Eur Respir J, 1996, 9, 2468–2473
DOI: 10.1183/09031936.96.09122468
Printed in UK - all rights reserved

Copyright ©ERS Journals Ltd 1996
European Respiratory Journal
ISSN 0903 - 1936

Role of thromboxane-A_2 and cholinergic mechanisms in bronchoconstriction induced by cigarette smoke in guinea-pigs

K. Matsumoto, H. Aizawa, H. Inoue, S. Takata, M. Shigyo, N. Hara

Role of thromboxane-A_2 and cholinergic mechanisms in bronchoconstriction induced by cigarette smoke in guinea-pigs. K. Matsumoto, H. Aizawa, H. Inoue, S. Takata, M. Shigyo, N. Hara. ©ERS Journals Ltd 1996.

ABSTRACT: Acute exposure to cigarette smoke provokes bronchoconstriction and increases the concentration of thromboxane (Tx) A_2 in bronchoalveolar lavage (BAL) fluid. The purpose of this study was to investigate the role of TxA_2 and cholinergic mechanisms in the airway response induced by exposure to cigarette smoke in guinea-pigs.

Anaesthetized animals were exposed to 200 puffs of smoke for 10 min. The amount of Evans blue dye extravasated into the bronchial tissue was then measured. BAL was performed to determine cell counts and the concentration of TxB_2, a stable metabolite of TxA_2. The effects of pretreatment with a Tx synthase inhibitor, OKY-046 (10 mg·kg^{-1}), and/or atropine (1 mg·kg^{-1}) were evaluated.

Exposure to cigarette smoke caused significant bronchoconstriction (284±33% of baseline pulmonary resistance (*R*L)) and plasma extravasation (30.0±3.8 *vs* 16.8±2.6 ng·mg^{-1} of sham control; main bronchi). OKY-046 or atropine significantly inhibited the bronchoconstriction to a similar extent, without affecting the plasma extravasation. Combined use of these compounds had no additive effect. The cigarette smoke caused significant increase in TxB_2 (48±10 *vs* 14±1 pg·mL^{-1} of sham control) in BAL fluid, which was abolished by OKY-046 but not by atropine. The cellularity in BAL fluid was not different among groups.

These results suggest that the bronchoconstriction induced by cigarette smoke is partially mediated by thromboxane A_2, which is dependent on a cholinergic pathway.
Eur Respir J., 1996, 9, 2468–2473.

Research Institute for Diseases of the Chest, Faculty of Medicine, Kyushu University, Higashiku, Fukuoka, Japan.

Correspondence: H. Aizawa
Research Institute for Diseases of the Chest
Faculty of Medicine
Kyushu University
3-1-1 Maidashi
Higashiku
Fukuoka 812
Japan

Keywords: Bronchoalveolar lavage
cholinergic pathway
plasma extravasation

Received: March 1996
Accepted after revision August 16 1996

Acute exposure to cigarette smoke provokes bronchoconstriction and the extravasation of plasma in the airways of rodents [1, 2]. Evidence suggests that the tachykinins are responsible for such acute airway responses [3–6], but the precise mechanisms are uncertain.

We found that acute exposure to cigarette smoke increases the concentration of thromboxane (Tx) B_2, a stable metabolite of TxA_2, in bronchoalveolar lavage fluid (BALF) in guinea-pigs, [7]. TxA_2 is known to contract the airway smooth muscle directly by stimulating the specific thromboxane (TP) prostanoid receptor on the muscle [8–10], or indirectly by potentiating the neuroeffector transmission of cholinergic contraction [11–14]. In addition, TxA_2 can produce plasma extravasation in the airways [15, 16]. It is, thus, reasonable to hypothesize that TxA_2 may be a key mediator that elicits bronchoconstriction and plasma extravasation induced by cigarette smoke.

Our objective was to clarify the role of TxA_2 and cholinergic mechanisms in the acute airway responses induced by cigarette smoke. We evaluated the effects of a Tx synthase inhibitor, OKY-046 [17], and atropine on the bronchoconstriction, plasma extravasation, cellularity in BALF and concentration of TxB_2 in BALF induced by acute exposure to cigarette smoke in the guinea-pig.

Methods

Study protocol

A total of 53 Hartley-strain male guinea pigs, weighing 450–550 g (Kyudo, Kumamoto, Japan), were anaesthetized with 50 mg·kg^{-1} of pentobarbital sodium administered intraperitoneally (*i.p.*). The animals were then intubated through a tracheostomy and mechanically-ventilated with a respirator (model 680; Harvard Apparatus, South Natick, MA, USA), at a tidal volume of 7 mL·kg^{-1} and a rate of 60 breaths·min^{-1}. A catheter was introduced into the jugular vein to administer drugs. To avoid effects on the adrenergic nervous system, propranolol (1 mg·kg^{-1}) was administered intravenously (*i.v.*) at the beginning of artificial ventilation.

Bronchoconstriction and plasma extravasation were studied in 28 of the animals. They were randomly divided into four groups (seven per group): 1) vehicle (0.9% saline, 1 mg·kg^{-1}), the control group; 2) OKY-046 (10 mg·kg^{-1}); 3) atropine (1 mg·kg^{-1}); and 4) OKY-046 (10 mg·kg^{-1}) and atropine (1 mg·kg^{-1}). The study protocol is shown in figure 1a. After determining the baseline value of total pulmonary resistance (*R*L), vehicle, OKY-046, atropine, or a combination of both drugs was

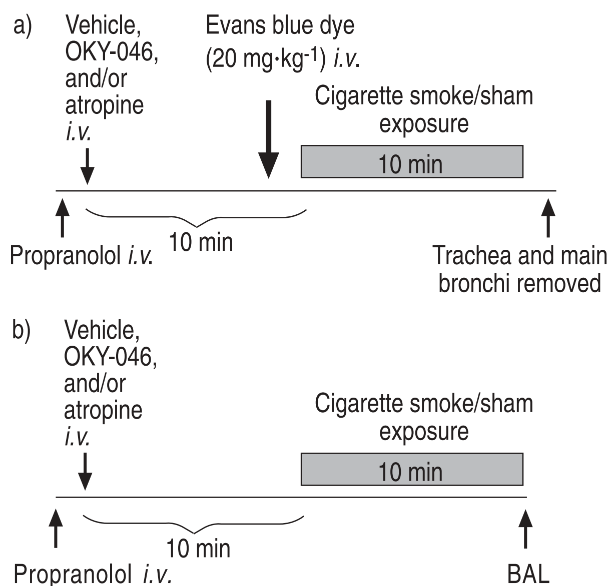

Fig. 1. – Study protocol. a) Measurement of total pulmonary resistance and Evans blue dye extravasation. All studies were performed in the presence of intravenously (*i.v.*) applied propranolol (1 mg·kg^{-1}). After determining the baseline value of total pulmonary resistance (R_L), vehicle (1 mL·kg^{-1}), OKY-046 (10 mg·kg^{-1}), atropine (1 mg·kg^{-1}), or a combination of both drugs was administered intravenously. Eight minutes after drug administration, Evans blue dye (20 mg·kg^{-1}) was administered intravenously. Two minutes later, the animals were exposed to cigarette smoke intermittently for 10 min. Immediately after the exposure, the lower portion of the trachea and the main bronchi were dissected, and the amount of Evans blue dye extravasated into the tissue was measured. b) Bronchoalveolar lavage (BAL). All studies were performed in the presence of intravenously applied propranolol. Vehicle, OKY-046, atropine, or a combination of both drugs was administered intravenously 10 min before the exposure to cigarette smoke or the sham exposure. Immediately after the exposure, animals were sacrified, and BAL was performed. The BAL fluid collected was processed for differential cell counts and the measurement of thromboxane (TxB_2) concentration.

administered intravenously. The doses of OKY-046 and atropine were based on previous studies [7, 17, 18]. Eight minutes after drug administration, Evans blue dye (20 mg·kg^{-1}) was administered intravenously, and 2 min later the animals were exposed to cigarette smoke for 10 min. Five other animals served as the sham-exposed control.

Cell counts and the concentration of TxB_2 in BALF were determined in 20 other animals. They were randomly divided into four groups (five per group). The study protocol is shown in figure 1b. The control group received the vehicle followed by sham exposure. In the other 15 animals, vehicle, OKY-046, or atropine were administered intravenously 10 min before exposure to cigarette smoke. Immediately after the exposure to smoke, animals were killed by an overdose of pentobarbital sodium (100 mg·kg^{-1}) and bronchoalveolar lavage (BAL) was performed as described below.

Measurement of total pulmonary resistance

A fluid-filled catheter was introduced into the oesophagus to estimate pleural pressure. Each animal was placed supine in a body plethysmograph. Airflow (V') was measured with a Fleisch pneumotachograph (TV-132T; Nihon Kohden, Tokyo, Japan) and a differential pressure transducer (TP-602T; Nihon Kohden, Tokyo, Japan). Transpulmonary pressure (P_{tp}) was estimated as the difference between the oesophageal and airway opening pressures, measured by a differential pressure transducer (TP-603T; Nihon Kohden, Tokyo, Japan). The method of AMDUR and MEAD [19] was used to calculate R_L from P_{tp} and V'.

Exposure to cigarette smoke

Exposure to cigarette smoke was achieved as described previously [6]. Briefly, cigarette smoke was supplied by a smoke generator using a volume-controlled respirator (model 681; Harvard Apparatus, South Natick, MA, USA), set at a constant tidal volume of 3.5 mL and a rate of 60 breaths·min^{-1}. Smoke from a cigarette directly connected to the ventilation circuit was delivered to the animal in 10 consecutive breaths, followed by 20 breaths of fresh air. This procedure was repeated for 10 min. Thus, the animals were intermittently exposed to a total of 200 puffs of cigarette smoke over 10 min. In a preliminary study, it was confirmed that the resistance remained unchanged throughout 10 min of sham exposure (100±2% of baseline R_L; n=7), and that this protocol for exposure did not produce serious hypoxaemia or circulatory disturbances. Cigarettes were purchased from Japan Tobacco Co. (Tokyo, Japan). Each cigarette contained 2.7 mg of nicotine and 26 mg of tar, according to the manufacture's specifications.

Measurement of plasma extravasation

Immediately after the exposure to smoke, the thorax was opened and a cannula was inserted into the ascending aorta through the left ventricle. The circulatory system was perfused with 500 mL of 0.9% saline at a pressure of 120 mmHg. The lower portion of the trachea and the main bronchi were dissected and incubated with 1 mL of formamide at 37°C for 18 h to extract Evans blue dye. Extravasation was quantified by measuring the optical density of the formamide extracts at a wavelength of 620 nm using a spectrophotometer (model UV-2200A; Shimadzu Scientific Instruments, Tokyo, Japan). The amount of dye that had extravasated into the tissues was interpolated from a standard curve, and expressed as nanograms per milligram of wet weight of tissue. Use of wet weight as the denominator for studies of extravasation might be inappropriate, as wet weight could increase substantially with capillary leakage. This would decrease the signal but might also decrease the ability to see changes with drug intervention. To avoid this, all the specimens were processed for the measurement as strictly in time as possible.

Bronchoalveolar Lavage

Before the lavage, the lung was hyperinflated (threefolds tidal volume) by manual occlusion of the expiratory outlet of the respirator, to decrease the potential occlusion of the airways and atelectasis of the lungs. The lung was gently lavaged three times *via* the tracheal cannula at a pressure of 2.45 kPa, with 0.9% saline with 5 mM indomethacin added to prevent further production of eicosanoids. The fluid was collected by gentle suctioning by a 20 mL syringe. Total cell counts

were determined under light microscopy using a standard haemocytometer. The lavage fluid was centrifuged at 200×g for 10 min at 4°C. The cell pellet was resuspended in normal saline to obtain a suspension of 10^5 cells·mL^{-1}. Cytospin preparations were made (Cytospin 3; Shandon, Pittsburgh, PA, USA) and the cells were visualized with a modified Wright-Giemsa stain (Diff-Quick; Baxter, McGaw Park, IL, USA). Differential counts per 500 cells were determined under light microscopy using a single-blind method.

Measurement of TxB$_2$

The remaining supernatant from the BALF was stored frozen at -80°C for the measurements of TxB_2 (stable metabolite of TxA_2). A 1 mL sample was extracted twice with a double volume of ethylacetate after acidification with 1 N HCl, and was evaporated to dryness under a stream of nitrogen. The residue was dissolved in benzene/ethylacetate (60:40). The solution was evaporated and processed for assay with radioimmunoassay kits (Daiichi Kagaku, Tokyo, Japan). Briefly, samples were incubated with ^{125}I-labelled TxB_2 and its antiserum for 16 h at 4°C. The antibody-bound fraction was then separated by centrifugation. The radioactivity of the antibody-bound fraction was determined with a gamma scintillation counter.

Materials

The drugs used in the present study were atropine sulphate (Tanabe Pharmaceutical, Osaka, Japan), propranolol hydrochloride (Zeneca Pharmaceutical, Osaka, Japan), indomethacin, formamide (Sigma Chemical, St. Louis, MO, USA), and pentobarbital sodium (Abbott Laboratories, North Chicago, IL, USA). In addition, OKY-046 was provided by Ono Pharmaceutical Co. (Osaka, Japan) and dissolved in 0.9% sterile saline at a concentration of 10 mg·mL^{-1}.

Statistical analysis

Data are expressed as mean±SEM. Baseline *R*L was compared among all groups by one-way analysis of variance (ANOVA). Values for *R*L are expressed as the percentage of the baseline *R*L. Time course curves for *R*L during the exposure to smoke between each drug-treated group and the control group were assessed by two-way ANOVA, followed by Dunnett's tests. The amount of Evans blue dye extravasated into the tissues, cell counts, and the concentration of TxB_2 in BALF were compared among all groups by the Kruskal-Wallis H-test, followed by the Mann-Whitney U-test. A p-value of less than 0.05 was accepted as statistically significant.

Results

Baseline pulmonary resistance

There were no significant differences in baseline *R*L values among the various groups. The values were as follows: 10.75±0.74, 11.72±1.05, 11.18±0.96, 10.03±1.00 (Pa·mL^{-1}·s^{-1}), for vehicle-treated, OKY-046-treated, atropine-treated, OKY-046 and atropine-treated, respectively.

Effects of OKY-046 and atropine on bronchoconstriction induced by cigarette smoke

Effects of the drugs on changes in *R*L in response to cigarette smoke are shown in figure 2a–c. In vehicle-treated animals, exposure to cigarette smoke caused a time-dependent bronchoconstriction. Treatment with OKY-046 significantly inhibited the bronchoconstriction induced by cigarette smoke (fig. 2a) ($p<0.05$). Atropine also significantly inhibited the bronchoconstriction ($p<0.05$) (fig. 2b). However, treatment with OKY-046 combined with atropine did not have any additive effect

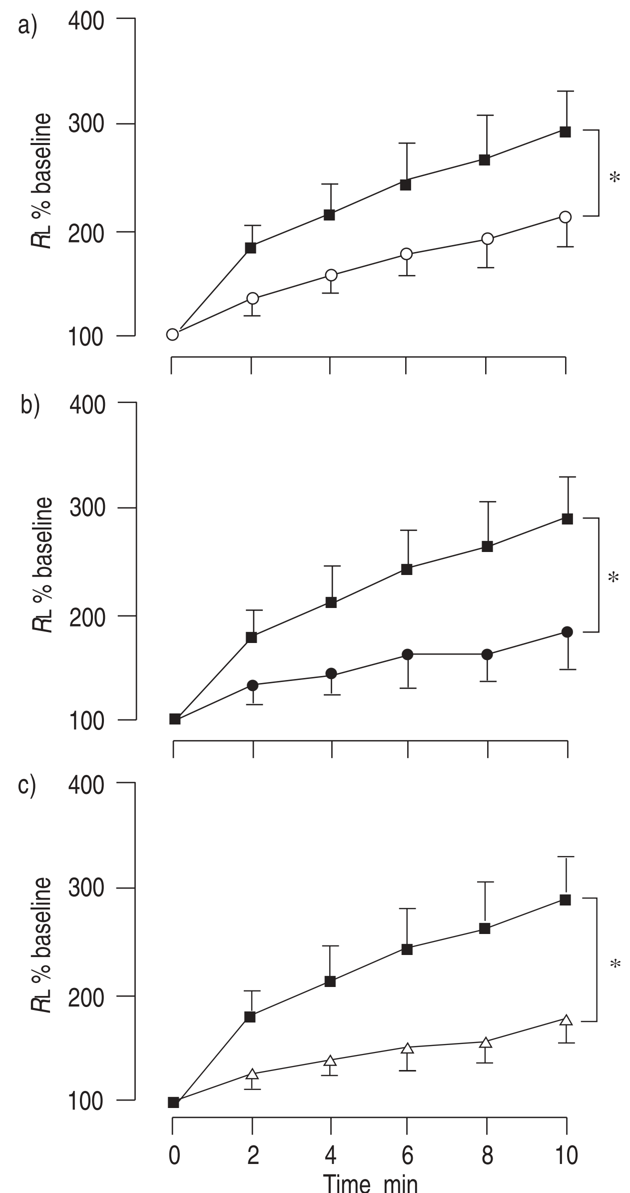

Fig. 2. – Effects of OKY-046 and/or atropine on bronchoconstriction induced by cigarette smoke. Vehicle-treated (—■—), cigarette smoke-exposed animals exhibited a time-dependent bronchoconstriction, as evidenced by increased total pulmonary pressure (*R*L). a) Treatment with OKY-046 (—○—) significantly inhibited the bronchoconstriction induced by cigarette smoke. b) Treatment with atropine (—●—) also significantly inhibited the bronchoconstriction. c) Treatment with OKY-046 combined with atropine (—Δ—), however, produced no additive inhibition on the bronchoconstriction. *: $p<0.05$, significance of comparison between treatments.

on the bronchoconstriction (fig. 2c). In our preliminary study, 1 mg·kg-1 of atropine completely prevented the bronchoconstriction induced by acetylcholine (60 µg·kg-1 *i.v.*), which increased *R*L by 493±56% (n=5), a greater response than that seen in the present study (the maximum *R*L change in the present study being 405%).

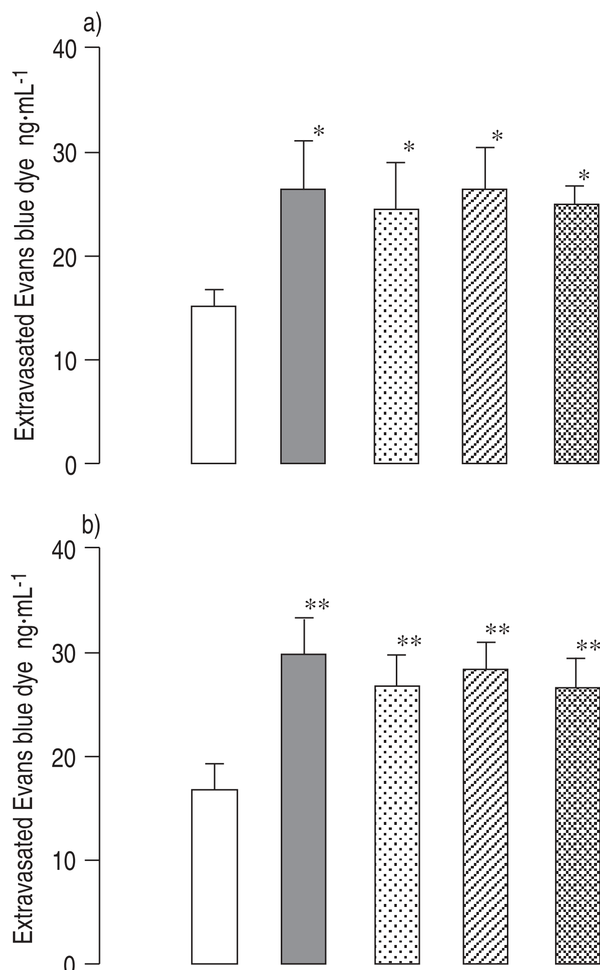

Fig. 3. – Effects of OKY-046 and/or atropine on the extravasation of Evans blue dye induced by cigarette smoke. A significant increase in the amount of extravasated Evans blue dye was noted both in: a) the trachea; and b) main bronchi of the cigarette smoke-exposed, vehicle-treated animals ( ) *versus* the sham-exposed animals ( ). Treatment with OKY-046 ( ), atropine ( ), or combined use of both drugs ( ) prior to cigarette exposure had no effect on the extravasation. *: p<0.05; **: p<0.01, compared with sham-exposed animals.

Table 1. – Bronchoalveolar lavage profile

	Recovery rate	BALF cell counts ×10⁵·mL-1			
	%	AM	Lymph	Neu	Eos
Sham	97±1	3.32±0.43	0.38±0.08	0.12±0.03	0.22±0.10
Vehicle	95±1	2.80±0.37	0.21±0.04	0.38±0.14	0.36±0.16
OKY-046	94±2	2.32±0.24	0.23±0.06	0.27±0.09	0.41±0.15
Atropine	94±1	2.46±0.55	0.23±0.09	0.23±0.12	0.30±0.13

Values are presented as mean±SEM. AM: alveolar macrophages; Lymph: lymphocytes; Neu: neutrophils; Eos: eosinophils; BALF: bronchoalveolar lavage fluid.

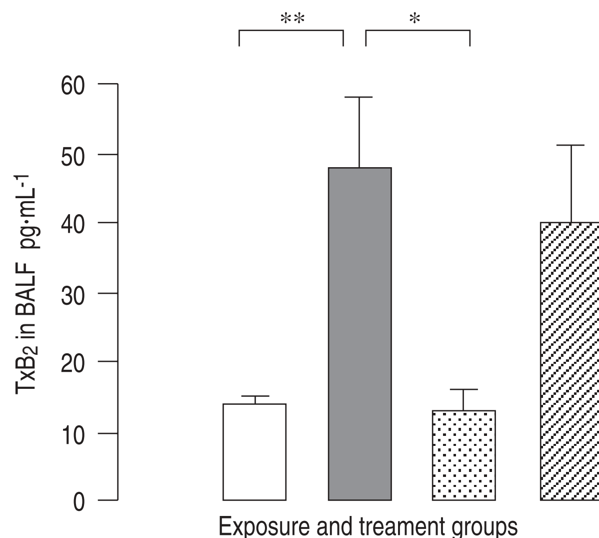

Fig. 4. – Effect of OKY-046 or atropine on concentration of thromboxane B_2 (TxB_2) in bronchoalveolar lavage fluid (BALF). The concentration of TxB_2 was significantly higher in the cigarette smoke-exposed, vehicle-treated group ( ) *versus* the sham-exposed, vehicle-treated group ( ). The concentration of TxB_2 was significantly lower in the cigarette smoke-exposed animals treated with OKY-046 ( ) as compared with the vehicle-treated group. Treatment with atropine ( ) had no significant effect on the concentration of TxB_2. *: p<0.05; **: p<0.01, significance of comparison between treatment groups.

Effects of OKY-046 and atropine on the extravasation of Evans blue dye

As shown in figure 3, a significant increase in the amount of extravasated Evans blue dye was noted both in the trachea and main bronchi of the cigarette-exposed, vehicle-treated animals compared with the sham-exposed animals (p<0.05 in the trachea, p<0.01 in main bronchi). Treatment with OKY-046, atropine, or the combination of drugs had no effect on the extravasation compared with treatment by vehicle alone in smoke-exposed animals.

Effects of OKY-046 and atropine on cell counts in BALF

The recovery rate of BALF did not differ significantly among the study groups, with the range being 94–97%. The cell counts also showed no significant differences among groups (table 1).

Effect of OKY-046 and atropine on concentration of TxB_2 in BALF

The concentration of TxB_2 in BALF was significantly higher in the smoke-exposed, vehicle-treated group compared with the sham-exposed, vehicle-treated group (p<0.01). The concentration of TxB_2 was significantly lower in the animals treated with OKY-046, as compared with the animals treated with vehicle and then exposed to cigarettes (p<0.05). Treatment with atropine had no significant effect on the concentration of TxB_2, compared with treatment with vehicle in smoke-exposed animals (fig. 4).

Discussion

In this study, the bronchoconstriction induced by cigarette smoke was partially inhibited by pretreatment with OKY-046 or with atropine, to a similar extent. This finding clearly indicates that the bronchoconstriction induced by cigarette smoke is partially caused by endogenous TxA_2 and acetylcholine. The combined use of OKY-046 and atropine showed no additive effect, which suggests that the effects of both compounds are mediated by the same pathway.

The novel finding of this study is that the inhibition of Tx generation by OKY-046 also inhibited the bronchoconstriction induced by cigarette smoke, suggesting a TxA_2-mediated component in this type of bronchoconstriction. It is known that TxA_2 can elicit bronchoconstriction by at least two mechanisms: 1) by potentiating the cholinergic neuroeffector transmission [11–14]; and 2) by direct stimulation of the TP prostanoid receptor on the airway smooth muscle, a cholinergic-independent mechanism [8–10]. The lack of additive effect of OKY-046 and atropine strongly suggests the contribution of a cholinergic-dependent mechanism in the TxA_2 action in the present model. Therefore, we consider that TxA_2 may cause bronchoconstriction *via* a cholinergic pathway, presumably by enhancing the release of acetylcholine from the vagus nerve terminals. SAROEA *et al.* [20] reported that the bronchoconstrictor effect of the inhaled Tx mimetic, U46619, is markedly reduced by a cholinergic antagonist in asthmatic airways. Their finding further suggests that the cholinergic pathway is an important component in TxA_2-induced bronchoconstriction in humans.

The contribution of a cholinergic component to the bronchoconstriction induced by cigarette smoke has been confirmed in numerous studies [5, 6, 21, 22]. We recently reported that the bronchoconstriction induced by cigarette smoke was completely abolished by pretreatment with FK224, a tachykinin antagonist at the dual neurokin 1 and neurokinin 2 (NK_1 and NK_2) receptors [6]. Exposure to cigarette smoke increases the concentration of TxB_2, a stable metabolite of TxA_2, in BALF, but pretreatment with FK224 prevents this increase [7]. Thus, it is likely that the TxA_2-mediated bronchoconstriction induced by exposure to cigarette smoke may depend on a tachykinin-mediated cholinergic pathway.

Unlike bronchoconstriction, the extravasation of plasma into the bronchial tissue was not affected by OKY-046 or atropine. The lack of effect of OKY-046 and atropine on the extravasation was also noted in the present study. Intravenous administration of U46619 has been shown to cause marked bronchoconstriction and plasma extravasation in the airways of guinea-pigs [15]. Another study demonstrated that the plasma extravasation induced by intratracheally instilled leukotriene D_4 (LTD_4) is partially mediated by Tx generation in guinea-pigs [16]. Our findings on extravasation are inconsistent with those of the previous investigators. Several studies have reported that thromboxane did not affect vascular permeability in the lung [23, 24]. The effect of thromboxane on vascular permeability is still controversial. The discrepancy may be, in part, explained by a dose-response relationship of thromboxane on vascular permeability. Indeed, LOTVALL *et al.* [15] reported that 2 $nmol \cdot kg^{-1}$ (*i.v.*) of U-46619 caused 3.5 fold increase in R_L without an effect on the extravasation of Evans blue dye, whereas 20 or 200 $nmol \cdot kg^{-1}$ of U-46619 elicited marked increases both in R_L and extravasation [15]. It seems likely that the amount of endogenous TxA_2 may have been insufficient to elicit an extravasation of plasma.

We previously showed that TxB_2 was increased in BALF collected 90–120 min after exposure to 3 min of cigarette smoke, and that the number of neutrophils in BALF was also increased [7]. In the present study, we found that TxB_2 increased immediately after exposure to cigarette smoke, but cell counts in BALF did not change. A similar report demonstrated that TxB_2 is increased in BALF immediately after acute exposure to acrolein, a component of cigarette smoke [25]. Although neutrophils are known to be a potent source of TxA_2 [26], the TxA_2 in the current study may have been generated from the resident cells other than inflammatory cells in the airway. Whilst we could not confirm the source of TxA_2 in the present study, it has been reported that TxA_2 can be generated from platelets, airway epithelium, endothelium, alveolar macrophages and fibroblasts, in response to various stimuli [27–29].

In summary, we conclude that the bronchoconstriction induced by acute exposure to cigarette smoke was partially mediated by endogenous thromboxane A_2, which was dependent on a cholinergic pathway.

Acknowledgements: The authors are grateful to Ono Pharmaceutical Co. (Osaka, Japan) for the donation of OKY-046. They are also grateful to S. Inutsuka for statistical analysis.

References

1. Lundberg JM, Martling CR, Saria A, Folkers K, Rosell S. Cigarette smoke-induced airway oedema due to activation of capsaicin-sensitive vagal afferent and substance P release. *Neuroscience* 1983; 10: 1361–1368.
2. Lee L-Y, Lou Y-P, Hong J-L, Lundberg JM. Cigarette smoke-induced bronchoconstriction and release of tachykinins in guinea-pig lungs. *Respir Physiol* 1995; 99: 173–181.
3. Delay-Goyet P, Franco-Cereceda A, Consalves SF, Cingan CA, Lowe JA, Lundberg JM. CP-96345 antagonism of NK_1 receptors and smoke-induced protein extravasation in relation to its cardiovascular effects. *Eur J Pharmacol* 1992; 222: 213–218.
4. Hirayama Y, Lei Y-H, Barnes PJ, Rogers DF. Effects of two novel tachykinin antagonists, FK224 and FK888, on neurogenic airway plasma exudation, bronchoconstriction and systemic hypotension in guinea-pigs *in vivo*. *Br J Pharmacol* 1993; 108: 844–851.
5. Hong J-L, Roger IW, Lee L-Y. Cigarette smoke-induced bronchoconstriction: cholinergic mechanisms, tachykinins, and cyclo-oxgenase products. *J Appl Physiol* 1995; 78: 2260–2266.
6. Matsumoto K, Aizawa H, Shigyo M, Inoue H, Takata S, Hara N. Role of tachykinins in airway narrowing induced by cigarette smoke in guinea-pigs. *Environ Toxicol Pharmacol* 1996; 1: 227–233.
7. Matsumoto K, Aizawa H, Inoue H, Shigyo M, Takata S, Hara N. Thromboxane causes airway hyperresponsiveness following neurogenic inflammation induced by cigarette smoke. *J Appl Physiol* 1996; (in press).

8. Svensson J, Strandberg K, Tuvemo T, Hamberg M. Thromboxane A_2: effects on airway and vascular smooth muscle. *Prostaglandins* 1977; 14: 425–436.
9. McKenniff MG, Rodger IW, Norman P, Gardiner PJ. Characterization of receptors mediating the contractile effects of prostanoids in guinea-pigs and human airways. *Eur J Pharmacol* 1988; 153: 149–159.
10. Norman P, Cuthbert NJ, McKenniff MG, Gardiner PJ. The thromboxane receptors of rat and guinea-pig lung. *Eur J Pharmacol* 1992; 229: 171–178.
11. Munoz NM, Shioya T, Murphy TM, *et al.* Potentiation of vagal contractile response by thromboxane mimetic U46619. *J Appl Physiol* 1986; 61: 1173–1179.
12. Tamaoki J, Sekizawa K, Osborne ML, Ueki IF, Graf PD, Nadal JA. Platelet aggregation increases cholinergic neurotransmission in canine airway. *J Appl Physiol* 1987; 62: 2246–2251.
13. Serio R, Daniel EE. Thromboxanes and other inflammatory modulators of canine trachealis neuromuscular function. *J Appl Physiol* 1988; 64: 1979–1988.
14. Aizawa H, Takata S, Shigyo M, *et al.* Effect of BAY u3405, a thromboxane A_2 receptor antagonist, on neuroeffector transmission in canine tracheal tissue. *Prostaglandin Leukot Essential Fatty Acids* 1995; 53: 213–217.
15. Lotvall J, Elwood W, Tokuyama K, Sakamoto T, Barnes PJ, Chung KF. A thromboxane mimetic, U-46619, produces plasma exudation in airways of the guinea-pigs. *J Appl Physiol* 1992; 72: 2415–2419.
16. Arakawa H, Lotvall J, Kawikova I, Lofdahl C-G, Skoogh B-E. Leukotriene D_4- and prostaglandin $F_{2\alpha}$-induced airflow obstruction and airway plasma exudation in guinea-pig: Role of thromboxane and its receptor. *Br J Pharmacol* 1993; 110: 127–132.
17. Iizuka K, Akahane K, Momose D, *et al.* Highly selective inhibitors of thromboxane synthetase. 1. Imidazole derivatives. *J Med Chem* 1981; 24: 1139–1148.
18. Inoue H, Aizawa H, Miyazaki N, Ikeda T, Shigematsu N. Possible roles of the peripheral vagal nerve in histamine-induced bronchoconstriction in guinea-pigs. *Eur Respir J* 1991; 4: 860–866.
19. Amdur MO, Mead J. Mechanics of respiration in unanesthetized guinea-pigs. *Am J Physiol* 1958; 192: 364–368.
20. Saroea G, Inman MD, O'Byrne PM. U46619-induced bronchoconstriction in asthmatic subjects is mediated by acetylcholine release. *Am J Respir Crit Care Med* 1995; 151: 321–324.
21. Hartiala J, Mapp C, Mitchell RA, Shields RL, Gold WM. Cigarette smoke-induced bronchoconstriction in dogs: vagal and extravagal mechanisms. *J Appl Physiol: Respirat Environ Exercise Physiol* 1984; 57: 1261–1270.
22. Nakamura M, Haga T, Sasaki H, Takishima T. Acute effects of cigarette smoke inhalation on peripheral airways in dogs. *J Appl Physiol* 1985; 58: 27–33.
23. Winn R, Harlan J, Nadir B, Harker L, Hildebrandt J. Thromboxane A_2 mediates lung vasoconstriction but not permeability after endotoxin. *J Clin Invest* 1983; 72: 911–918.
24. Wurts MM, Stephenson AH, Sprague RS, Lonigro AJ. Enhanced microvascular permeability of PMA-induced acute lung injury is not mediated by cyclo-oxygenase products. *J Appl Physiol* 1992; 73: 2135–2141.
25. Leikauf GD, Leming LM, O'Donnell JR, Doupnik CA. Bronchial responsiveness and inflammation in guinea–pigs exposed to acrolein. *J Appl Physiol* 1989; 66: 171–178.
26. Higgs GA, Moncada S, Salmon JA, Seager K. The source of thromboxane and prostaglandins in experimental inflammation. *Br J Pharmacol* 1983; 79: 863–868.
27. Bunting S, Moncada S, Vane JR. The prostacyclin-thromboxane A_2 balance: pathophysiological and therapeutic implications. *Br Med Bull* 1983; 39: 271–276.
28. Leikauf GD, Ueki IF, Nadel JA, Widdicombe JH. Release of cyclo-oxygenase products from cultured epithelium derived from human and dog trachea (Abstract). *Fed Proc* 1985; 4: 1920.
29. Shirahase H, Kanda M, Kurahashi K, Nakamura S, Usui H, Shimizu Y. Endothelium-dependent contraction in intrapulmonary arteries: mediation by endothelial NK_1 receptors and TxA_2. *Br J Pharmacol* 1995; 115: 1215–1220.