Effects of a thromboxane synthetase inhibitor (OKY-046) and a lipoxygenase inhibitor (AA-861) on bronchial responsiveness to acetylcholine in asthmatic subjects

MASAKI FUJIMURA, FUMIHIKO SASAKI, YASUTO NAKATSUMI, YOSHIFUMI TAKAHASHI, SENSU HIFUMI, KUNIAKI TAGA, JUN-ICHIRO MIFUNE, TAKASHI TANAKA, TAMOTSU MATSUDA

From the Division of Pulmonary Disease, Fukui Cardiovascular Center, Fukui, and the Third Department of Internal Medicine, Kanazawa University School of Medicine, Kanazawa, Japan

ABSTRACT The effect of a selective thromboxane synthetase inhibitor, OKY-046, and a selective 5-lipoxygenase inhibitor, AA-861, on bronchial responsiveness to acetylcholine was studied in 23 asthmatic subjects. The provocative concentration of acetylcholine producing a 20% fall in forced expiratory volume in one second (PC₂₀ FEV₁) was measured before and after oral administration of OKY-046 (3000 mg over four days) and AA-861 (1100 mg over four days) and inhalation of OKY-046 (30 mg) in 10, 10, and nine asthmatic subjects respectively. Baseline values of FEV₁ and forced vital capacity (FVC) were not altered by oral OKY-046, oral AA-861, or inhaled OKY-046. The geometric mean value of PC₂₀ FEV₁ increased significantly from 0.55 to 2.24 mg/ml after oral OKY-046, but was unchanged after inhalation of OKY-046 and after oral administration of AA-861. These results suggest that thromboxane A₂ may play a part in bronchial hyperresponsiveness to acetylcholine.

One of the major clinical features of bronchial asthma is the increased bronchial responsiveness to various specific and non-specific stimuli. Thromboxane A₂, a metabolite of arachidonic acid, is a potent bronchoconstrictor.¹ OKY-046 ((E)-3-[p-(1H-imidazole-1-ylmethyl)phenyl]-2-propenoic acid hydrochloride monohydrate) is a selective thromboxane A₂ synthetase inhibitor.² In guinea pigs it has been shown to suppress bronchoconstriction induced by allergen³ or by leukotrienes, prostaglandin F_{2α}, histamine, and acetylcholine.⁴ Indomethacin, a cyclo-oxygenase inhibitor, has been shown to reduce bronchial responsiveness to histamine in patients with asthma⁵ and the increase in bronchial responsiveness seen in dogs exposed to ozone.⁶ These findings implicate cyclo-oxygenase products such as thromboxane A₂ in bronchial hyperresponsiveness.

The lipoxygenase pathway is the other major route

of arachidonic acid metabolism, producing the potent bronchoconstrictors leukotriene C₄, D₄, and E₄,^{7,8} the main components of slow reacting substance of anaphylaxis (SRS-A).⁹ These 5-lipoxygenase products are important chemical mediators in immediate type hypersensitivity reactions,^{7,10} but it is not clear whether they play a part in bronchial hyperresponsiveness. AA-861 (2,3,5-trimethyl-6-(12-hydroxy-5,10-dodecadienyl)-1,4-benzoquinone) is a selective 5-lipoxygenase inhibitor.¹¹ In the present study we gave OKY-046 and AA-861 to investigate the role of thromboxane A₂ and 5-lipoxygenase products in the bronchial hyperresponsiveness of patients with asthma.

Subjects and methods

The subjects were 15 men and eight women with asthma attending the hospital, with a mean age of 57 (range 37-75) years. All showed an improvement of 15% or more in forced expiratory volume in one second (FEV₁) after inhalation of 300 µg salbutamol sulphate. They all had intrinsic asthma with no familial

Address for reprint requests: Dr Masaki Fujimura, Third Department of Internal Medicine, Kanazawa School of Medicine, 13-1 Takara-machi, Kanazawa 920, Japan.

Accepted 30 June 1986

history of allergic diseases and no increased levels of specific IgE antibodies. The test was performed when their symptoms were mild and stable while they were having oral bronchodilators (theophylline retard and β_2 stimulants) and mucolytic agents but not steroids. All medication was stopped at 9.00 pm the previous day to allow a washout time of 18 hours before the measurement of bronchial responsiveness at 3.30 pm on the test day.

Bronchial responsiveness was evaluated with acetylcholine. Acetylcholine chloride was dissolved in physiological saline to make solutions of 0.08, 0.16, 0.31, 0.63, 1.25, 2.5, 5, 10, and 20 mg/ml. Saline and acetylcholine were inhaled from a DeVilbiss 646 nebuliser (DeVilbiss Co, Somerset, Pennsylvania) operated by compressed air at 5 l min^{-1} . Saline was inhaled first for two minutes and FEV_1 was measured (Autospirom HI-409, Chest Co Ltd, Japan). If the change in FEV_1 from the baseline after inhalation of saline was 10% or less, inhalation of acetylcholine was started. When inhaled saline caused a larger change in FEV_1 the test was stopped or postponed. Acetylcholine was inhaled for two minutes by tidal breathing with a nose clip, and this was followed immediately by spirometry. It was given in increasing concentrations until a fall of 20% or more in FEV_1 was noted. The measured values were plotted on semilogarithmic graph paper and the acetylcholine concentration ($\text{PC}_{20} \text{ FEV}_1$) producing a 20% fall in FEV_1 was calculated.

OKY-046 (Kissei Pharmaceutical Co Ltd, Matsumoto, and Ono Pharmaceutical Co Ltd, Osaka, Japan) was given orally in a dose of 200 mg 4 times a day for three days plus 200 mg in the morning, at noon and at 3.00 pm on the 4th day (test day). Bronchial responsiveness was then measured at 3.30 pm.

On a separate occasion OKY-046 solution (30 mg/ml) was inhaled with a DeVilbiss 646 nebuliser for two minutes, 10 minutes before the measurement of bronchial responsiveness to acetylcholine. About 30 mg OKY-046 was inhaled.

AA-861 (Central Research Division, Takeda Chemical Industries Ltd, Osaka, Japan) was given at a dose of 100 mg three times a day orally plus 100 mg in the morning and at 1.30 pm on the 4th day (test day). An acetylcholine challenge test was carried out at 3.30 pm.

Informed consent was obtained from all patients after the purpose of the test had been explained. No information on the test drugs and their pharmacological actions was given to the patients or the technical staff who performed the acetylcholine inhalation test.

DATA ANALYSIS

Acetylcholine $\text{PC}_{20} \text{ FEV}_1$ values are expressed as

Fig 1 Effect of inhaled OKY-046 on acetylcholine $\text{PC}_{20} \text{ FEV}_1$ (the provocative concentration of acetylcholine reducing FEV_1 by 20%) in nine asthmatic subjects.

Fig 2 Effects of oral administration of OKY-046 on acetylcholine $\text{PC}_{20} \text{ FEV}_1$ (see fig 1 legend) in 10 asthmatic subjects.

	Inhaled OKY-046 (n = 9)		Oral OKY-046 (n = 10)		Oral AA-861 (n = 10)	
	Control	Pretreatment	Control	Pretreatment	Control	Pretreatment
FVC(l)	2.99 (0.2)	3.12 (0.3)	2.51 (0.2)	2.49 (0.2)	2.65 (0.3)	2.62 (0.3)
FVC (% pred)	95 (4)	99 (6)	83 (4)	83 (5)	86 (6)	85 (9)
FEV ₁ (l)	1.54 (0.2)	1.66 (0.2)	1.50 (0.2)	1.45 (0.2)	1.59 (0.2)	1.56 (0.2)
FEV ₁ (% pred)	63 (5)	67 (4)	62 (7)	62 (8)	65 (6)	64 (8)
FEV ₁ /FVC (%)	53 (6)	55 (5)	57 (5)	56 (6)	59 (3)	59 (4)
Age (y)		57 (4)		57 (3)		56 (4)
Male:female		6:3		6:4		6:4

Fig 3 Effects of oral administration of AA-861 on acetylcholine PC₂₀ FEV₁ (see fig 1 legend) in 10 asthmatic subjects.

geometric means with the geometric standard error of the mean (GSEM) expressed as a factor. Values for baseline FVC and FEV₁ are reported as arithmetic means and standard errors of the mean (SEM).

Geometric mean PC₂₀ FEV₁ values were compared by the paired *t* test. A *p* value of 0.05 was taken as significant.

Results

Mean baseline values of FVC and FEV₁ are shown before and after the administration of OKY-046 and AA-861 in the table. There was no significant difference in the FEV₁ or FVC baseline values before and after each drug.

PC₂₀ FEV₁ values before and after inhalation of OKY-046 are shown in figure 1. The geometric mean values, 0.60 (GSEM 1.32) mg/ml before and 0.59 (GSEM 1.48) mg/ml after inhalation, did not differ significantly.

PC₂₀ FEV₁ values before and after oral dosing with OKY-046 are shown in figure 2. There was a significant increase in PC₂₀ FEV₁ (*p* < 0.01) after oral OKY-046 from 0.55 (GSEM 1.48) mg/ml to 2.24 (GSEM 1.51) mg/ml.

PC₂₀ FEV₁ values before and after oral dosing with AA-861 are shown in figure 3. There was no significant difference—0.56 (GSEM 1.32) mg/ml before and 0.39 (GSEM 1.32) mg/ml after treatment.

There were no adverse effects from either oral OKY-046 or oral AA-861.

Discussion

Thromboxane synthetase inhibitors have been found to suppress bronchoconstriction caused by various bronchoconstrictive agents (histamine, serotonin, acetylcholine, bradykinin, and prostaglandin F_{2α}) in guinea pig tracheal strips.¹² The thromboxane synthetase inhibitor OKY-046 has been shown to suppress airway anaphylaxis induced by antigen inhalation³ and non-specific bronchoconstriction caused by various bronchoconstrictive agents.⁴ These findings suggest that thromboxane A₂ plays a part in the development of bronchial hyperresponsiveness in guinea pigs. In man, however, thromboxane A₂ has not as yet been shown to be concerned in bronchial hyperresponsiveness, although thromboxane A₂ is released from lung parenchyma at the time of anaphylaxis.¹³

Walters *et al*⁵ reported that bronchial hyperresponsiveness to histamine in patients with asthma was suppressed by the cyclo-oxygenase inhibitor indomethacin. In dogs exposed to ozone the increase in bronchial responsiveness can be suppressed by indomethacin.⁶ These findings show a close connection between bronchial hyperresponsiveness and

cyclo-oxygenase products, and would be consistent with the participation of thromboxane A₂, a potent bronchoconstrictor, in bronchial hyperresponsiveness. This study set out to determine whether thromboxane A₂ plays a part in bronchial hyperresponsiveness to acetylcholine in patients with asthma by using OKY-046,¹ a selective thromboxane synthetase inhibitor. The concentration of OKY-046 that gave a 50% inhibition (IC₅₀) of thromboxane synthetase in rabbit platelets was 1.1×10^{-8} mol/l; the values for cyclooxygenase and prostaglandin I₂ synthetase were more than 1×10^{-4} mol/l² and no inhibitory effect was seen at 1×10^{-3} mol/l on 5-lipoxygenase in cytosol of RBL-1 (personal communication). When given orally to healthy adults¹⁴ 25 mg OKY-046 suppressed the ability of blood platelets to synthesise thromboxane A₂, while with higher doses (200–400 mg) this suppression lasted for more than 12 hours and after the drug had disappeared from the blood. In the present study OKY-046 was given at a dose of 800 mg/day for three days plus 600 mg on the test day. This dose did not affect baseline pulmonary function, but bronchial hyperresponsiveness to acetylcholine was reduced. Suppression of thromboxane A₂ production is associated with increased production of prostaglandin I₂ (PGI₂).^{2,15} PGI₂ has a potent blood vessel dilating effect, but no consistent bronchodilating effect in either normal subjects or asthmatic patients.¹⁶ It is likely therefore that the inhibitory effect of OKY-046 on bronchial hyperresponsiveness is due to a reduction in thromboxane A₂ concentrations rather than increased PGI₂ concentrations. In the present study OKY-046 given by inhalation caused no change in bronchial hyperresponsiveness. This may be a problem of bioavailability, possibly because the dose was too small or because it was inhaled only once, 10 minutes before the acetylcholine inhalation.

When Walters *et al.*¹⁷ gave prostaglandin E₂ (PGE₂) to normal subjects by inhalation bronchodilation occurred with a reduction in bronchial responsiveness. When bronchodilation ceased bronchial responsiveness was raised. Inhalation of prostaglandin F_{2α} (PGF_{2α}) suppresses bronchial hyperresponsiveness in addition to causing bronchoconstriction.¹⁸ These findings with PGE₂ and PGF_{2α} show some dissociation between their effect on bronchial calibre and their effect on bronchial responsiveness.

The 5-lipoxygenase pathway is the other major route of arachidonic acid metabolism. The metabolic products include SRS-A, which mainly consists of leukotriene C₄, D₄, and E₄.^{7,10} all potent bronchoconstrictors. Leukotriene D₄ increased bronchial responsiveness to histamine in guinea pigs,¹⁹ sug-

gesting that leukotrienes might promote bronchial responsiveness at the time of allergic and inflammatory reactions. In the present study AA-861, a 5-lipoxygenase inhibitor, caused no change in bronchial responsiveness in these asthmatic patients. AA-861 has a dose dependent inhibitory effect on 5-lipoxygenase in guinea pig leucocytes (IC₅₀ of 3×10^{-6} mol/l) but little effect on cyclo-oxygenase of bovine seminal vesicle gland (IC₅₀ of 1×10^{-3} mol/l or higher¹⁰). AA-861 also shows a dose dependent inhibitory effect on the release of LTC₄ from human peripheral blood leucocytes induced by Ca⁺⁺-ionophore A23187 (IC₅₀ 1×10^{-6} mol/l: unpublished data). Possibly when asthma symptoms are stable the spontaneous release of leukotrienes is not large enough to affect bronchial responsiveness, and this may explain why in the present study the 5-lipoxygenase inhibitor had no effect on bronchial responsiveness.

We wish to thank Kissei Pharmaceutical Company Ltd, Matsumoto; Ono Pharmaceutical Company Ltd, Osaka; and Takeda Chemical Industries Company Ltd, Osaka, Japan, for kindly supplying OKY-046 and AA-861, and also to express thanks to the participating technical staff, Mrs Fusako Yamada and Mrs Setsuko Matsugashita, for performing the measurements of bronchial hyperresponsiveness.

References

- 1 Svensson J, Strandberg K, Tuvemo T, Hamberg M. Thromboxane A₂: effects on airway and vascular smooth muscle. *Prostaglandins* 1977;14:425–36.
- 2 Naito J, Komatsu H, Ujije A, Hamano S, Kubota T, Tsuboshima M. Effects of thromboxane synthetase inhibitors on aggregation of rabbit platelets. *Eur J Pharmacol* 1983;91:41–8.
- 3 Fujimura M, Koshino K, Nishioka S, Matsuda T. Involvement of thromboxane A₂ in SRS-A-mediated bronchoconstriction induced by aerosol antigen in the guinea pig. *Kokyu* 1984;3:1066–71.
- 4 Nishioka S, Kanamori K, Okafuji K, *et al.* Effects of OKY-046 on bronchoconstriction induced by inhalation of acetylcholine, histamine, prostaglandin F_{2α} and leukotriene C₄ in guinea pigs. *Jap J Allergol* 1985;34:706–6.
- 5 Walters EH. Prostaglandins and the control of airways responses to histamine in normal and asthmatic subjects. *Thorax* 1983;38:188–94.
- 6 O'Byrne PM, Walters EH, Aizawa H, *et al.* Indomethacin inhibits the airway hyperresponsiveness but not the neutrophil influx induced by ozone in dogs. *Am Rev Respir Dis* 1984;130:220–24.
- 7 Murphy RC, Hammarström S, Samuelsson B. A slow reacting substance from murine mastocytoma cells. *Proc Natl Acad Sci* 1979;76:4275–9.

- 8 Samuelsson B, Hammarström S. Nomenclature for leukotrienes. *Prostaglandins* 1980;**19**:645–8.
- 9 Kellaway CH, Trethewie ER. The liberation of a slow-reacting smooth muscle-stimulating substance in anaphylaxis. *Quant J Exp Physiol* 1940;**30**:121–45.
- 10 Brocklehurst WE. The release of histamine and formation of a slow reacting substance (SRS-A) during anaphylactic shock. *J Physiol* 1960;**151**:416–53.
- 11 Yoshimoto T, Yokoyama C, Ochi K, *et al.* 2,3,5-Trimethyl-6-(12-hydroxy-5,10-dodecadinyl)-1,4-benzoquinone (AA-861), a selective inhibitor of the 5-lipoxygenase reaction and the biosynthesis of slow reacting substance of anaphylaxis. *Biochim Biophys Acta* 1982;**713**:470–3.
- 12 Kitamura S, Ishihara Y, Takaku F. Effect of thromboxane synthetase inhibitors (OKY-046, OKY-1580) on the action of bronchoactive agents in guinea pig tracheal strips and on arachidonate metabolism in guinea pig lung lobes. *Prostaglandins, Leukotrienes and Medicine* 1984;**14**:341–50.
- 13 Shulman ES, Newball HH, Demers LM, Fitzpatrick FA, Adkinson NF. Anaphylactic release of thromboxane A₂, prostaglandin D₂ and prostacycline from human lung parenchyma. *Am Rev Respir Dis* 1981;**124**:402–6.
- 14 Ito T, Ogawa K, Sakai K. Effects of a selective inhibitor of thromboxane synthetase in humans. *Advances in Prostaglandin, Thromboxane and Leukotriene Research* 1983;**11**:245–50.
- 15 Uyama O, Nagatsuka K, Nakabayashi S, *et al.* The effect of a thromboxane synthetase inhibitor, OKY-046, on urinary excretion of immunoreactive thromboxane B₂ and 6-keto-prostaglandin F_{1α} in patients with ischemic cerebrovascular disease. *Stroke* 1985;**16**:241–4.
- 16 Hardy C, Robinson C, Lewis RA, Tattersfield AE, Holgate ST. Airway and cardiovascular responses to inhaled prostacyclin in normal and asthmatic subjects. *Am Rev Respir Dis* 1985;**131**:18–21.
- 17 Walters EH, Bevan C, Parrish RW, Davies BH, Smith AP. Time-dependent effect of prostaglandin E₂ inhalation on airway responses to bronchoconstrictor agents in normal subjects. *Thorax* 1982;**37**:438–42.
- 18 Fish JE, Jameson LS, Albright A, Norman PS. Modulation of the bronchomotor effects of chemical mediators by prostaglandin F_{2α} in asthmatic subjects. *Am Rev Respir Dis* 1984;**130**:571–4.
- 19 Stewart AG, Thompson DC, Fennessy MR. Leukotriene D₄ potentiates histamine-induced bronchoconstriction in guinea-pigs. *Agents Actions* 1984;**15**:146–52.