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

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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 (PC_{20} FEV_1) 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 FEV_1 and forced vital capacity (FVC) were not altered by oral OKY-046, oral AA-861, or inhaled OKY-046. The geometric mean value of PC_{20} FEV_1 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 A_2 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 A_2, 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 A_2 synthetase inhibitor.[2] In guinea pigs it has been shown to suppress bronchoconstriction induced by allergen[3] or by leukotrienes, prostaglandin $F_{2\alpha}$, histamine, and acetylcholine.[4] Indomethacin, a cyclo-oxygenase inhibitor, has been shown to reduce bronchial responsiveness to histamine in patients with asthma[5] and the increase in bronchial responsiveness seen in dogs exposed to ozone.[6] These findings implicate cyclo-oxygenase products such as thromboxane A_2 in bronchial hyperresponsiveness.

The lipoxygenase pathway is the other major route of arachidonic acid metabolism, producing the potent bronchoconstrictors leukotriene C_4, D_4, and E_4,[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-dodecadiynyl)-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 A_2 and 5-lipoxygenase products in the bronchial hyperresponsiveness of patients with asthma.

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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 (FEV_1) after inhalation of 300 μg salbutamol sulphate. They all had intrinsic asthma with no familial

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 (Autospiror 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 (PC_{20} 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 PC_{20} FEV_1 values are expressed as

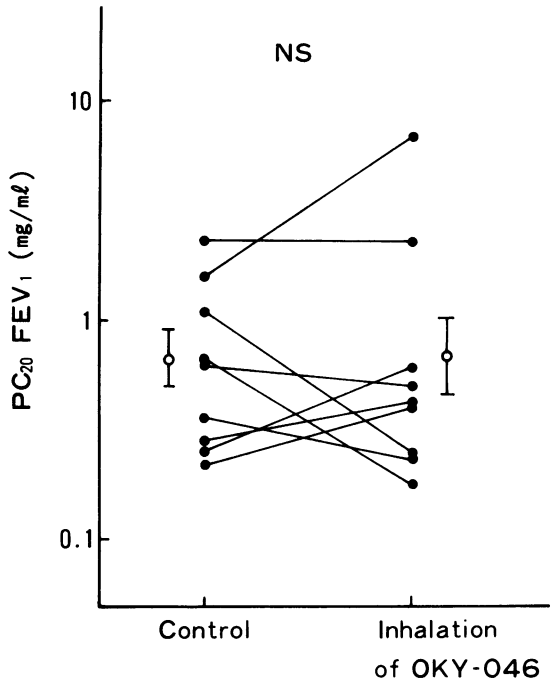

Fig 1 *Effect of inhaled OKY-046 on acetylcholine PC_{20} FEV_1 (the provocative concentration of acetylcholine reducing FEV_1 by 20%) in nine asthmatic subjects.*

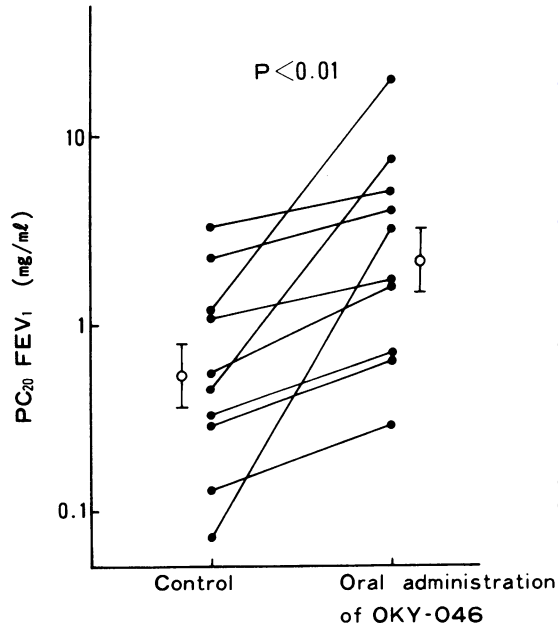

Fig 2 *Effects of oral administration of OKY-046 on acetylcholine PC_{20} FEV_1 (see fig 1 legend) in 10 asthmatic subjects.*

Details of the patients and their baseline forced vital capacity (FVC) and FEV_1 (means with standard errors in parentheses)

	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	

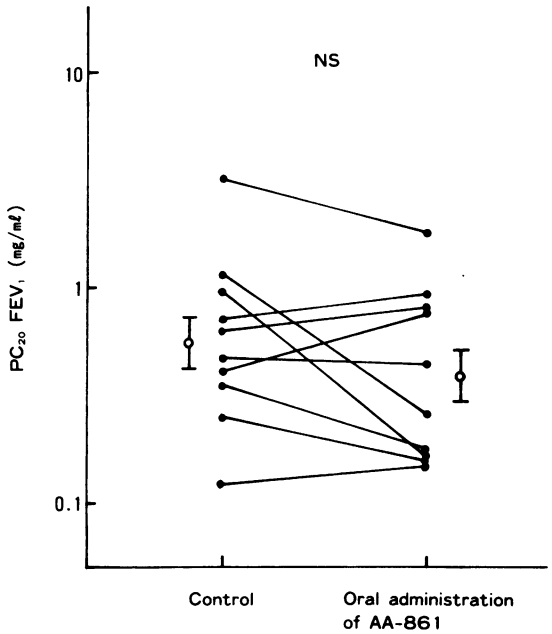

Fig 3 *Effects of oral administration of AA-861 on acetylcholine PC_{20} FEV_1 (see fig 1 legend) in 10 asthmatic subjects.*

geometric means with the geometric standard error of the mean (GSEM) expressed as a factor. Values for baseline FVC and FEV_1 are reported as arithmetic means and standard errors of the mean (SEM).

Geometric mean PC_{20} FEV_1 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 FEV_1 are shown before and after the administration of OKY-046 and AA-861 in the table. There was no significant difference in the FEV_1 or FVC baseline values before and after each drug.

PC_{20} FEV_1 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.

PC_{20} FEV_1 values before and after oral dosing with OKY-046 are shown in figure 2. There was a significant increase in PC_{20} FEV_1 ($p < 0·01$) after oral OKY-046 from 0·55 (GSEM 1·48) mg/ml to 2·24 (GSEM 1·51) mg/ml.

PC_{20} FEV_1 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 $F_{2\alpha}$) in guinea pig tracheal strips.[1,2] The thromboxane synthetase inhibitor OKY-046 has been shown to suppress airway anaphylaxis induced by antigen inhalation[3] and non-specific bronchoconstriction caused by various bronchoconstrictive agents.[4] These findings suggest that thromboxane A_2 plays a part in the development of bronchial hyperresponsiveness in guinea pigs. In man, however, thromboxane A_2 has not as yet been shown to be concerned in bronchial hyperresponsiveness, although thromboxane A_2 is released from lung parenchyma at the time of anaphylaxis.[1,3]

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 A_2, a potent bronchoconstrictor, in bronchial hyperresponsiveness. This study set out to determine whether thromboxane A_2 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 (IC_{50}) of thromboxane synthetase in rabbit platelets was $1{\cdot}1 \times 10^{-8}$ mol/l; the values for cyclooxygenase and prostaglandin I_2 synthetase were more than 1×10^{-4} mol/l[2] 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 adults[14] 25 mg OKY-046 suppressed the ability of blood platelets to synthesise thromboxane A_2, 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 A_2 production is associated with increased production of prostaglandin I_2 (PGI_2).[2 15] PGI_2 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 A_2 concentrations rather than increased PGI_2 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 E_2 (PGE_2) to normal subjects by inhalation bronchodilation occurred with a reduction in bronchial responsiveness. When bronchodilation ceased bronchial responsiveness was raised. Inhalation of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) suppresses bronchial hyperresponsiveness in addition to causing bronchoconstriction.[18] These findings with PGE_2 and $PGF_{2\alpha}$ 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 C_4, D_4, and E_4,[7 10] all potent bronchoconstrictors. Leukotriene D_4 increased bronchial responsiveness to histamine in guinea pigs,[19] suggesting 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 (IC_{50} of 3×10^{-6} mol/l) but little effect on cyclo-oxygenase of bovine seminal vesicle gland (IC_{50} of 1×10^{-3} mol/l or higher[10]). AA-861 also shows a dose dependent inhibitory effect on the release of LTC_4 from human peripheral blood leucocytes induced by Ca^{++}—ionophore A23187 (IC_{50} 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.

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