

Inhibitory effect of inhalation of a thromboxane synthetase inhibitor on bronchoconstriction induced by aerosolized leukotriene C₄ and thromboxane A₂ analogue in anesthetized guinea pigs

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Effect of aerosol administration of a thromboxane synthetase inhibitor (OKY-046) on bronchoconstriction induced by aerosol leukotriene C₄, histamine and a thromboxane A₂ analogue (STA₂) was studied in anesthetized, artificially ventilated guinea pigs in order to evaluate the effectiveness of inhalation of OKY-046 on an unfavorable mechanism of secondary release of thromboxane A₂. 0.01-1.0 µg/ml leukotriene C₄, 25-400 µg/ml histamine and 0.033-1.0 µg/ml STA₂ inhaled from an ultrasonic nebulizer developed for small animals caused a dose-dependent increase of pressure at the airway opening (Pao), which is considered to be an index representing bronchial response. Pretreatment of the animals with aerosol OKY-046 (0.035 and 0.35 mg/animal) significantly reduced the airway responses produced by inhalation of leukotriene C₄ and STA₂, in a dose-dependent manner, while the pretreatment did not affect the histamine dose-response curve. These findings suggest that aerosol leukotriene C₄ and STA₂ activate thromboxane synthesis in the airway, and inhalation of OKY-046 may be useful for preventing the secondary release of thromboxane A₂, which is an unfavorable mechanism in asthma.

Key words: guinea pig *in vivo*; inhalation; leukotriene C₄; thromboxane A₂ analogue (STA₂); thromboxane synthetase inhibitor (OKY-046).

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Slow-reacting substance of anaphylaxis (SRS-A) was originally reported to be released from sensitized guinea pig lung (14) and it produces potent and long-lasting bronchoconstriction in these animals and humans (1). Since leukotriene C₄, D₄ and E₄ (LTC₄, LTD₄ and LTE₄) (21, 23), the main components of SRS-A, are regarded as putative mediators of asthma (24), guinea pigs have been used for studies on the biological activities of LT. In guinea pigs, it has been shown that systemically administered indomethacin, one of the cyclooxygenase inhibitors, potentiated the bronchoconstriction induced by aerosol administration of LTC₄ (16) and LTD₄ (10). It has been reported, however, that the airway responses to

intravenously administered LTC₄ (17, 25) and LTD₄ (10, 17, 25) were reduced by systemic administration of indomethacin and thromboxane synthetase inhibitors (20, 25). According to these findings, it has been considered that secondary thromboxane A₂ release is important in intravenous LTC₄ and LTD₄-induced bronchoconstrictions but not in the airway responses to aerosol LTC₄ and LTD₄. However, we previously demonstrated that a thromboxane synthetase inhibitor, OKY-046 (22), inhibited the bronchoconstriction mainly mediated by SRS-A when aerosol antigen was administered to passively sensitized guinea pigs (4). Recently, we also found that aerosol LTC₄-induced bronchocon-

striction was reduced by intravenously administered OKY-046 in guinea pigs (5). So we have considered the role of thromboxane A₂ in the bronchoconstriction induced by LTC₄, one of the putative mediators of asthma.

Clinically, we showed that bronchial hyperresponsiveness to acetylcholine was reduced by oral and aerosol administration of OKY-046 in patients with bronchial asthma (6, 7) and now a double blinded clinical trial of oral OKY-046 for treatment of asthma is going on in Japan. However, systemic administration of OKY-046 inhibits thromboxane synthesis, not only in the bronchopulmonary system, but also in the other systemic organs, especially circulating platelets (11). Aerosol administration of antiasthmatic drugs, for example, β_2 -stimulants and steroids, is widely used to avoid systemic side effects of an oral administration. Therefore, if aerosol OKY-046 inhibits the secondary release of thromboxane A₂ by LTC₄, it may be more useful for asthma than oral OKY-046. In addition, our previous study (8) showed that bronchoconstriction induced by a thromboxane A₂ analogue, STA₂ (13), was inhibited by intravenous administration of OKY-046 but potentiated by indomethacin.

In this study, we examined the effects of inhaled OKY-046 on bronchoconstriction induced by aerosol histamine, LTC₄ and STA₂ in order to evaluate the effectiveness of OKY-046 inhalation on the unfavorable mechanism of secondary synthesis and release of thromboxane A₂.

MATERIAL AND METHODS

Male albino Hartley strain guinea pigs (340–370 g) were anesthetized intraperitoneally by an injection of 75 mg/kg of sodium pentobarbital. They were placed in the supine position and the trachea was cannulated with a polyethylene tube (outside diameter, 2.5 mm; inside diameter, 2.1 mm).

After surgery, the guinea pig was artificially ventilated by a small animal respirator (Model 1680, Harvard Apparatus Co., Inc., South Natick, MA) adjusted to a tidal volume of 10 ml/kg at a rate of 60 strokes/min. The changes in lung resistance to inflation, the lateral pressure of the

tracheal tube (pressure at the airway opening; Pao) (cmH₂O) were measured by the modified method of Konzett & Rössler (15) described by Hamel and co-workers (12) with a pressure transducer (Model TP-603T, Nihon Koden Kogyo Co., Ltd., Tokyo). Since the change in Pao following inhalation of LTC₄ represented the average of changes in pulmonary resistance (R_L) and reciprocal dynamic lung compliance (1/C_{dyn}) (9), we have used Pao as an overall index of bronchial response to bronchoactive agents.

When all preparations were completed, the animals were overinflated by doubling the tidal volume for 2 breaths by clamping the outlet of the respirator to equalize with the lung volume. The animals were given aerosolized 0.9 % w/v saline with or without OKY-046 (10 and 100 mg/ml), a selective thromboxane A₂ synthetase inhibitor (22). The aerosol was generated during a 30 s period by an ultrasonic nebulizer developed for small animals at our institution. The amount of aerosol was 15.2 μ l/min, and 46.4 % of the aerosol was deposited in the lung as measured by the radioaerosol technique (19). It was therefore calculated that approx. 0.35 mg of OKY-046 was deposited in the lung during the 30-s period when 100 mg/ml of OKY-046 was inhaled.

Ten minutes later, when Pao had been stabilized, ascending doses of LTC₄, (0.01, 0.033, 0.1, 0.33, 1.0 μ g/ml) or histamine (25, 50, 100, 200, 400 μ g/ml) were inhaled for 30 s at 5-min intervals without interrupting the constant ventilation. Ascending doses of STA₂ (13) (0.033, 0.1, 0.33, 1.0 μ g/ml) were inhaled for 30 s at 10-min intervals. Solutions of all the agonists were prepared in 0.9 % w/v saline and placed in an electric refrigerator (4°C). All experiments with LTC₄, histamine or STA₄ were completed within 60 min. Statistical differences were determined by Mann-Whitney's U-test, with a *P* value of ≤ 0.05 considered to be significant.

The following chemicals were used: sodium pentobarbital (Abbott Laboratories, North Chicago, IL); leukotriene C₄ (Takeda Pharmaceutical Ind., Osaka, Japan); histamine (Wako Pure Chemical Ind., Osaka, Japan); STA₂ (9, 11-epithio-11, 12-methano-TXA₂) (Ono Pharma-

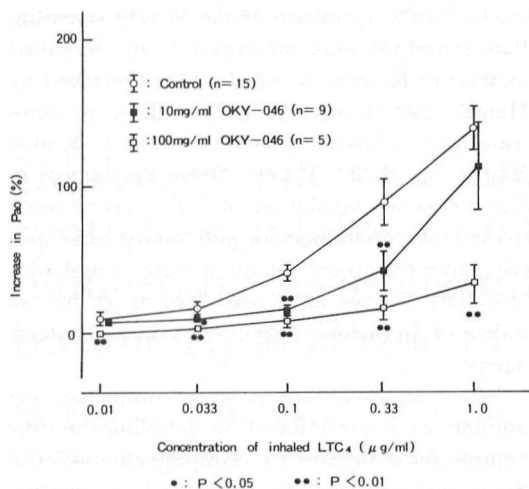


Fig. 1. Percent increase in Pao by aerosol LTC₄ in anesthetized guinea pigs pretreated with inhaled OKY-046 and saline. Each point represents the mean value ($\pm$ SEM).

((E)-3 (4-(1-imidazolylmethyl) phenyl)-2-propenoic acid hydrochloride monohydrate) (Kissei Pharmaceutical Co., Ltd., Matsumoto, and Ono Pharmaceutical Co., Ltd., Osaka, Japan).

RESULTS

The Pao values before the first challenge of LTC₄ were 8.3 ± 0.6 (mean $\pm$ SEM), 8.8 ± 0.3

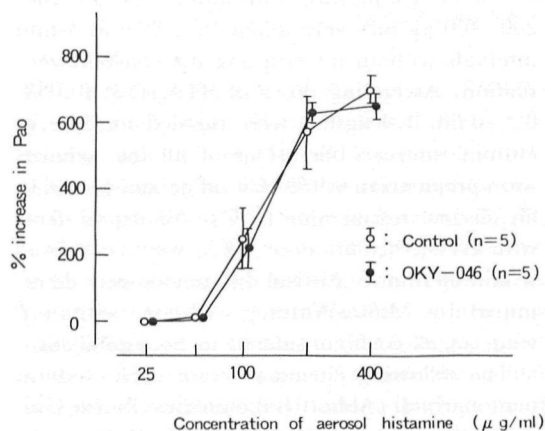


Fig. 2. Percent increase in Pao by aerosol histamine in anesthetized guinea pigs pretreated with inhaled OKY-046 and saline. Each point represents the mean value ($\pm$ SEM).

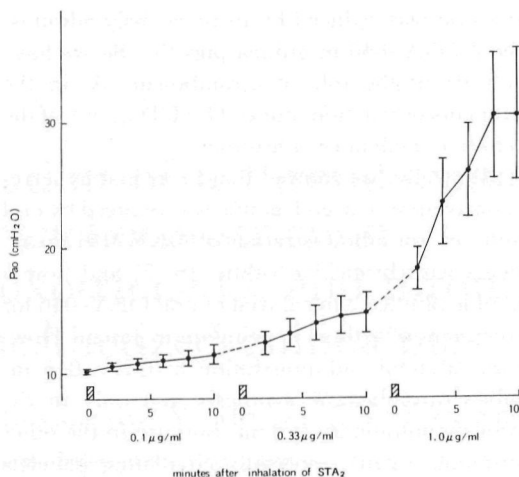


Fig. 3. Time course of Pao after inhalation of each concentration of STA₂ in anesthetized guinea pigs. Each point represents the mean value ($\pm$ SEM).

and 7.7 ± 0.1 cmH₂O in the animals pretreated with aerosolized saline ($n = 15$), 10 mg/ml OKY-046 ($n = 9$) and 100 mg/ml OKY-046 ($n = 5$), and there was no difference among them. Dose-response curves of % increases in Pao by inhaled LTC₄ (0.01–1.0 μg/ml) are shown in Fig. 1. Pretreatment of the animals with aerosol OKY-046 significantly reduced the airway responses produced by inhalation of LTC₄ in a dose-dependent manner.

The Pao values before histamine provocation were 9.6 ± 0.2 and 9.6 ± 0.3 cmH₂O in the animals pretreated with aerosolized saline ($n = 5$) and 100 mg/ml OKY-046 ($n = 5$), and there was no difference between these. Dose-response curves of % increases in Pao by aerosol histamine (25–400 μg/ml) are shown in Fig. 2. Pretreatment with OKY-046 did not affect the histamine dose-response curve.

The Pao values before inhalation of STA₂ were 10.2 ± 0.2 , 10.0 ± 0.3 and 9.7 ± 0.4 cmH₂O in the animals pretreated with aerosolized saline ($n = 13$), 10 mg/ml OKY-046 ($n = 7$) and 100 mg/ml OKY-046 ($n = 7$), and there was no difference among them. Fig. 3 shows time course of Pao after inhalation of each concentration of aerosol STA₂ in anes-

tized guinea pigs pretreated with inhaled saline. The Pao values after inhalation of 0.33 and 1.0 $\mu\text{g}/\text{ml}$ of STA₂ reached maximum about 8 to 10 min and 6 to 10 min after, respectively, and lasted for at least 10 min.

Dose-response curves of % increases in Pao by STA₂ (0.033–1.0 $\mu\text{g}/\text{ml}$) are shown in Fig. 4. Pretreatment of the animals with inhaled OKY-046 significantly reduced the airway responses produced by inhalation of STA₂ in a dose-dependent manner.

DISCUSSION

Leukotriene C₄, D₄ and E₄, the main components of SRS-A, are thought to be putative

mediators of asthma, and many clinical and experimental studies have been carried out. Recently, it has been possible to give LT receptor antagonists and 5-lipoxygenase inhibitors to human subjects and the role of LT in bronchial asthma has been evaluated using the agents in asthmatic patients. However, up to the present, none of the researchers has investigated whether LT receptor antagonists or 5-lipoxygenase inhibitors have significant anti-asthmatic effects in humans. Mann and co-workers reported that inhaled piriprost (U-60, 257), a novel leukotriene inhibitor, had no effect on allergen and exercise-induced bronchoconstriction in asthma (18). Britton et al. found that an oral leukotriene D₄ antagonist, L-649923, showed a small degree of protection against the early response to antigen but no effect on the late response in asthma (2). We previously reported that an oral leukotriene synthesis inhibitor, AA-861, did not alter bronchial hyperresponsiveness to acetylcholine in asthmatics (6). Although these investigations suggest that leukotrienes do not play a major role in asthma, further studies with more potent specific inhibitors and antagonists are needed since the dose and activity of the examined drugs might be inadequate to inhibit the action of leukotrienes *in vivo*. Recently, Cloud and co-workers reported that a specific LTD₄/LTE₄-receptor antagonist, LY171883, improved pulmonary function in patients with mild, chronic asthma (3).

We have previously shown that oral administration of a thromboxane synthetase inhibitor, OKY-046, reduced bronchial hyperresponsiveness to acetylcholine in patients with asthma (6) and have investigated the role of thromboxane A₂ in asthma. In double blinded clinical trials in Japan, oral OKY-046 has been proved to have excellent anti-asthmatic effect (personal communication by Terumasa Miyamoto).

In guinea pigs *in vivo*, it is suggested that secondary release of cyclooxygenase products, particularly thromboxane A₂, contributes to the bronchoconstriction caused by intravenous administration of leukotriene C₄ and D₄ because the bronchoconstriction has been inhibited by cyclooxygenase inhibitors and thromboxane synthetase inhibitors (10, 17, 20, 25). On the

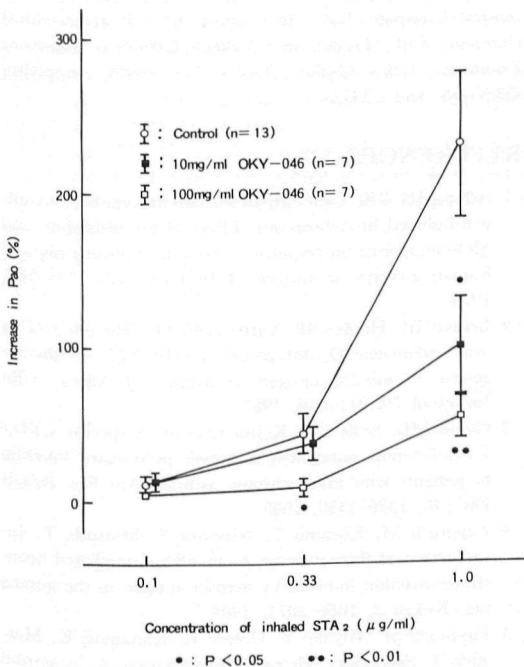


Fig. 4. Percent increase in Pao by aerosol STA₂ in anesthetized guinea pigs pretreated with inhaled OKY-046 and saline. Each point represents the mean value ($\pm$ SEM).

the bronchoconstriction has been inhibited by cyclooxygenase inhibitors and thromboxane synthetase inhibitors (10, 17, 20, 25). On the other hand, inhaled LTC₄ and D₄-induced bronchoconstriction has been inhibited by thromboxane synthetase inhibitors (5) while potentiated by cyclooxygenase inhibitors (10, 16). Consequently, we speculate that thromboxane A₂ may be secondarily released by aerosol LTC₄ and D₄ while the bronchoaction of the cyclooxygenase products may be bronchodilating as a whole. Thus, to control bronchoconstriction it may be useful to inhibit thromboxane A₂ and preserve bronchodilating prostaglandins.

OKY-046 is a selective thromboxane synthetase inhibitor (22). The concentration of OKY-046 to give 50% inhibition (IG₅₀) 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/l (22), no inhibitory effect was seen at 1×10^{-3} mol/l on 5-lipoxygenase in cytosol of RBL-1 and no antagonistic effect was found against leukotrienes and thromboxane A₂ (personal communication by Tetsuro Yoshimura, Kissei Pharmaceutical Company Ltd., Matsumoto, Japan).

Systemic administration of OKY-046 strongly inhibited platelet aggregation. When given orally to healthy adults 25 mg OKY-046 suppressed the ability of blood platelets to synthesize thromboxane A₂, while with higher doses (200–400 mg) this suppression lasted for more than 12 h and continued after the drug had disappeared from the blood (11). Such an effect is unfavorable as a side effect, for example systemic bleeding tendency. If aerosol administration of OKY-046 is as effective as systemic administration it should be favorable. In practice, steroids and β_2 -adrenergic agonists are usually administered by inhalation. We recently found that inhalation of OKY-046 (25 mg q.i.d.) significantly improved bronchial hyperresponsiveness without inhibiting platelet aggregation in patients with asthma (7).

This study was therefore designed to examine whether inhalation of OKY-046 had an inhibitory effect on aerosol LTC₄-induced bronchoconstriction or not. The results showed that both aerosol OKY-046 and intravenous

OKY-046 (100 mg/kg) inhibited the bronchoconstriction in a dose-dependent manner (5). In addition, this study showed an inhibitory effect of aerosol OKY-046 on the bronchoconstriction induced by aerosol STA₂, which is a thromboxane A₂ analogue (13). We previously showed that bronchoconstriction induced by aerosolized STA₂ was inhibited by intravenous administration of OKY-046 (100 mg/kg) while potentiated by intravenously administered indomethacin (8). Because it is an unfavorable mechanism for asthmatics that secondary release of thromboxane A₂ by both thromboxane A₂ and LTC₄ in the airway could potentiate bronchoconstriction, control of thromboxane A₂ might be useful for treatment of asthma. The results of this study suggest that inhalation of OKY-046 may be more favorable than oral dosing because the inhalation dose is smaller and systemic side effects can be avoided.

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