

The Modulatory Effect of Antigen- and PAF-Induced Asthmatic Reaction by Aerosol Administration of OKY-046 in Guinea Pigs

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Inhalation of antigen, ovalbumin (OVA), in the absence of adjuvant has been demonstrated to induce airway obstruction and airway hyperresponsiveness in guinea pigs.^{1,2} The airway spasms result from the release of preformed and newly generated mediators from activated mast cells in the bronchial mucosa. This immediate hypersensitivity response reaches a maximum 15 to 30 minutes after challenge and lasts for as long as 2 hours. It is followed by a second wave of air-flow limitation, or late phase response 6 to 12 hours afterward.³ This late phase response may occur with diminishing intensity over the following 2 to 3 days.⁴ The late phase reactions are accompanied by an increase in the responsiveness of the airways to a wide variety of stimuli as reflected by increased reactivity to inhaled methacholine and histamine.^{5,6}

(E)-3-[p-(1H-imidazole-1-ylmethyl) phenyl]-2-propenoic acid (OKY-046), an imidazol derivative, has been reported to inhibit the generation of thromboxane A₂ (TXA₂), a potent constrictor of smooth muscle and a potent inducer of platelet aggregation, by inhibiting TXA₂ synthetase in various animal

SUMMARY The therapeutic effect of a thromboxane A₂ (TXA₂) synthetase inhibitor on asthma is still controversial. This study was aimed at clarifying its effect on asthmatic reactions in guinea pigs. Both ovalbumin (OVA)- and platelet activating factor (PAF)-induced dual phase airway spasm and hyperreactivity in guinea pigs were used as the asthma model.

Our results demonstrated that aerosol administration of OKY-046 could inhibit both OVA- and PAF-induced late phase bronchoconstriction and airway hyperreactivity to methacholine in OVA sensitized guinea pigs. PAF administration could also induced dual phase bronchoconstriction in normal guinea pigs. Similarly, late phase airway spasm and airway hyperreactivity after PAF exposure was also blocked by OKY-046.

In conclusion, aerosol administration of OKY-046 is a safe and effective way to modulate OVA- and PAF-induced asthmatic reactions. The protective effect of OKY-046 on OVA- and PAF-induced late phase bronchoconstriction and airway hyperreactivity indicates that TXA₂ might play an important role in the late phase asthmatic reaction and airway hyperreactivity. The normalization of PAF-induced airway hyperreactivity by OKY-046 also indicates that PAF induced airway inflammation might be through the generation of TXA₂.

species.^{7,8} Previous reports suggest the involvement of TXA₂ not only in airway smooth muscle constriction but also in increased airway hyperresponsiveness.⁹⁻¹² Numerous studies have also demonstrated that thromboxane-derived products increased markedly in asthmatics and that intravenously administrated TXA₂ synthetase inhibitor prevents airway hyperresponsiveness.^{13,14} Recently, a randomized, double-blind, placebo-controlled, cross-over study on the effect of one week

treatment with a potent selective thromboxane synthetase inhibitor showed that there is lack of short-

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term effect on airway reactivity to methacholine in asthmatic individuals.¹⁵ These reports indicate that the role of TXA₂ in the development or maintenance of hyperresponsiveness in asthma is still controversial.

Platelet-activating factor (PAF) can induce bronchoconstriction,¹⁶ intravascular platelet aggregation, and platelet and neutrophil diapedesis immediately after its systemic administration to the guinea pigs.¹⁷ Inhalation of PAF can also produce bronchoconstriction and increase airway reactivity in normal and asthmatic individuals.^{18,19} It is of interest to clarify the effect of TXA₂ synthetase inhibitor on both antigen (OVA)- and PAF-induced airway spasm and airway hyperreactivity in a guinea pig asthma model. Aerosol administration of OKY-046 was used as TXA₂ synthetase inhibitor in this study.

MATERIALS AND METHODS

Materials

Mepyramine malate, OVA Grade V, PAF, and methacholine were purchased from Sigma Chemical Co. (St. Louis MO, USA). Ketamine was obtained from Parke-Davis Inc. (Elk Grove Village, IL, USA). OKY-046 was kindly supplied by ONO Pharmaceutical Co., Ltd. (Osaka, Japan).

Sensitization by repeated inhalation of OVA aerosols

Male Dunkin-Hartley guinea pigs weighing 450 to 550 g were sensitized by exposure to aerosolized OVA (1% w/v in 0.9% sterile sodium chloride) on 2 occasions separated by 7 days. The aerosol was generated by a Mefar MB3 dosimeter (Mefar Ele, Brescia, Italy) with driving pressure: 1.65 kg/cm, flow rate 70 l/min, particle size of aerosol 4.0 μ m. The aerosol was generated into 5-liter plastic chamber in which the animals were placed for 10 minutes on each occasion. All animals were

studied with 2% w/v OVA in 0.9% sterile sodium chloride seven days after the second exposure. Thirty minutes before OVA challenge, the animals were injected intraperitoneally with antihistamine, mepyramine (10 mg/kg), to avoid a fatal anaphylactic reaction.

Measurement of pulmonary function and bronchial hyperreactivity

The airway responses to OVA were determined, in conscious nose-breathing guinea pigs, by measuring pulmonary resistance, dynamic lung compliance and tidal volume. After intramuscular administration of Ketamine (30 mg/kg), guinea pigs were placed in a supine position. The pharynx and epiglottis were swabbed with 1% Lidocaine to prevent gagging and a water-filled CH50 feeding tube was placed into the esophagus to measure intrathoracic pressure.

Lung volume and intrathoracic pressure were measured with a "LAB" pulmonary evaluation and diagnostic system (Hatfield, PA, USA). The concentrations of methacholine used for airway provocation were 0.075, 0.15, 0.3, 0.625, 1.25, and 5 mg/ml. The data of methacholine PD₁₀₀ pulmonary resistance were defined as a provocative dose causing a 100% increase in pulmonary resistance. At the end of the procedure, the animals were given fenoterol hydrobromide nebulization (Boehringer Ingelheim, Germany) until the pulmonary function returned to normal. Methacholine was prepared in normal saline, and delivered through a Mefar MB3 dosimeter (Mefar Ele Brescia, Italy). Prior to methacholine challenge, all animals received five inhalations of normal saline, pulmonary function tests were performed and the results were taken as controls. If the pulmonary function did not differ from the baseline, animals were accepted for methacholine challenge. Five breaths

of serial dilutions of methacholine were given to each guinea pig, with pulmonary function measurements taken three minutes at each challenge. PAF and lyso-PAF were also used to induce airway spasm in the same way as described above. Both mediators were dissolved in 0.9% normal saline containing 0.25% bovine serum albumin at a concentration of 10⁻⁵ M. OKY-046, dissolved in 0.9% normal saline at the concentration ranging from 1 mg/ml to 10 mg/ml, was aerosolized through the Mefar MB3 dosimeter immediately before challenge with OVA and PAF to test its effect on OVA- and PAF-induced airway spasm and hyperreactivity.

RESULTS

Effect of OKY-046 on OVA-induced bronchoconstriction and airway hyperresponsiveness in guinea pigs.

OVA challenge could induce dual phase bronchoconstriction (early phase followed by late phase) and bronchial hyperreactivity in guinea pigs presensitized with OVA. Aerosol administration of OKY-046 was able to inhibit late phase bronchoconstriction and bronchial hyperreactivity to methacholine.

The study of the effect of 2% OVA challenge on OVA sensitized guinea pigs over time showed that there was an immediate increase in airway resistance and decrease of tidal volume after OVA exposure. The late phase reaction occurred 6 hours later and gradually returned to normal over the following 48 hours (Fig. 1).

Aerosol administration of OKY-046 to OVA presensitized guinea pigs showed that OKY-046 treatment could significantly suppress OVA-induced late phase reaction. Its effect on early phase reaction, however, was negligible (Fig. 2).

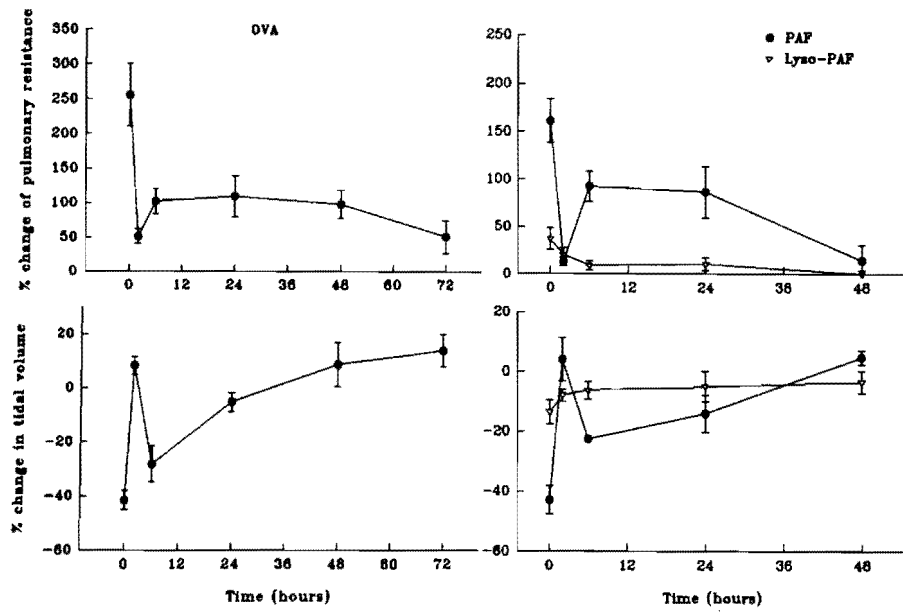


Fig. 1. Time course study of the effect of OVA (left panel), PAF and lyso-PAF (right panel) on pulmonary resistance and tidal volume in OVA-sensitized guinea pigs. Each point represents the mean \pm SEM of 10 animals.

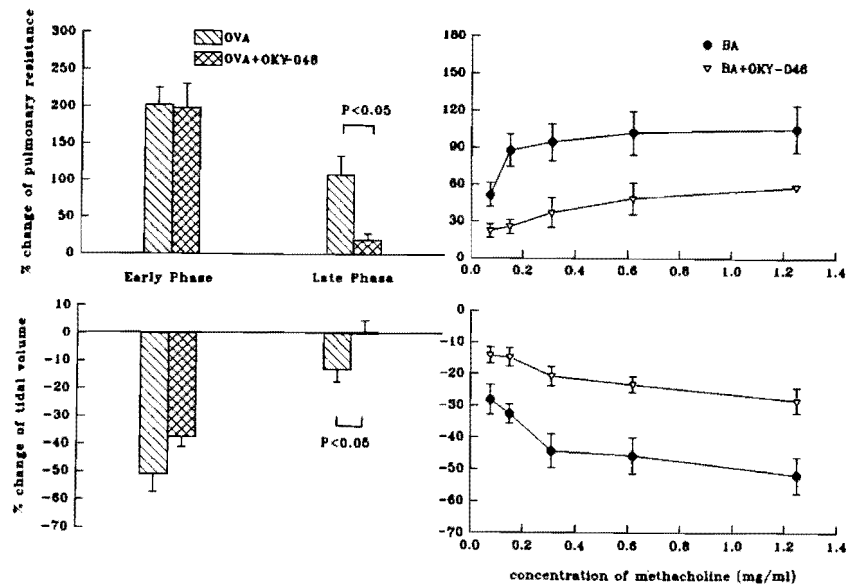


Fig. 2. Effect of OKY-046 on OVA-induced dual phase bronchoconstriction (left panel) and bronchial hyperreactivity (right panel) in OVA sensitized bronchial asthmatic (BA) guinea pigs. OKY-046 aerosol (10 mg/ml) was administered immediately before OVA challenge. For bronchial hyperreactivity to methacholine, OKY-046 aerosol (10 mg/ml) was administered immediately before methacholine challenge. Each point represents the mean \pm SEM of 10 animals.

In the airway hyperreactivity study, the airways were sensitive to low dose methacholine in OVA sensitized guinea pigs. The pulmonary resistance increased markedly after methacholine exposure with the PD₁₀₀ of methacholine 0.31 mg/ml. However, the airway hyperreactivity could return to normal after OKY-046 inhalation (Fig. 2).

Effect of OKY-046 on PAF-induced bronchoconstriction and airway hyperreactivity in guinea pigs

PAF administration can cause bronchoconstriction and airway hyperreactivity in both normal and OVA sensitized asthmatic guinea pigs. Dual phase reactions was noted after PAF exposure. Aerosol administration of OKY-046 was able to inhibit PAF-induced late phase reaction and airway hyperreactivity to methacholine in both normal and OVA-sensitized asthmatic guinea pigs.

The early phase bronchoconstriction occurred immediately after PAF exposure and would return to baseline 1 hour later. The late phase reaction occurred 6 hours later with a return to baseline over the following 48 hours (Fig. 1). OKY-046 pretreatment could prevent PAF-induced late phase bronchoconstriction dose-dependently, however, it has no protective effect on early phase reaction at any dose tested (Fig. 3).

Both normal and OVA-sensitized asthmatic guinea pigs were administered with aerosolized OKY-046, then followed by PAF inhalation. The PAF-induced immediate bronchoconstriction could not be inhibited by OKY-046 in both groups, however, a significant improvement of late phase reaction was noted in both groups after OKY-046 exposure (Fig. 4).

In the airway hyperreactivity study, methacholine challenge was performed 1 hour before and 6 hours after PAF inhalation. Airway hyper-

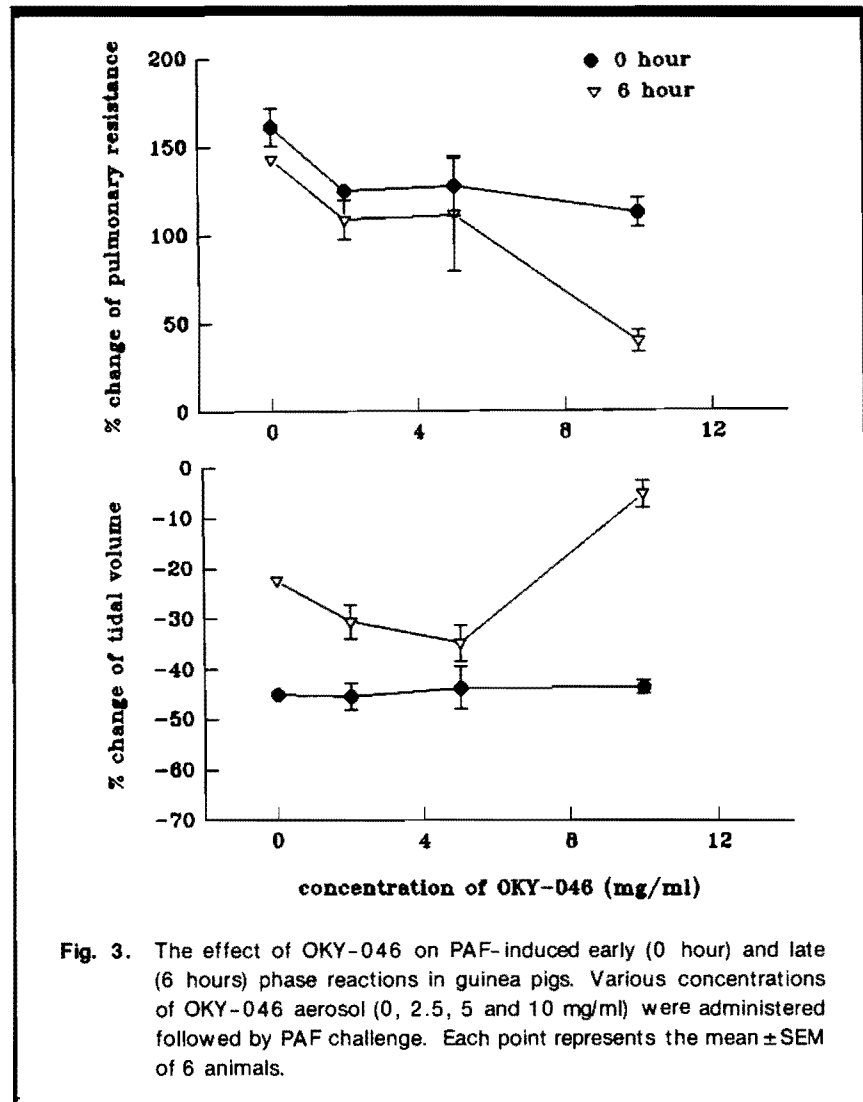


Fig. 3. The effect of OKY-046 on PAF-induced early (0 hour) and late (6 hours) phase reactions in guinea pigs. Various concentrations of OKY-046 aerosol (0, 2.5, 5 and 10 mg/ml) were administered followed by PAF challenge. Each point represents the mean \pm SEM of 6 animals.

reactivity increased greatly after PAF inhalation (Fig. 5). Aerosol administration of OKY-046 was performed immediately before PAF inhalation to test its effect on airway hyperreactivity induced by PAF. Results showed that OKY-046 could inhibit PAF-induced airway hyperreactivity only 6 hours after PAF exposure (Fig. 5).

DISCUSSION

In this study, we have demonstrated that aerosol administration of OKY-046 can not only prevent OVA- and PAF-induced late phase reaction but also reduce the airway

hyperreactivity to methacholine in both groups of guinea pigs.

Both OVA- and PAF-induced late phase bronchoconstriction can be blocked by OKY-046, which indicates that antigen and PAF-induced late phase reactions are caused by the generation of TXA₂. It has been reported that TXA₂-derived product markedly increases during an asthma attack.¹⁴ The generation and release of TXA₂ has also been reported to be from mast cells, monocytes and platelets,^{20,21} which indicate that the effect of OKY-046 may be through the stabilization of these inflammatory

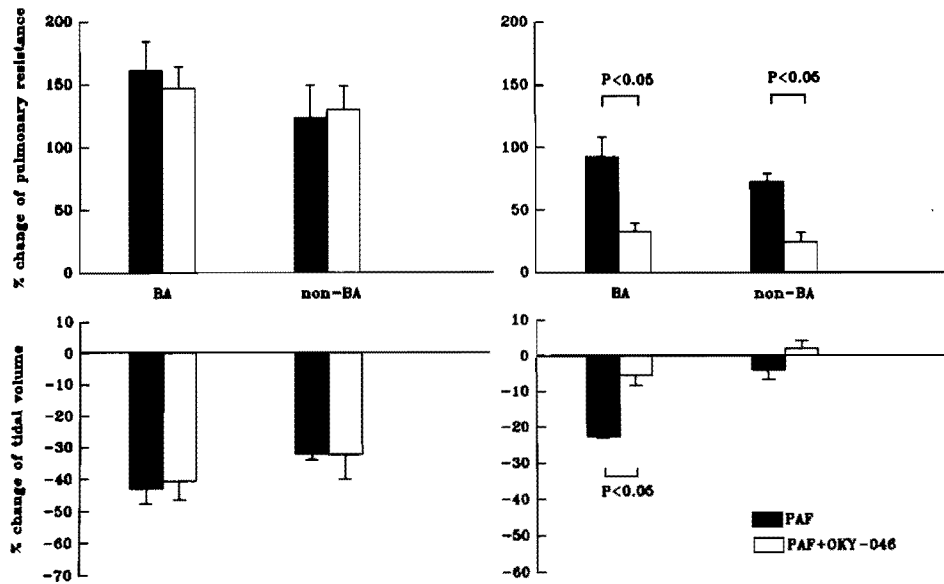


Fig. 4. Effects of OKY-046 on PAF induced early phase and late phase pulmonary function changes in guinea pigs. Both OVA-sensitized asthmatic (BA) and normal (non-BA) guinea pigs were administered with aerosolized OKY-046, then followed by PAF inhalation. Each column represents the mean \pm SEM of 10 animals.

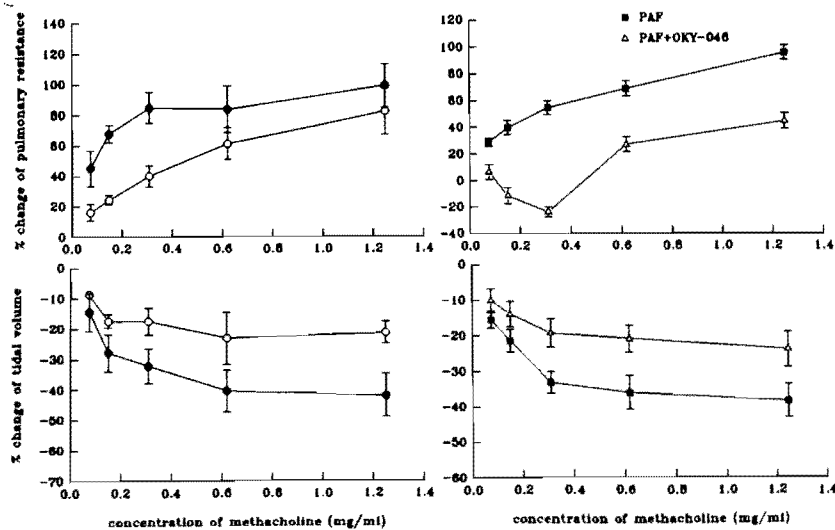


Fig. 5. The effects of PAF on airway hyperreactivity to methacholine (left panel) and the effects of OKY-046 on PAF-induced airway hypersensitivity to methacholine (right panel) in normal guinea pigs. Methacholine challenge was performed 1 hour before (●) and 6 hours after (○) PAF inhalation (left panel). Aerosol administration of OKY-046 was performed immediately before PAF inhalation, then followed by methacholine challenge. Each point represents the mean \pm SEM of 6 animals.

cells. The identification of OKY-046 target cells requires further investigation in this guinea pig animal model.

The immediate bronchoconstriction induced by OVA and PAF cannot be inhibited by OKY-046, suggesting that TXA2 might not be the only one mediator relevant to the early reaction. There are many reports indicating that several inflammatory mediators are involved in the pathogenesis of early allergic reaction such as histamine, sulfido-peptide leukotrienes and PAF.^{22,23} It is conceivable that the prevention of antigen induced immediate reactions should include many kinds of mediator antagonist in addition to TXA2.

Airway hyperreactivity has been considered to be an indicator of airway inflammation.²⁴ Our results showed that PAF could induce long term airway hyperreactivity and dual phase bronchoconstriction in both normal and OVA-sensitized asthmatic guinea pigs and indicates that PAF is a potent inflammatory mediator in airway diseases. Similar studies have reported that PAF inhalation can cause bronchoconstriction in man.¹⁶

The aerosol administration of OKY-046 was shown to inhibit both OVA- and PAF-induced airway hyperreactivities to methacholine indicating that TXA2 probably plays a role in the pathogenesis of airway hyperreactivity. These results, therefore, suggest that OKY-046 is a potent antiinflammatory agent. The lack of effect of TXA2 synthetase inhibitor in the previous report¹⁵ might either be due to different TXA2 synthetase inhibitors (UK 38, 485, vs OKY-046) or due to different administration routes (oral vs aerosol administration).

In conclusion, aerosol administration of OKY-046 can modulate antigen-induced late phase bronchoconstriction and airway hyperreactivity. Inhalation therapy of

TXA2 synthetase inhibitor might be an efficient and safe alternative way to treat allergen-induced asthma.

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