bronchoprovocation

Acetaldehyde bronchoprovocation was performed using the same method previously described. Briefly, acetaldehyde was dissolved in physiologic saline solution to make concentrations of 5, 10, 20, and 40 mg/ml. Saline and acetaldehyde solutions were inhaled from a nebulizer (DeVilbiss 646, DeVilbiss Co, Somerset, Pa) operated by compressed air at 5 L/min. Saline solution was inhaled first for 2 min and forced inspiratory volume in 1 s (FEV1) (Chestac 55, Chest Ltd., Nagoya, Japan) was measured. If the change in FEV1 from the baseline after inhalation of saline solution was 10 percent or less, inhalation of acetaldehyde was started. When inhaled saline solution caused a greater change in FEV1, the test was stopped or postponed. Acetaldehyde was inhaled for 2 min by mouth tidal breathing with the patient wearing a nose-clip; this was followed immediately by measurements of FEV1. All concentrations of acetaldehyde were inhaled to measure the dose-response curves. After completion of the acetaldehyde provocation, each subject inhaled 0.5 percent salbutamol solution for 2 min. FEV1 was measured three times and the best FEV1 value of three attempts was recorded each time.

Study Protocol

This study was performed in a double-blind, randomized, placebo-controlled, crossover fashion. Dose-response curves for bronchial response to inhaled acetaldehyde were measured on two occasions separated by 2 weeks. OKY-046 was given orally in a dose of 200 mg twice a day for 3 days and at 7 AM on the fourth day (test day). Placebo was administered by the same procedure as OKY-046. All medication expect for pretreatment with OKY-046 or placebo was stopped at 12 pm on the previous test day to allow a washout time of 23 h. The bronchial reactivity to inhaled acetaldehyde was then measured at 11 AM.

Data Analysis

Bronchoconstrictive responses were analyzed as percentage falls in FEV1 from the values measured immediately after inhalation of saline solution. Baseline forced vital capacity (FVC) and FEV1 values and percentage fall in FEV1 induced by acetaldehyde were expressed as mean ± standard error of the mean (SEM), and these values were compared between placebo and OKY-046 using Wilcoxon signed-rank test. The measured values of FEV1 were plotted on semilogarithmic graph paper and acetaldehyde concentration producing a 20 percent fall in FEV1 (PC20-Ac-CHO) was determined. After the pretreatment with OKY-046, a 20 percent fall in FEV1 was not obtained in eight of nine subjects at the highest concentration of acetaldehyde administered, and therefore, a minimal estimate was obtained by calculating the cumulative PC20-Ac-CHO on the next doubling concentration beyond the highest administered. Logarithmic transformation was applied to PC20-Ac-CHO values before analysis, and values were expressed as geometric mean (geometric SEM). Because of these censored data, PC20-Ac-CHO values were compared between placebo and OKY-046 using Wilcoxon signed-rank test. Significance was based on a 95 percent confidence level (p<0.05).

Results

The mean baseline values of FVC and FEV1 in asthmatics before inhalation of saline solution on the placebo day were 3.61±0.31 and 2.79±0.29 L, and those on the OKY-046 day were 3.62±0.31 and 2.75±0.29 L. There were no significant differences in the FVC and FEV1 between the 2 test days.

Individual dose-response curves are shown in Fig-

CHEST / 106 / 5 / NOVEMBER, 1994 1415
Figure 1. Individual dose-response curves for inhaled acetaldehyde-induced bronchoconstriction after administration of placebo (open circles) and OKY-046 (closed circles). Data are shown as change in FEV₁ values. S = inhalation of saline solution; B₁ = inhalation of B₁ agonist salbutamol.

Figure 2. Dose-response curve for percent change in FEV₁ produced by aerosolized acetaldehyde in nine asthmatics pretreated with OKY-046 (closed circles) or placebo (open circles) in a double-blind, randomized, placebo-controlled, crossover manner. Asterisk = p<0.05 and two asterisks = p<0.01 compared with placebo.

Discussion

This study shows that OKY-046, a selective thromboxane synthetase inhibitor, has a significant and marked inhibitory effect on acetaldehyde-induced bronchoconstriction in asthmatic subjects. Since we⁹ already reported that inhalation of acetaldehyde by the same method causes no significant bronchoconstriction in healthy nonasthmatic subjects, no control subject participated in this study.

In our previous studies, 3,000 mg over 4 days¹⁴ and 2,600 mg over 4 days¹⁵ of OKY-046 did not affect baseline pulmonary function. Since baseline FVC and FEV₁ of the present study were also similar between 2 test days, changes in baseline airway caliber¹⁷ can be ruled out as an explanation for the
inhibitory effect of OKY-046. It suggests that the inhibitory effect of OKY-046 on acetaldehyde-induced bronchoconstriction is associated with its pharmacologic actions as a thromboxane synthetase inhibitor.

Inhibition of thromboxane synthetase leads to production of prostaglandin I₂ (PGI₂).15,19 PGI₂ has a potent blood vessel dilating effect, but no consistent bronchodilating effect in normal subjects and asthmatic patients.20 It is likely that the inhibitory effect of OKY-046 is due to a reduction in thromboxane A₂ synthesis rather than production of PGI₂.

Our previous report9 demonstrated that inhaled acetaldehyde produces bronchoconstriction, which is caused mainly via release of histamine. The origin of histamine released by acetaldehyde in asthmatic airways is not clear, although acetaldehyde causes dose-dependent histamine release from leukocytes of asthmatics in vitro.1 Thromboxane A₂ is also produced by mononuclear leukocytes and mast cell.21-25 It is possible that acetaldehyde produces thromboxane A₂ in asthmatic airways. However, since terfenadine, a selective histamine H₁ antagonist, almost completely inhibits acetaldehyde-induced bronchoconstriction,9 simple production of thromboxane A₂ cannot explain the marked inhibitory effect of OKY-046 on acetaldehyde-induced bronchoconstriction.

Jones et al12 reported that inhaled thromboxane mimetic U46619 is a potent bronchoconstrictor in normal and asthmatic subjects, and that subthreshold concentrations of U46619 cause methacholine airway hyperresponsiveness in asthmatic subjects. Moreover, oral administration of OKY-046 reduces bronchial hyperresponsiveness to acetylcholine14 and methacholine.15 It is possible that preventing effect of OKY-046 on acetaldehyde-induced bronchoconstriction may result from reduction of nonspecific bronchial hyperresponsiveness. In the present study, however, a lower dose of OKY-046 was administered compared with those used in our previous studies.14,15 Indeed, the provocative concentration of methacholine producing a 20 percent fall in FEV₁ (PC20-MCh) after the relatively high dose of OKY-046 (800 mg/d for 3 days plus 200 mg on the test day) was just a little (2.4-fold) higher than the baseline PC20-MCh.15 Therefore, it is unlikely that reduction of nonspecific bronchial hyperresponsiveness is a major mechanism for the marked inhibitory effect of OKY-046 on the acetaldehyde-induced bronchoconstriction.

Another possible mechanism is that endogenous histamine-induced bronchoconstriction by inhaled acetaldehyde may be mediated in part via thromboxane production, because histamine induces the formation of thromboxanes in the lungs of sensitized and nonsensitized guinea pigs.26,27 Thromboxane formation occurs mainly in response to the stimulation of H₁-receptors.26 Indeed, four types of thromboxane synthetase inhibitors (imidazol, dazoxiben, CGS 13090, and SQ 80358) showed marked depressory effects on histamine-induced thromboxane formation in the guinea pig lung.28 To our knowledge, the role of thromboxane A₂ in endogenous histamine-induced bronchoconstriction in human subjects has not yet been studied. In order to draw a conclusion, it is necessary to investigate the role of thromboxane A₂ in another endogenous histamine-mediated bronchoconstriction such as hyperventilation,29 exercise,30 ultrasonically nebulized distilled water,31 adenosine 5’ monophosphate,32 and cold air33 in asthmatic subjects.

In conclusion, thromboxane A₂ is one of contributors to acetaldehyde-induced bronchoconstriction in asthmatics. It suggests that thromboxane A₂ plays a important role in endogenous histamine-induced bronchoconstriction caused by acetaldehyde in asthmatic airways. Furthermore, we believe that this is a first report suggesting the interaction between endogenous histamine and thromboxane A₂ in asthmatic airways.

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