

Effect of a thromboxane A₂ receptor antagonist (AA-2414) on bronchial hyperresponsiveness to methacholine in subjects with asthma

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Bronchial hyperresponsiveness (BHR) to various stimuli is one of the major clinical features of bronchial asthma. In this study, the effect of a thromboxane A₂ (TXA₂) receptor antagonist, AA-2414, on BHR to methacholine was evaluated in 15 patients with asthma. The methacholine inhalation test was performed before and after oral administration of AA-2414 for 4 days (20 or 40 mg/day). The provocative concentration of methacholine producing a 20% fall in FEV₁ (PC₂₀) was measured as an index of BHR. There was a significant increase in PC₂₀ ($p < 0.01$) from 0.43 (geometric SEM, 1.42) mg/ml to 0.93 (geometric SEM, 1.43) mg/ml after 40 mg/day of AA-2414, whereas baseline values of FVC and FEV₁ were not changed by the treatment. Twenty milligrams per day of AA-2414 did not alter the PC₂₀ value nor the parameter of baseline pulmonary functions. These findings might support our hypothesis that the subthreshold concentration of TXA₂ in the bronchial tissues, which has no effect on bronchomotor tone per se, may be involved in BHR in asthma. Further studies with more potent and specific TXA₂ receptor antagonists are needed to confirm the conclusion. (J ALLERGY CLIN IMMUNOL 1991;87:23-7.)

One of the major clinical features of bronchial asthma is the increased bronchial responsiveness to various specific and nonspecific stimuli.¹ TXA₂, a metabolite of arachidonic acid, is a potent bronchoconstrictor.² We previously demonstrated that oral or aerosol administration of a thromboxane synthetase inhibitor, OKY-046 ((E)-3-[4-(1-imidazolymethyl) phenyl]-2-propenoic acid hydrochloride monohydrate),³ reduced BHR to acetylcholine but did not alter the baseline pulmonary functions in subjects with asthma^{4, 5} and that the attenuating effect of OKY-046 on the BHR could be demonstrated only in patients with asthma but not in normal, bron-

Abbreviations used

TXA ₂ :	Thromboxane A ₂
BHR:	Bronchial hyperresponsiveness
PGD ₂ , PGI ₂ , PGF ₂ :	Prostaglandin D ₂ , I ₂ , and F _{2α}
LTC ₄ , LTD ₄ :	leukotriene C ₄ and D ₄
PC ₂₀ :	Provocative concentration of methacholine producing a 20% fall in FEV ₁
GSEM:	Geometric SEM

chitic, or bronchiectatic subjects.⁶ Thus, our working hypothesis is that subthreshold concentration of TXA₂ in bronchial tissues, which has no effect on bronchomotor tone per se, may be involved in the development of BHR in asthma. However, it has been reported that OKY-046 has a potentiating effect on the production of prostacyclin (PGI₂), a potent bronchodilator, as well as an inhibitory effect of thromboxane synthesis.³ Therefore, an involvement of increased PGI₂ could not be excluded in the reduction of the BHR caused by the OKY-046 treatment. This study was designed to clarify the involvement of TXA₂ in BHR. For that purpose we examined the effect of a

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TABLE I. Clinical characteristics of subjects

No.	Dose of AA-2414 (mg/day)	Age (yr)	Sex	Height (cm)	Type	Severity	IgE		Skin test	Peripheral eosinophil (%)	Complication	Family history of allergic diseases	Treatment
							RIST (IU/ml)	RAST					
1	20	54	F	152	Ext	Mild	237	JC	NT	3	AR	None	Pro
2	20	68	M	162	Int	Mild	54	—	—	2	HT	None	Pro, theo
3	20	66	F	152	Int	Mild	23	—	—	67	None	None	Pro, theo
4	20	30	F	154	Ext	Mild	256	HD, mite	HD	8	AR	Asthma	Pro, theo
5	20	28	M	176	Int	Mild	57	—	—	14	None	None	Pro, theo
6	40	63	M	160	Int	Mild	116	—	—	7	None	None	Pro
7	40	40	F	155	Ext	Mild	48	JC	HD, JC	1	None	None	Pro, theo
8	40	72	M	150	Ext	Mild	960	HD	HD	2	None	None	Pro, theo
9	40	38	M	167	Int	Mild	180	—	—	7	UR	None	Pro, theo
10	40	64	F	152	Int	Mild	149	—	—	8	None	None	—
11	40	32	M	163	Ext	Mild	728	HD, mite	HD	6	AR	None	Pro, theo
12	40	59	M	157	Int	Mild	87	—	NT	8	None	None	Pro, theo
13	40	28	M	176	Int	Mild	57	—	—	14	None	None	Pro, theo
14	40	61	M	162	Int	Mild	377	—	—	11	None	None	Pro, theo
15	40	67	F	150	Int	Mild	63	—	—	9	None	None	Pro

Ext, Extrinsic; Int, intrinsic; JC, Japanese cedar; HD, house dust; NT, not tested; AR, allergic rhinitis; HT, hypertension; UR, urticaria; Pro, inhaled procaterol; theo, theophylline.

TXA₂ receptor antagonist, AA-2414 ((±)-7-(3,5,6-Trimethyl-1,4-benzoquinon-2-yl)-7-phenylheptanoic acid),⁷ on BHR to methacholine in patients with asthma. AA-2414 is a novel, long-active TXA₂ receptor antagonist that competitively inhibited the contractile response to U-46619, a TXA₂ mimetic, in guinea pig trachea and parenchymal strips and dog saphenous vein strips with pA₂ values of 7.69, 8.29, and 6.79, respectively.⁷

SUBJECTS AND METHODS

Fifteen patients with asthma and with a mean age of 51.4 (range, 28 to 72 years) years, participated in the study (Table I). All the patients demonstrated an improvement of 15% or more in the FEV₁ after inhalation of 300 µg of salbutamol sulfate. Two men and three women who demonstrated positive in allergen skin tests and/or increased specific IgE antibodies in the serum had extrinsic asthma. Seven men and three women with no familial history of allergic diseases and no increased level of specific IgE antibodies had intrinsic asthma. The study was performed when their symptoms were mild and stable and while they were taking slow-released theophylline, β₂-adrenergic stimulants, and/or mucolytic agents. None of these patients had experienced corticosteroid therapy or viral infection for at least 4 weeks before the study. All these patients were nonsmokers. Concomitant medication, except for steroid treatment, was maintained without any modification during the study period, although all medications were stopped during the washout period of 13 hours from 9 PM of the previous day to 10 AM on the day of methacholine challenging and until the end of the methacholine challenge test.

Bronchial responsiveness was evaluated by methacholine challenge.⁸ Methacholine chloride (Sigma Chemical Co., St. Louis, Mo.) was dissolved in physiologic saline to make solutions of 0.04, 0.08, 0.16, 0.31, 0.63, 1.25, 2.5, 5, 10, 20, 40, 80, and 160 mg/ml. Saline and methacholine was inhaled from a DeVilbiss 646 nebulizer (DeVilbiss Co., Somerset, Pa.) operated by compressed air at 5 L/min. The nebulizer output was 0.28 ml/2 min. Saline was inhaled first for 2 minutes, and FEV₁ was measured on a dry-wedge spirometer (transfer test, P. K. Morgan, Ltd., Chatham, England). After the change in FEV₁ from the baseline after inhalation of saline to be 10% or less in all subjects was confirmed, inhalation of methacholine was started. Methacholine was inhaled for 2 minutes by tidal breathing with a noseclip and was immediately followed by spirometry. Methacholine of increasing concentrations was successively inhaled, with an interval of 10 minutes, until a fall of 20% or more in the FEV₁ occurred. Measured values were plotted on semilogarithmic graph paper, and the PC₂₀ was calculated.

AA-2414 (Takeda Chemical Industries, Ltd., Osaka, Japan) was administered orally in a dose of 20 or 40 mg/day, every evening for 3 days, and 20 or 40 mg at 8 AM on the fourth day (test day). Bronchial responsiveness to methacholine was then measured at 10 AM.

Informed consent was obtained from all subjects after the purpose of the study had been explained. No information on the test drug and the pharmacologic actions was provided to subjects or technical staffs who performed the methacholine inhalation test and calculated the PC₂₀. This study was approved by the ethics committee of our university hospital.

PC₂₀ values were presented as geometric means with the

TABLE II. Baseline FVC and FEV₁ before and after oral administration of AA-2414 (mean $\pm$ SEM)

	AA-2414 (20 mg/day)		AA-2414 (40 mg/day)	
	Before	After	Before	After
FVC (L)	3.54 $\pm$ 0.37	3.58 $\pm$ 0.41	3.44 $\pm$ 0.24	3.50 $\pm$ 0.25
% FVC (%)	122 $\pm$ 12.1	123 $\pm$ 12.1	113 $\pm$ 5.0	116 $\pm$ 6.0
FEV ₁ (L)	2.47 $\pm$ 0.31	2.34 $\pm$ 0.19	2.33 $\pm$ 0.22	2.40 $\pm$ 0.20
% FEV ₁ (%)	98 $\pm$ 9.9	97 $\pm$ 9.7	90 $\pm$ 4.8	94 $\pm$ 4.5
FEV _{1%} (%)	70 $\pm$ 3.4	68 $\pm$ 4.1	67 $\pm$ 2.7	69 $\pm$ 2.4

GSEM expressed as factor, and values for baseline FVC and FEV₁ as arithmetic means and SEM.

Geometric mean PC₂₀ values before and after AA-2414 were compared by the Student's paired *t* test. The difference with a *p* value of 0.05 or less was considered statistically significant.

RESULTS

Mean baseline values of FVC and FEV₁ before and after the administration of 20 mg/day and 40 mg/day of AA-2414 are presented in Table II. There was no significant difference in the FVC or FEV₁ baseline values before and after each dose of the drug.

PC₂₀ values before and after the oral dosing with AA-2414 are plotted in Fig. 1. There was a significant increase in PC₂₀ (*p* < 0.01) from 0.43 (GSEM, 1.42) mg/ml to 0.93 (GSEM, 1.43) mg/ml after the administration of 40 mg/day of AA-2414, whereas the PC₂₀ values before and after 20 mg/day of AA-2414, 0.58 (GSEM, 1.84) mg/ml, and 0.47 (GSEM, 2.01) mg/ml did not differ significantly.

There were no adverse effects, including bleeding tendency.

DISCUSSION

A selective inhibitor of thromboxane synthesis (OKY-046)³ has been demonstrated to suppress the bronchoconstriction induced by antigen,⁹ LTC₄, and LTD₄^{10, 11} in the guinea pig in vivo. Aizawa et al.¹² demonstrated that OKY-046 inhibited the increase of airway responsiveness to acetylcholine induced by ozone and that a TXA₂ mimetic, U-46619, in the subthreshold concentration caused an increase in airway responsiveness to acetylcholine in dogs. We¹³ also demonstrated that the subthreshold dose of inhaled STA₂, a TXA₂ mimetic,¹⁴ potentiated bronchial responsiveness to aerosol histamine in guinea pigs, where the subthreshold dose was determined by a dose-response curve of bronchoconstriction by inhaled STA₂. Repeated inhalation of the dose of STA₂ did not produce any bronchoconstriction. In addition,

TXA₂ has been demonstrated to be released from the human lung parenchyma at the time of anaphylaxis.¹⁵ Wenzel et al.¹⁶ demonstrated that TXB₂, a stable metabolite of TXA₂ recovered by bronchoalveolar lavage, increased by antigen provocation in subjects with asthma. These findings suggest that TXA₂ plays an important role in bronchial asthma, especially in the development of BHR.

Our previous studies^{4,6} demonstrated that the oral and aerosol administration of OKY-046 significantly reduced BHR to acetylcholine and methacholine in patients with intrinsic asthma. Yukawa et al.¹⁷ also reported the inhibitory effect of OKY-046 on BHR to acetylcholine and LTD₄ in subjects with extrinsic asthma. In contrast, it has been reported that OKY-046 has a potentiating effect on prostacyclin (PGI₂) production as well as an inhibitory effect on thromboxane synthesis.³ This study was designed to re-evaluate the involvement of TXA₂ in BHR of asthma, with the exception of the effect of PGI₂ by use of a TXA₂ receptor antagonist, AA-2414.⁷ AA-2414 is a novel TXA₂ receptor antagonist that has been demonstrated to inhibit bronchoconstriction induced by a TXA₂ mimetic, U-46619.⁷ However, it has also been reported that AA-2414 reduces bronchoconstriction produced by PGD₂, platelet activating factor, and LTD₄ in guinea pigs in vivo.⁷ Since in vitro studies have demonstrated that AA-2414 inhibits the contractile response to U-46619, PGD₂, and PGF_{2 α} in guinea pig tracheal strips with pA₂ value of 7.69, 7.20, and 5.71, respectively.⁷ It is believed that AA-2414 is a receptor antagonist of TXA₂ and PGD₂ but not PGF_{2 α} . Beasley et al.¹⁸ reported that, in atopic subjects with asthma, a TXA₂ receptor antagonist, GR32191, in a single oral dose of 80 mg, did not affect the baseline airway caliber or the methacholine-induced bronchoconstriction, but caused significant inhibition of PGD₂-induced bronchoconstriction, displacing the concentration-response curves to the right by more than tenfold. They also reported that pA₂ value for GR32191 against U-46619, PGD₂, and PGF_{2 α} was

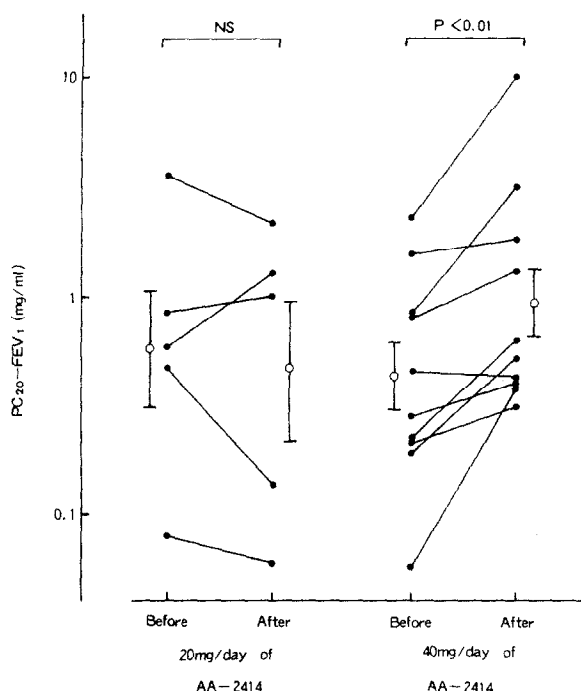


FIG. 1. Effect of oral administration of a TXA₂ receptor antagonist (AA-2414) on BHR to methacholine in subjects with asthma; NS, not significant.

8.26, 8.79, and 7.92, respectively, in isolated guinea pig trachea and that the values did not differ significantly from each other. From these data, AA-2414 is believed to be more specific to TXA₂ and PGD₂ receptors than GR32191. In the study by Beasley et al.,¹⁸ GR32191 was administered in a single dose 1 hour before the methacholine challenge. We previously reported that a single inhalation of OKY-046 failed to reduce BHR⁴ but that a 4-day inhalation of OKY-046 significantly attenuated BHR in subjects with asthma.⁵ This may be responsible for the discrepancy between the results of this study and the study by Beasley et al.¹⁸

It has been established that bronchoconstriction caused by LTC₄, LTD₄, and platelet activating factor depends in part on the release of TXA₂ in the guinea pig,^{10, 11, 19} whereas it has been unknown in humans. It has been controversial whether or not PGD₂ can potentiate bronchial responsiveness in humans. Fuller et al.²⁰ demonstrated that PGD₂ potentiated airway responsiveness to histamine and methacholine in subjects with asthma, but Black et al.²¹ reported that PGD₂ did not potentiate bronchial responsiveness to histamine in normal humans. In contrast, it is unknown whether TXA₂ can potentiate bronchial responsiveness in humans because TXA₂ is chemically unstable, with a half-life of 30 seconds.²² Accordingly, we can-

not determine which receptor blockade of TXA₂, PGD₂, or both contributed to the attenuating effect of AA-2414 on BHR of asthma presented in this study. However, these results may partly support our working hypothesis that a subthreshold concentration of TXA₂ in the bronchial tissues, which has no effect on bronchomotor tone per se, is involved in the development of BHR in asthma. However, further studies with more potent and specific antagonists of TXA₂ are needed to confirm the hypothesis.

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Secretion of granule proteins from eosinophils and neutrophils is increased in asthma

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The activity of eosinophil and neutrophil granulocytes with respect to secretion of granule proteins was studied in 30 patients with asthma and with varying severity of their disease. Granulocytes were stimulated with serum-opsonized Sephadex particles, and the released amount of eosinophil cationic protein (ECP), eosinophil protein X (EPX), and myeloperoxidase was measured by means of specific radioimmunoassays. Eosinophils from patients with asthma released significantly more ($p < 0.001$) ECP and EPX after 20 minutes of incubation than cells from control subjects without asthma. The release of myeloperoxidase from neutrophils was also somewhat higher ($p < 0.03$). The serum concentrations of ECP and EPX were also significantly increased ($p < 0.001$) in the group with asthma. No significant relationships were found between clinical variables and the secretory activity of either eosinophils or neutrophils. We conclude that eosinophils and, to some extent, neutrophils from subjects with asthma have an increased propensity to release their granule proteins, which we suggest is a consequence of priming of these cells. (J ALLERGY CLIN IMMUNOL 1991;87:27-33.)

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During recent years, a role for the eosinophil granulocyte in asthma has been suggested by a number of studies. Thus, the eosinophil contains several highly cytotoxic proteins, such as ECP, EPX (eosinophil-derived neurotoxin), eosinophil peroxidase (EPO), and major basic protein, which may cause damage to airway epithelium resembling the histopathologic findings in asthma.^{1,2} The eosinophils also produce the potent bronchoconstrictory agents, leukotriene C₄ and platelet-activating factor.^{3,4} Moreover, eosinophil accumulation is a common finding in the lung of patients