

Inhibitory effect of aerosol WP871 on SRS-A mediated bronchoconstriction in the guinea pig *in vivo*

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Slow-reacting substance of anaphylaxis (SRS-A) is an important factor mediating bronchoconstriction in asthma. We developed a guinea pig model for SRS-A mediated bronchoconstriction induced by antigen inhalation. Using this model, we investigated the effect of inhaled WP871, a new anti-allergic drug, on bronchoconstriction. Aerosol WP871 (0.01 and 0.033%) to some extent inhibited the antigen-induced bronchoconstriction in a dose-dependent fashion, but high-dose WP871 (0.1%) inhalation itself produced a non-specific bronchoconstriction. However, aerosol WP871 (0.033%) showed no inhibitory effect on bronchoconstriction caused by direct inhalation of leukotriene C₄, a component of SRS-A. These findings indicate that aerosol WP871 does not antagonize SRS-A, but inhibits synthesis and/or release of SRS-A and has some non-specific bronchoconstrictive effect in high concentration.

Key words: anti-allergic drug, WP871; allergic bronchoconstriction; guinea pig; LTC₄; SRS-A.

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A newly synthesized compound, 3-(1H-tetrazol-5-yl) oxanilic acid (WP871) is a major metabolic product of an anti-allergic drug, WP833 (butyl 3' [1H-tetrazol-5-yl]) (1), and has been found potentially effective for allergic rhinitis (personal communication). The inhibitory activity of this compound on allergic rhinitis was suggested to be shown through the suppression of mediator release following the antigen-antibody reaction. In this study we investigated the anti-allergic actions of WP871 on aerosol antigen-induced bronchoconstriction and bronchoconstriction produced by aerosol histamine and leukotriene C₄ (LTC₄) in anesthetized and mechanically ventilated guinea pigs.

MATERIAL AND METHODS

Male albino Hartley strain guinea pigs weighing 300 to 450 g were used in this study.

Allergic bronchoconstriction

Guinea pig homocytotropic antiserum was made by the method of Santives et al. (5). 500 µg ovalbumin (OA) was emulsified in Freund's complete adjuvant and injected intradermally into each guinea pig at multiple sites. Boosting was carried out in the same manner 2 weeks later. Serum was collected from each animal 2 weeks after the booster, pooled and kept frozen until use. Normal guinea pigs were passively sensitized with 1 ml/kg of the antiserum administered intraperitoneally.

Twelve to 24 h after passive sensitization, the guinea pigs were anesthetized with an intraperitoneal injection of 75 mg/kg sodium pentobarbital. They were placed in the supine position, the trachea was cannulated with a polyethylene tube (o.d. 2.5 mm; i.d. 2.1 mm) and one jugular vein was cannulated for the adminis-

tration of drugs. Pressure at the airway opening (Pao), the lateral pressure of the tracheal tube, was measured to estimate the overall bronchoconstriction (Model TP-603T, Nihon Koden Kogyo Co., Ltd., Tokyo) (3). The animals were artificially ventilated by a small animal ventilator (Model 1680, Harvard Apparatus Co. Inc., South Natick, MA.) adjusted to a tidal volume of 10 ml/kg at a rate of 60 breaths/min. Mechanical dead space in this system was 0.5 ml.

When all procedures were completed, the animals were given 60 mg/kg diphenhydramine hydrochloride intraperitoneally to block the action of histamine completely (2), and received a vital capacity maneuver in order to unify the volume history of the lung.

Fifteen min later, when Pao was stable, the animals were challenged with nebulized ovalbumin dissolved in saline (1 mg/ml) without interrupting the constant ventilation. The ovalbumin aerosol was generated for 30 s by an apparatus equipped with an ultrasonic nebulizer, developed for small animal experiments (4). Since the amount of aerosolized ovalbumin solution (1 mg/ml) was 15.2 μ l/min, and 46.4 % of the aerosol was deposited in the lung (4), approximately 3.5 μ g ovalbumin was considered to be deposited in the lung during the 30 s.

WP871 (0.01, 0.033 and 0.1 %) was inhaled for 30 s 10 min before the antigen challenge and Pao was observed for 20 min. Control animals were given 0.9 % saline in the same manner.

Histamine-induced bronchoconstriction

Normal guinea pigs were prepared as described above. Ascending doses of histamine (25, 50, 100, 200 μ g/ml) were inhaled for 20 sec at 5 min intervals without interrupting the constant ventilation and Pao was observed. The normal guinea pigs were given 30 s inhalation of 0.1 % WP871 10 min before histamine provocation. Control animals were given aerosol saline in the same manner.

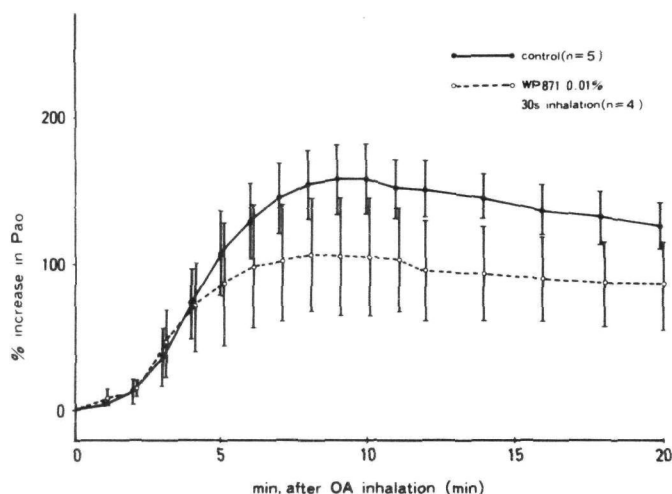


Fig. 1. Inhibitory effect of 30 s inhalation of 0.01 % WP871 on allergic bronchoconstriction. Each value represents mean $\pm$ SEM.

LTC₄-induced bronchoconstriction

Normal guinea pigs were prepared as described above. 0.033, 0.1, 0.33, 1.0 μ g/ml LTC₄ were given by aerosol and Pao were observed.

The normal guinea pigs were given 30 s inhalation of 0.033 % WP871 before LTC₄ provocation. Control animals were given saline in the same manner.

Statistical analysis. Statistical differences were determined by Mann-Whitney's U test, with a *P* value of 0.05 or less as significant.

Chemicals. The following chemicals were used: ovalbumin (Sigma, St. Louis, USA); diphenhydramine hydrochloride (Sigma, St. Louis, USA); Freund's complete adjuvant (Difco Laboratories, Detroit, MI, USA); sodium pentobarbital (Abbot Laboratories, North Chicago, IL, USA); WP871 (Wakamoto Pharmaceutical Co. Ltd., Kanazawa, Japan); leukotriene C₄ (Ono Pharmaceutical Co., Ltd., Osaka, Japan).

RESULTS

Aerosol antigen challenge produced gradual and long-lasting increase in Pao. Maximal increase in this index was observed from 6 to 10

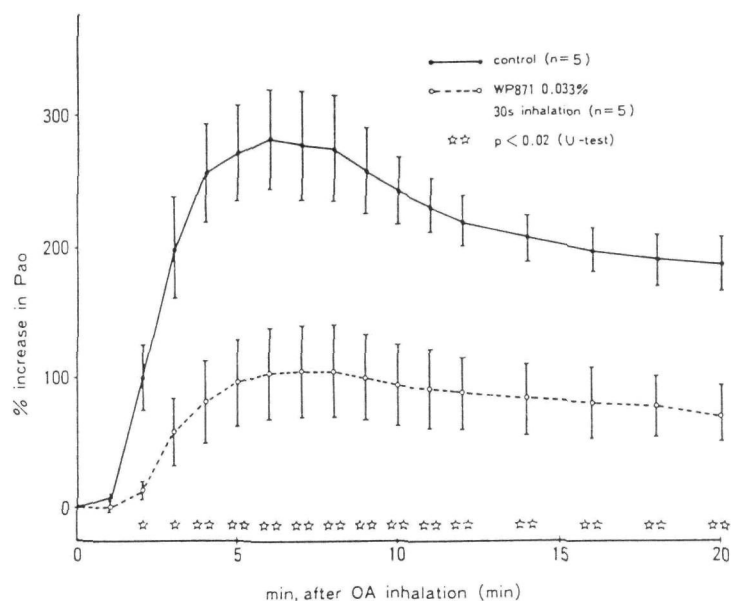


Fig. 2. Inhibitory effect of 30 s inhalation of 0.033% WP871 on allergic bronchoconstriction. Each value represents mean $\pm$ SEM.

min after the challenge. The increase in Pao induced by antigen challenge was significantly inhibited by WP871 in a dose-dependent fashion (Figs 1, 2). Pretreatment with 0.01% WP871 significantly inhibited the increase in Pao at 5 to 20 min after the challenge (Fig. 1). The mean inhibitory rate was 31.7%. 0.033% WP871 significantly inhibited the Pao increase throughout the observation period (Fig. 2). The mean inhibitory rate was 63.5%.

However, high-dose (0.1%) WP871 inhalation showed no effect on antigen-induced bronchoconstriction (Fig. 3). So we tried the next experiment. High-dose (0.1%) WP871 significantly enhanced the non-specific histamine-induced bronchoconstriction (Fig. 4).

On the other hand, inhaled LTC₄ produced an increase in Pao in a dose-dependent fashion. Pretreatment of the animals with 0.033% WP871 inhalation did not affect the increase in Pao by aerosol LTC₄ at all (Fig. 5).

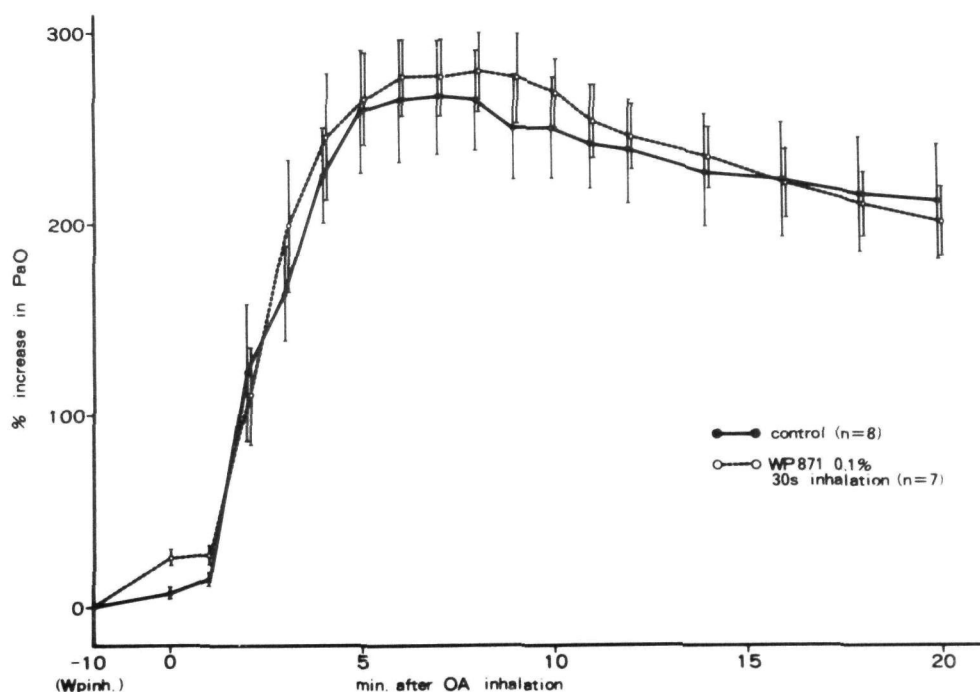


Fig. 3. Inhibitory effect of 30 s inhalation of 0.1% WP871 on allergic bronchoconstriction. Each value represents mean $\pm$ SEM.

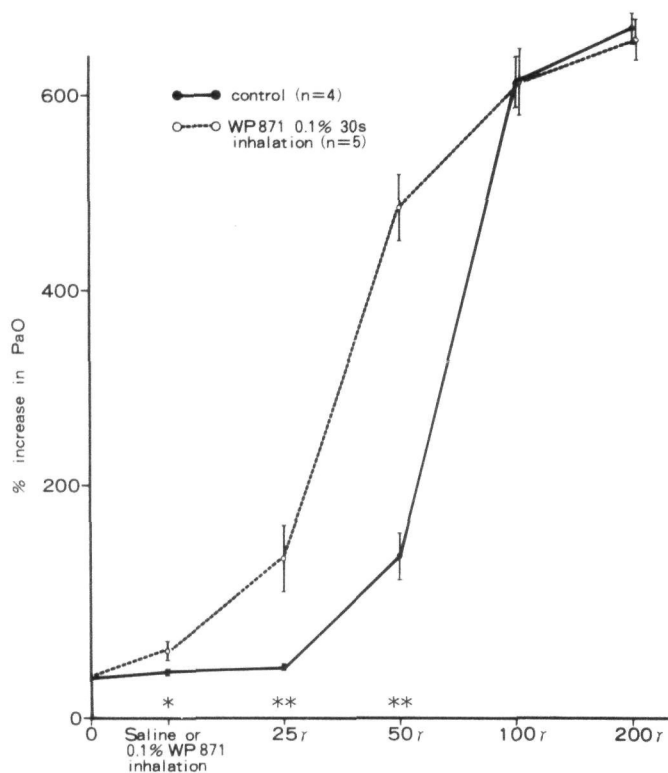


Fig. 4. Influence of 30 s inhalation of 0.1% WP871 on histamine-induced bronchoconstriction. Each value represents mean $\pm$ SEM.

DISCUSSION

Bronchial asthma is produced by a local allergic reaction with inhaled antigen. Anti-allergic drugs given in aerosol form accumulate in the bronchial wall in high concentration, and relatively few of them transmit to the whole body, thus being more effective for asthma and the avoidance of systemic side effects.

The guinea pigs used in this study were passively sensitized with homocytotropic antiserum and pretreated with a high dose of diphenhydramine hydrochloride. The hypersensitivity reaction occurred when animals were challenged with the aerosol antigen. In our previous study (3), this type of allergic bronchoconstriction was inhibited for the most part by FPL55712, a selective inhibitor of SRS-A (6). These findings indicate that the allergic bronchoconstriction in this study is mediated mainly by SRS-A.

WP871 is a newly synthesized anti-allergic drug and a major metabolic product of WP833 (6). The effect of WP833 has already been established (6, 7) and, in a previous study (8),

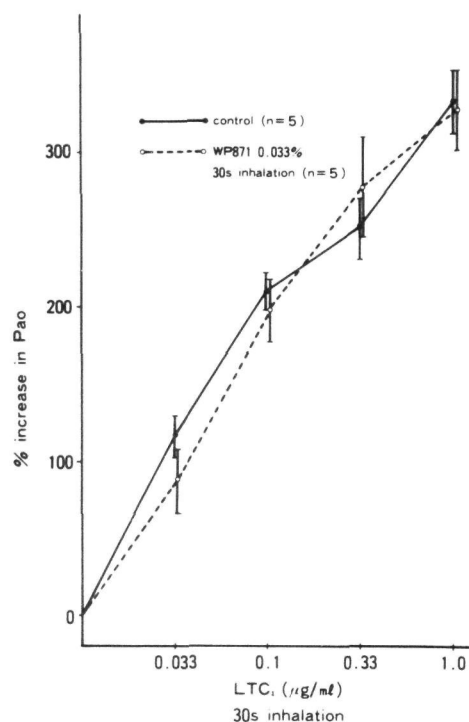


Fig. 5. Influence of 30 s inhalation of 0.033% WP871 on LTC₄-induced bronchoconstriction. Each value represents mean $\pm$ SEM.

the anti-allergic action of WP833 was suggested responsible for the inhibition of chemical mediator-release from mast cells. Thus, the effect of WP871 is thought to be same as WP833 and DSCG, but little has been done to elucidate the mechanisms of the anti-allergic action of this drug. The results of this study revealed that pretreatment with WP871 apparently inhibited aerosol antigen-induced bronchoconstriction which was mediated mainly by SRS-A. In contrast, bronchoconstriction caused by inhalation of LTC₄ was not inhibited by the pretreatment. These facts indicate that the anti-allergic action of WP871 is partly due to inhibition of synthesis and/or release of SRS-A.

Finally, WP871 is water soluble and is most suitable for clinical use. However, as high-dose (0.1%) WP871 enhanced the non-specific histamine-induced bronchoconstriction, it is important to use adequate dosage in bronchial asthma.

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