Inhibitory Effects of OKY-046 on Spasmogen-Induced Bronchoconstrictions in Sensitized and Non-Sensitized Guinea Pigs

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Abstract—We examined the effect of thromboxane A₂ (TXA₂) synthetase inhibitor, OKY-046, on bronchoconstriction induced by antigen and various spasmogenic mediators in guinea pigs *in vivo*. Further, inhibitory activities of OKY-046 on contractions of isolated tracheae and lung parenchymal strips induced by various contractile agents were also investigated *in vitro*. OKY-046, but not indomethacin, significantly inhibited antigen-induced bronchoconstriction in a dose-dependent manner. Moreover, OKY-046 attenuated bronchoconstrictions induced by peptide leukotrienes (LTs) and platelet activating factor (PAF), but not those by histamine, prostaglandin D₂ (PGD₂) and STA₂ (a stable TXA₂ mimetic agent). Although contractile responses induced by spasmogens such as peptide LTs, PAF and histamine were not influenced by OKY-046 in isolated tracheae, OKY-046 elicited significant and concentration-dependent inhibitions against contractile responses induced by peptide LTs and PAF in isolated lung parenchymal strips. These results suggest the possible involvement of TXA₂ in the development of anaphylactic bronchoconstriction in sensitized guinea pigs.

The release of eicosanoids from lung tissues in response to immunological stimuli has been described previously (1, 2). Among eicosanoids, thromboxane A₂ (TXA₂), which is produced by platelets, mononuclear leukocytes, lung mast cells and other cells, has been considered as one of the important mediators (3–7) in inflammatory respiratory diseases such as asthma, since it has potent contractile activities on pulmonary airways and blood vessels (4, 8) beside promoting platelet aggregation. However, the activity of TXA₂ on airway function *in vivo* has been difficult to assess due to the short half-life of this mediator in blood (9).

In the present study, we examined the role of TXA₂ in anaphylactic bronchoconstriction by using a potent and selective TXA₂ synthetase inhibitor, (E)-3-[p-(1H-imidazol-1-ylmethyl) phenyl]-2-propenoic acid (OKY-

046) (10), in the guinea pig. Furthermore, inhibitory activities of OKY-046 on bronchoconstriction responses induced by various spasmogenic mediators such as histamine, peptide leukotrienes (LTs) and platelet activating factor (PAF) were also investigated both *in vivo* and *in vitro*.

Materials and Methods

Animals

Male Hartley guinea pigs weighing 250 to 500 g were used throughout the experiments.

Drug

Either the sodium or hydrochloride salt of OKY-046 was used. Leukotriene (LT) C₄, LTD₄, LTE₄, PGD₂, 9,11-epithio-11,12-methano-TXA₂ (STA₂, a stable TXA₂ mimetic agonist) and platelet activating factor (PAF) were synthesized by Ono. LTC₄, LTD₄ and LTE₄ (dissolved in 50% ethanol) and PGD₂

(dissolved in absolute ethanol) were diluted with 1/15 M phosphate-buffered solution (pH 7.4). STA₂ was dissolved in 1/15 M phosphate-buffered solution (pH 7.4), and PAF was dissolved in absolute ethanol and diluted accordingly with physiological saline containing 0.25% bovine serum albumin (BSA, Fraction V, Sigma). Histamine dihydrochloride (Sigma), acetylcholine chloride (Ovisot®, Daiichi Seiyaku), carbachol (Sigma), ovalbumin (OVA, Grade III, Sigma) and BSA were dissolved in physiological saline. Indomethacin and killed *Bordetella pertussis* were purchased from Sigma and Chemo-Sero-Therapeutic Research Institute, respectively. The composition of Krebs-Henseleit solution was as follows: 118 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl₂, 1.2 mM MgSO₄, 1.2 mM KH₂PO₄, 25 mM NaHCO₃ and 5.6 mM glucose.

Drug administration

For in vivo studies, OKY-046 and indomethacin were dissolved in physiological saline and 7% sodium bicarbonate, respectively. Both were administered i.v. 2 min prior to challenge with antigen or various other spasmogens. For in vitro studies, OKY-046 was dissolved in physiological saline and added into the organ bath 20 min before challenge with various contractile agents.

In vivo experiment

Sensitization procedure: The animals were injected intraperitoneally with 1 mg OVA containing 5×10^9 killed *Bordetella pertussis* on day 0. The injection volume was 0.5 ml. Sensitized animals were challenged by i.v.-administration of OVA on days 15 to 17. The antibody produced in this sensitization procedure belonged to the IgG class when assessed by 4 hr- and 8 day-homologous passive cutaneous anaphylactic reactions (11).

Measurement of bronchoconstriction: A small cannula was inserted into the surgically exposed trachea of the pentobarbital-anesthetized (75 mg/kg, i.p.) animal and secured tightly with ligatures. Two other cannulae were inserted into the carotid artery and jugular vein separately for monitoring systemic blood pressure and administration of drugs and antigen, respectively. The tracheal cannula was connected to a constant volume

respirator, and the animal was artificially ventilated at a constant volume of 5 ml at a frequency of 70 strokes/min. Changes in insufflation pressure at a constant airflow were measured by a pressure transducer connected to the side-arm of the tracheal cannula and expressed as a percentage of the maximal increase in insufflation achieved by clamping off the trachea at the end of the experiment.

Effects on antigen- and spasmogen-induced bronchoconstriction: A single bolus i.v.-injection of OVA (0.2 mg/kg) produced approximately 70% of the maximal bronchoconstriction in preliminary experiments. Bronchoconstriction after OVA challenge was monitored for 15 min. LTC₄ (2.0 µg/kg), LTD₄ (2.0 or 0.5 µg/kg), LTE₄ (5.0 µg/kg) PAF (0.3 µg/kg) and PGD₂ (100 µg/kg) were intravenously administered. Histamine (10 µg/kg, i.v.) and STA₂ (3.0 µg/kg, i.v.) were administered several times until constant airway responses were obtained.

In vitro experiment

Preparation of guinea pig tracheal and lung parenchymal strips: The animals were sacrificed by a sharp blow to the head, exanguinated, and the tracheae and lungs were isolated. Tracheae were carefully trimmed of excess fatty and connective tissues and cut into zigzag strips of equal number of segments. The lung parenchymal strips were removed from the outer edge of the right and left lower lobes of the lung. Each preparation was suspended under 0.5 g of passive tension in a 10-ml organ bath containing Krebs-Henseleit solution, which was maintained at 37°C, pH 7.4, and continuously aerated with 95% O₂-5% CO₂. Contractions of these tissues were measured by using an isotonic transducer (Nihon Kohden, TD112S) for the tracheal strip and a force-displacement transducer (Nihon Kohden, TB611T) for the lung parenchymal strip. After each tissue was equilibrated for 0.5 to 1 hr before commencement of the experiment, constant responses to carbachol (10^{-6} M) for tracheal strips and acetylcholine (10^{-5} M) for lung parenchymal strips were obtained at least twice.

Effect of spasmogen-induced contractile responses

After a maximally effective concentration of carbachol (10^{-5} M) for tracheal strips or

acetylcholine (10^{-3} M) for parenchymal strips was obtained, cumulative concentration-response curves for contractile agonists, i.e., histamine, LTC₄, LTD₄, STA₂ and PGD₂, were constructed by successive increases in the bath concentration of these agonists. In the case of PAF, all strips obtained from the same animal were challenged with only one concentration of this agonist because of its tachyphylactic effect (12). To minimize the variation between tissues of animals, contractile responses were expressed as a percentage of the maximal response with carbachol (10^{-5} M) or acetylcholine (10^{-3} M).

Statistical evaluation

Statistical evaluations were made by Student's unpaired or paired *t*-test, with a probability value of $P < 0.05$ regarded as significant.

Results

In vivo

Inhibitory activities on antigen-induced bronchoconstrictions: Administration of antigen in sensitized guinea pigs produced a bronchoconstriction with rapid onset (within 30 sec) and long-lasting action (more than 15 min) characterized by large increases in insufflation pressure ($77.3 \pm 4.8\%$ of maximal increase at 4 min) and a temporal but an abrupt hypertension (34.4 ± 8.4 mmHg at 2 min) followed by slight hypotension (-6.3 ± 2.2 mmHg at 15 min). The peak in bronchoconstriction and hypertension occurred approximately 2 to 5 min and 1 to 3 min after antigen challenge, respectively. OKY-046 significantly inhibited both bronchoconstriction and hypertension induced by antigen in a dose-dependent manner at doses ranging

Fig. 1. Effects of OKY-046 and indomethacin on antigen-induced bronchoconstrictions and blood pressure responses in sensitized guinea pigs. Each point represents the mean of each group. ○—○: Control (N=10); ●—●: OKY-046, 1 mg/kg, i.v. (N=7); ▲—▲: OKY-046, 3 mg/kg, i.v. (N=7); ■—■: OKY-046, 10 mg/kg, i.v. (N=7); △—△: indomethacin, 2 mg/kg, i.v. (N=8). *, ** and *** indicate significant differences compared with the control where $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

from 1 to 10 mg/kg. On the other hand, indomethacin significantly inhibited this airway response only within 1 min after challenge, although it almost prevented the pressor response (Fig. 1).

Inhibitory activities on responses induced by various spasmogens: OKY-046, when given as a single bolus i.v.-injection, had no inhibitory effects on histamine (10 μ g/kg. i.v.)-, PGD₂ (100 μ g/kg. i.v.)- and STA₂ (3 μ g/kg. i.v.)-induced bronchoconstrictions at

the dose of 30 mg/kg (Table 1).

Intravenous administration of LTC₄ (2 μ g/kg) produced a biphasic bronchoconstriction with peaks that appeared at 30–60 sec and 3–4 min, and elicited a transient hypertensive effect followed by hypotension 2 to 4 min after administration (Fig. 2). OKY-046 preferentially inhibited the initial phase at doses ranging from 0.1 to 1 mg/kg, but had no influence on the latter phase of bronchoconstriction. Indomethacin also inhibited the air-

Table 1. Effects of OKY-046 on histamine-, STA₂- and PGD₂-induced bronchoconstrictions in guinea pigs

Spasmogens	Dose (μ g/kg. i.v.)	N	Increase in insufflation pressure (% max.)		P value
			Control	OKY-046	
Histamine	10	5	63.8 $\pm$ 4.3	66.1 $\pm$ 3.9	>0.1
STA ₂	3	5	60.5 $\pm$ 3.1	60.9 $\pm$ 2.3	>0.1
PGD ₂	100	5	45.6 $\pm$ 5.4	55.4 $\pm$ 9.6	>0.1

OKY-046 was intravenously administered 2 min prior to injection of each agonist at the dose of 30 mg/kg. Each value represents the mean $\pm$ S.E.M. Significant difference compared to the control was determined using Student's paired *t*-test for histamine and STA₂ or the unpaired *t*-test for PGD₂.

Fig. 2. Time-related changes in insufflation pressure and blood pressure after injection of LTC₄ (2 μ g/kg. i.v.).

way response within 1 min, but augmented the response at 3 and 4 min after LTC₄ challenge. Pressor responses induced by LTC₄ were abolished and turned into depressor responses by OKY-046 (Fig. 3). OKY-046 elicited similar inhibitory effects on LTD₄ (2 µg/kg, i.v.)- and LTE₄ (5 µg/kg, i.v.)-induced responses that resembled those of LTC₄ (data not shown). Pretreatment of indomethacin abolished LTD₄ (0.5 µg/kg)-induced rapid airway response when compared with the response without indomethacin pretreatment. OKY-046 (10 mg/kg,

i.v.) did not show any inhibitory effects on bronchoconstrictions induced by LTD₄ in the presence of indomethacin (Fig. 4).

Intravenous administration of PAF (0.3 µg/kg) elicited temporal bronchoconstrictions with rapid onset (within 30 sec). An increase in insufflation pressure of $61.9 \pm 7.9\%$ was accompanied by a transient increase followed by a sustained fall (3 to 10 min) in blood pressure. OKY-046 showed a dose-dependent attenuation against bronchoconstriction and hypertension at doses ranging from 1 to 10 mg/kg. Indomethacin also

Fig. 3. Effects of OKY-046 and indomethacin on LTC₄ (2 µg/kg, i.v.)-induced bronchoconstrictions and blood pressure responses in guinea pigs. Each point represents the mean of each group. ●—●: Control (N=10); ○—○: OKY-046, 0.1 mg/kg, i.v. (N=7); △—△: OKY-046, 0.3 mg/kg, i.v. (N=7); □—□: OKY-046, 1 mg/kg, i.v. (N=7); ▲—▲: indomethacin, 2 mg/kg, i.v. (N=3). *, ** and *** indicate significant differences compared with the control where $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

Fig. 4. Time-related changes in insufflation pressure and blood pressure in response to LTD₄ (0.5 µg/kg, i.v.) in the presence (○—○, N=3) or absence (●—●, N=3) of indomethacin (2 mg/kg, i.v.) and the effect of OKY-046 (△—△, 10 mg/kg, i.v., N=3) on LTD₄-induced responses in indomethacin-treated guinea pigs. Each point represents the mean of each group. ** and *** indicate significant differences in comparison with the non-treated control where $P < 0.01$ and $P < 0.001$, respectively.

significantly attenuated the spasmogenic and hypertensive activities of PAF (Fig. 5).

In vitro

In isolated tracheal strips, OKY-046 (10^{-4} M) did not show any inhibitory activities against contractile responses induced by histamine, PGD_2 , STA_2 , LTC_4 and LTD_4 (Table 2).

In isolated lung parenchymal strips, OKY-046 (10^{-4} M) similarly did not show any inhibitory effects on the contractile responses induced by histamine, PGD_2 and STA_2 (Table 2). However, OKY-046 (10^{-5} – 10^{-4} M) produced significant and concentration-dependent inhibitions on LTD_4 -induced con-

tractions and shifted the concentration-response curve towards the right in lung parenchymal strips (Fig. 6). Indomethacin also showed significant inhibitions on LTD_4 -induced contractions in lung parenchymal strips (Fig. 6). OKY-046 or indomethacin inhibited PAF-induced contraction in a concentration-dependent manner (Fig. 7).

Discussion

We investigated the effect of OKY-046 on experimental asthma in sensitized guinea pigs. The present study confirmed that OKY-046, but not indomethacin, elicited a dose-dependent inhibitory effect on antigen-

Fig. 5. Effects of OKY-046 (A) and indomethacin (B) on PAF ($0.3 \mu\text{g}/\text{kg}$, i.v.)-induced bronchoconstrictions and blood pressure responses in guinea pigs. Each point represents the mean of each group. A: ●—●, Control ($N=5$); ○—○, OKY-046, $1 \text{ mg}/\text{kg}$, i.v. ($N=5$); △—△, OKY-046, $.3 \text{ mg}/\text{kg}$, i.v. ($N=5$); □—□, OKY-046, $10 \text{ mg}/\text{kg}$, i.v. ($N=5$). B: ■—■, Control ($N=5$); ▲—▲, indomethacin, $2 \text{ mg}/\text{kg}$, i.v. ($N=5$). *, **, and *** indicate significant differences in comparison with the control where $P<0.05$, $P<0.01$ and $P<0.001$, respectively.

Table 2. Effects of OKY-046 on contractile responses induced by various spasmogens in guinea pig trachea and lung parenchymal strips

Spasmogenis	Doses of spasmogens (M)	Doses of OKY-046 (M)	Effects of OKY-046	
			Trachea	Lung parenchyma
Histamine	10^{-8} – 10^{-3}	10^{-4}	No Effect (5)	No Effect (5)
STA_2	10^{-10} – 10^{-6}	10^{-4}	No Effect (5)	No Effect (5)
PGD_2	10^{-8} – 10^{-4}	10^{-4}	No Effect (5)	No Effect (5)
LTC_4	10^{-10} – 10^{-6}	10^{-4}	No Effect (5)	ND ^a
LTD_4	10^{-10} – 10^{-6}	3×10^{-5} – 10^{-4}	No Effect (5)	Inhibition ^b

Numbers in parentheses indicate the number of preparations used. ^a not done. ^b See Fig. 6.

Fig. 6. Effects of OKY-046 and indomethacin on contractile response induced by LTD₄ in guinea pig lung parenchymal strips. Each point represents the mean value. Numbers in parentheses represent the numbers of preparations used. *, ** and *** indicate significant differences compared with the control where $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

Fig. 7. Effects of OKY-046 and indomethacin on contractile response induced by PAF in guinea pig lung parenchymal strips. Each point represents the mean value of 4 to 7 preparations. * and ** indicate significant differences compared with the control where $P < 0.05$ and $P < 0.01$, respectively.

induced bronchoconstriction. Since various spasmogens are considered to play important roles in mediating anaphylactic reactions, we examined the effect of OKY-046 on spasmogen-induced bronchoconstrictions.

In our in vitro studies, OKY-046 showed no effect on the contractile responses induced by histamine, STA₂ and PGD₂ in both tracheae and lung parenchymal strips. The findings suggest that such spasmogen-induced air-

way tissue contractions are not mediated via the actions of TXA_2 , and OKY-046 has no direct antagonism against the actions of these spasmogens.

LTD_4 -induced contractions of lung parenchymal strips were significantly attenuated by either OKY-046 or indomethacin. Weichman et al. (13) reported that peptide LTs-induced contractions may be mediated in part by contractile cyclooxygenase metabolites in lung parenchymal strips. Furthermore, evidences that TXA_2 is released from lung parenchyma when stimulated with peptide LTs have been documented (13, 14). Therefore, the present in vitro studies suggest that TXA_2 is a strong candidate for the contractile cyclooxygenase metabolite in mediating peptide LTs-induced contractions of lung parenchymal strips. However, the origin of TXA_2 in lung parenchyma is unclear, although TXA_2 is produced by platelets, mononuclear leukocytes, mast cells and other cells (3–7). On the other hand, OKY-046 did not show any inhibitory effects on the contractile responses induced by LTC_4 or LTD_4 in guinea pig tracheae. This suggests that the involvement of TXA_2 on peptide LTs-induced tracheal contractions can be discounted. Although guinea pig trachea is capable of synthesizing TXA_2 , the major cyclooxygenase product produced by this tissue is PGE_2 (15). On the other hand, Ally et al. (16) demonstrated that guinea pig lung parenchyma synthesizes predominantly TXA_2 . Therefore, it is conceivable that different effects of OKY-046 on trachea and lung parenchyma may be due to the difference in the amounts of TXA_2 produced by peptide LTs in these two tissues.

It has been shown that peptide LTs are released from sensitized guinea pig lung fragment stimulated with antigen (17) and have potent contractile activities on airway tissues in vivo and in vitro (18–21). Also Andersson (22) reported that peptide LTs are involved in anaphylactic bronchoconstriction using a specific LT antagonist FPL 55712. In the present in vivo study, we showed that OKY-046 attenuated transient bronchoconstriction and hypertension induced by peptide LTs. OKY-046 showed no inhibitory effect on LTD_4 -induced bronchoconstriction with indomethacin treatment,

and elicited significant inhibition on contractile responses induced by LTD_4 in lung parenchymal but not tracheal strips. Furthermore, evidences to indicate peptide LTs can release TXA_2 from guinea pig lung parenchymal strips (14) and perfused lung (23) have been documented. Therefore, it is conceivable that the inhibitory effect of OKY-046 on LTs-induced bronchoconstriction is attributable to inhibition of TXA_2 generation in peripheral airways. An attenuation of the initial phase of bronchoconstriction by indomethacin (similar to OKY-046) is probably due to suppression of TXA_2 production in peripheral airways.

PAF is released from perfused guinea pig lungs in response to antigen (24). Furthermore, Lagente et al. (25) demonstrated that Ro 19-3704, a PAF antagonist, inhibits anaphylactic bronchoconstriction. These evidences suggest the possible involvement of PAF in antigen-induced bronchoconstriction. We confirmed the potent spasmogenic activity of PAF (Fig. 5). Although PAF-induced bronchoconstriction was inhibited by OKY-046 and indomethacin, it seems unlikely that the inhibitory effects of these two compounds were attributed to direct antagonisms against PAF since the hypotensive responses induced by i.v. administration of PAF were not affected by these two compounds. PAF induces platelet-dependent bronchoconstriction (26) and stimulates the release of TXA_2 from guinea pig lung tissue (27). Furthermore, PAF causes platelet aggregation, platelet and neutrophil diapedesis immediately after its systemic administration in guinea pigs (25, 28). Therefore, it is possible that the inhibitory effects of OKY-046 and indomethacin against PAF-induced bronchoconstrictions are attributable to the inhibition of PAF-induced TXA_2 generation through platelet aggregation.

OKY-046 attenuated the pressor response induced by peptide LTs, PAF and antigen in accordance with the inhibitory activity on the airway response. In addition, Katsura et al. (29) reported that STA_2 caused both bronchoconstriction and the pressor response. Therefore, TXA_2 is most likely to be involved in peptide LTs-, PAF- and antigen-induced responses if assessed by responses of the blood pressure.

Histamine is considered as a potent mediator in bronchoconstriction following antigen challenges in guinea pigs (30, 31). OKY-046 had no inhibitory effects against the contractile responses induced by exogenous histamine. Accordingly, it is unlikely that the anti-asthmatic effect of OKY-046 is due to antagonism on the contractile action of histamine.

Indomethacin did not elicit any effect on antigen-induced bronchoconstriction except 1 min after antigen challenge (Fig. 1). Krell et al. (32) and Hitchcock (33) reported that indomethacin potentiates the contractile responses to peptide LTs and antigen in isolated guinea pig tracheae. There are several explanations for the failure of indomethacin to inhibit antigen-induced bronchoconstriction. First, indomethacin potentiates histamine release from basophils and airway tissues (34, 35). Secondly, indomethacin suppresses the synthesis of bronchodilating PGs, such as PGI₂ (9), and/or causes the shunt of arachidonic acid to the 5-lipoxygenase pathway (36).

In conclusion, we demonstrated that OKY-046 inhibited antigen-induced anaphylactic bronchoconstrictions in sensitized guinea pigs in vivo. The inhibitory effect of OKY-046 is probably due to the inhibition of TXA₂ production directly from an antigen-antibody reaction and/or indirectly via peptide LTs or PAF in the peripheral airways.

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