

Effects of aerosol administration of a thromboxane receptor antagonist (S-1452) on experimental asthma in guinea pigs

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The importance of thromboxane A_2 (TXA₂), one of the arachidonate metabolites, in the pathogenesis of bronchial asthma has been emphasized recently. Because aerosolized administration of antiasthmatic drugs is effective and safe, this study examined the effect of aerosolized TXA₂ receptor antagonist (S-1452) on allergic bronchoconstriction in passively sensitized and mechanically ventilated guinea pigs. Under the cover of antihistamine, antigen-induced bronchoconstriction was markedly inhibited by pretreatment with aerosolized S-1452 inhalation in a dose-dependent manner. Although aerosolized S-1452 itself provoked weak bronchoconstriction for its partial agonist effect, bronchial responsiveness to inhaled histamine did not change 10 min after S-1452 inhalation. These results indicate that aerosolized S-1452 may be useful in treating bronchial asthma.

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Many kinds of chemical mediators are implicated in the pathogenesis of bronchial asthma. Among them, thromboxane A_2 (TXA₂), one of the arachidonate metabolites and a potent bronchoconstrictor itself (18), is thought to play a major role in bronchial asthma. We have been paying attention to the role of TXA₂ in asthma because 1) bronchial hyperresponsiveness in asthmatic patients is reduced by a thromboxane synthetase inhibitor (OKY-046) (5, 8) or a thromboxane receptor antagonist (AA-2414) (7), 2) OKY-046 inhibits the antigen-induced bronchoconstriction in both guinea pigs (4) and man (10), 3) OKY-046 improves symptoms in asthmatic patients (15), and 4) aerosolized administration of subthreshold concentration of a TXA₂ analog (STA₂) promotes bronchial hyperresponsiveness in guinea pigs (6). S-1452 is one of the new and most potent TXA₂ antagonists (9). We investigated the effects of aerosol administration of S-1452 on allergic bronchoconstriction in passively sensitized guinea pigs.

Material and methods

Animals

Male albino Hartley strain guinea pigs weighing 350–400 g were used in this study.

Passive sensitization of guinea pigs

Guinea pig homocytotropic antiserum was made according to the method of Santives et al. (17). Briefly, 500 µg ovalbumin (OA) was dissolved in 0.625 ml of normal saline, emulsified in the same amount of Freund's complete adjuvant, and injected intradermally (i.d.) into each guinea pig at multiple sites. Boosting was done in the same manner 2 weeks later. Serum was collected from each animal 2 weeks after the booster, pooled, and kept frozen until use. The antibody titers of this serum were 1:12 800, 1:6400, and 1:512, as estimated by passive cutaneous anaphylaxis (PCA) at 4 h, 24 h, and 7 d, respectively. Normal guinea pigs were passively sensitized with 1.0 ml antiserum/kg intraperitoneally.

Preparation of animals

Between 24 and 48 h after passive sensitization, the animals were anesthetized intraperitoneally by injection of 75 mg/kg 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, South Natick, MA, USA), adjusted to a tidal volume of 10 ml/kg at a rate of 60 strokes/min. The changes in lung resistance to inflation and the lateral pressure of the tracheal tube (pressure at the airway opening (P_{ao}) (cmH₂O) were measured by the modified method of Konzett & Roessler (13) described by Jones et al. (11), with a pressure transducer (Model TP-603T, Nihon Koden Kogyo Co, Tokyo, Japan). Since the change in P_{ao} after inhalation of leukotriene C₄ (LTC₄) represented the average of the changes in pulmonary resistance (R_L) and reciprocal dynamic lung compliance ($1/C_{dyn}$) (3), we have used P_{ao} as an overall index of bronchial response to bronchoactive agents. Immediately after the cannulation was finished, the animals were overinflated by twice the tidal volume for two breaths by cramping the outlet port of the respirator to unify the volume history of the lung.

Effect of inhaled S-1452 on antigen-induced bronchoconstriction

The animals were sensitized passively, as described above, 24–48 h before experiments. On the experimental day, after surgery, the animals were given diphenhydramine hydrochloride (60 mg/kg i.p.) to block the action of histamine, and then 0.9% w/v saline, or each concentration of S-1452 dissolved in 0.9% w/v saline was inhaled for 30 s, and 10 min later the animals were challenged with 0.1% ovalbumin solution. Antigen inhalation was performed for 30 s with an ultrasonic nebulizer developed for small animals at our institution (14). The amount of aerosol was 15.2 µl/min, and 46.4% of the aerosol was deposited in the lung, as measured by the radioaerosol method (14). The concentrations of inhaled S-1452 were 0.01, 0.033, and 0.1 mg/ml.

Effect of S-1452 on bronchial responsiveness to inhaled histamine

Nonsensitized guinea pigs were used in this experiment. After surgery, 0.9% w/v saline or each concentration of S-1452 (0.01, 0.033 mg/ml) was inhaled for 30 s. Ten minutes later, ascending doses of histamine (25, 50, 100, 200 µg/ml) were inhaled for 20 s at 5-min intervals.

Statistical analysis

Statistical differences were determined by the Mann-Whitney U-test; a P value of 0.05 or less was considered to be significant.

Chemicals

The following chemicals were used: sodium pentobarbital (Abbott Laboratories, North Chicago, IL, USA); histamine (Wako Pure Chemical, Osaka, Japan); S-1452 (calcium 5(z)-1R, 2S, 3S, 4S-7-[3-phenylsulfonylaminobicyclo {2.2.1} hept-2-yl]5-heptenoate hydrate) (Shionogi Pharmaceutical, Osaka, Japan); ovalbumin (Sigma Chemical Co, St Louis, MO, USA); diphenhydramine hydrochloride (Sigma); Freund's complete adjuvant (Difco Laboratories, Detroit, MI, USA).

Results

Airway response to inhaled S-1452 (Fig. 1)

Inhaled S-1452 induced bronchoconstriction in a dose-dependent manner. Percent increases in P_{ao} were 2.0 ± 0.6 , 7.0 ± 1.6 , 15.6 ± 2.7 , and $17.8 \pm 2.8\%$ after inhalation of 0.9% w/v saline and each concentration (0.01, 0.033, and 0.1 mg/ml) of S-1452, respectively. There were significant differences between the control group and each S-1452-inhaled group.

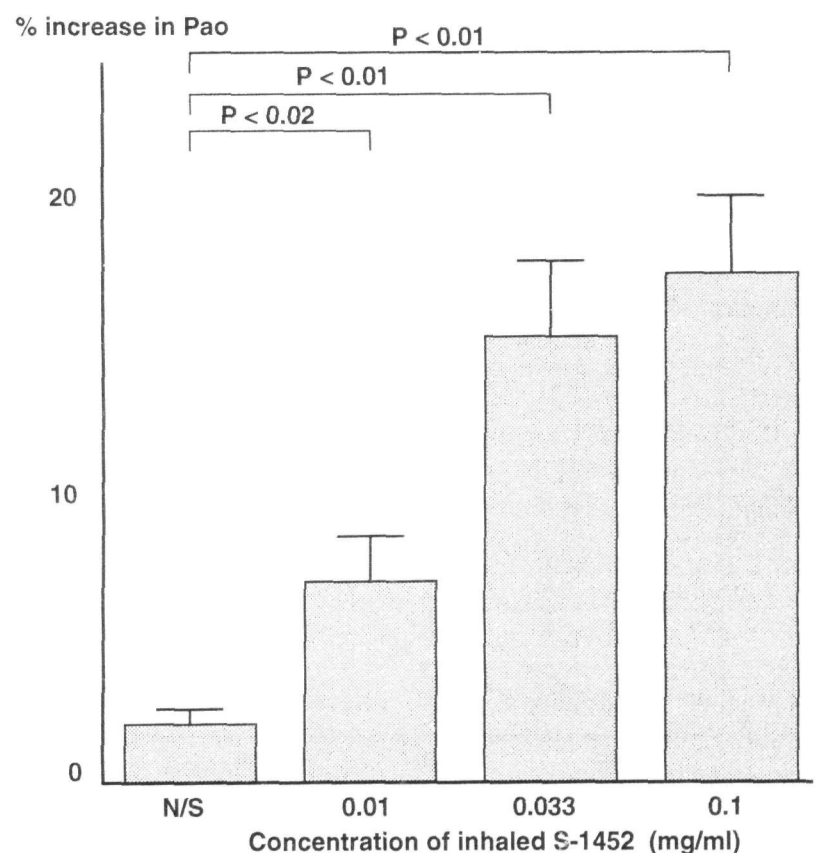


Fig. 1. Airway response to inhaled S-1452. S-1452 inhalation provoked bronchoconstriction because of its partial agonist effect. Degree of bronchoconstriction measured as P_{ao} (pressure at the airway opening) increased significantly in dose-dependent manner. N/S = normal saline. $n = 8$ in each group.

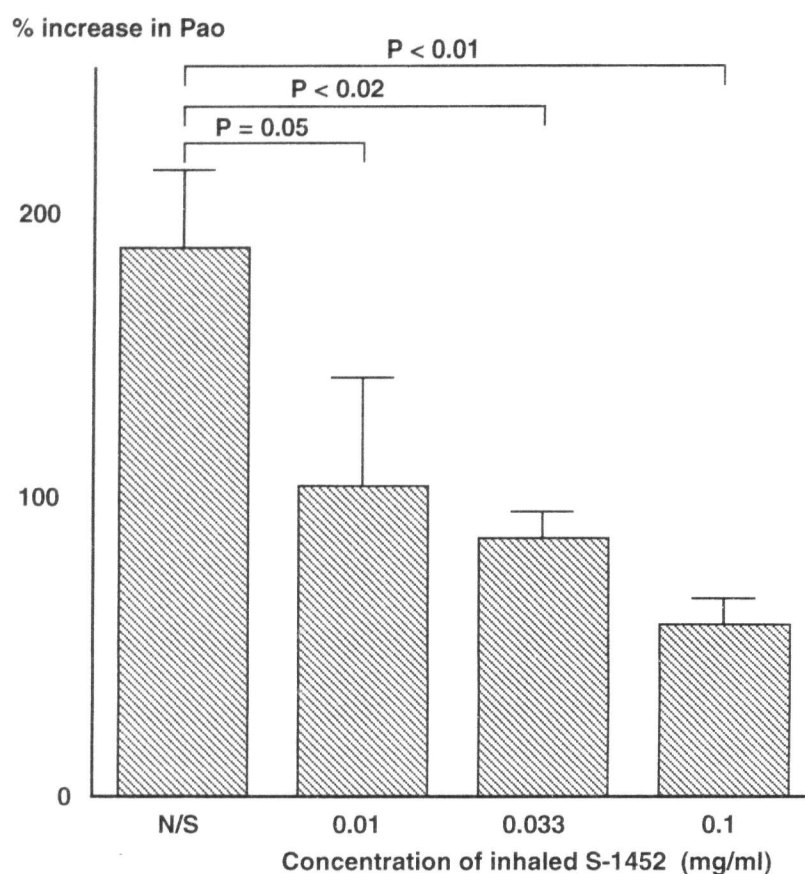


Fig. 2. Airway response to inhaled antigen. Animals passively sensitized with intraperitoneal injection of anti-OA serum showed immediate asthmatic response when aerosolized OA was challenged. Inhaled S-1452 showed significant inhibitory effect on bronchoconstriction in dose-dependent manner. N/S = normal saline. $n = 8$ in each group.

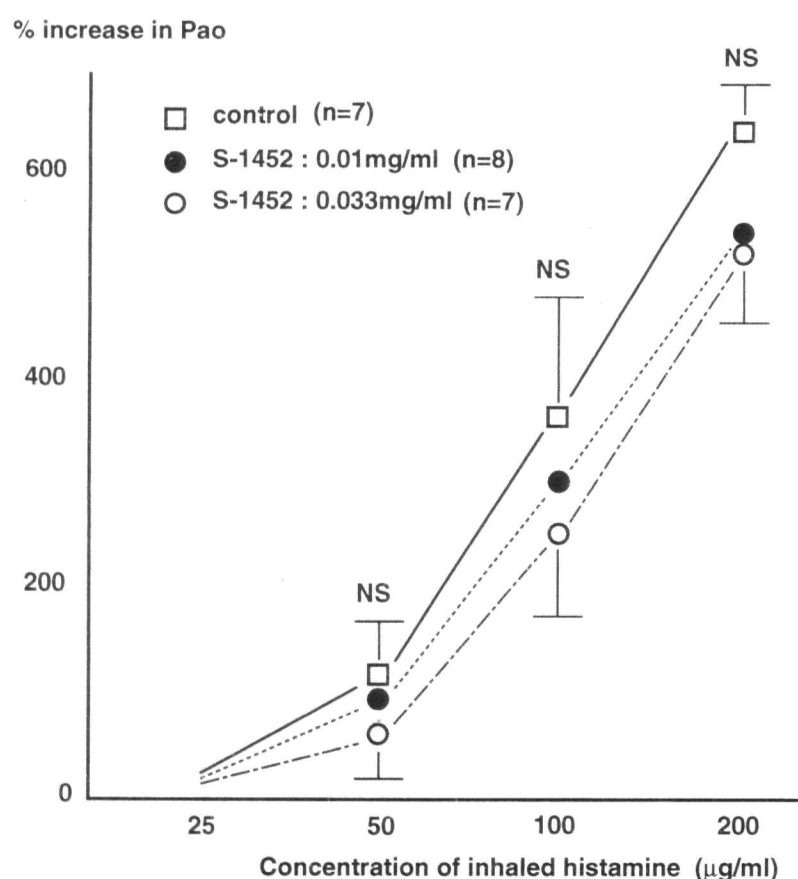


Fig. 3. Airway response to inhaled histamine after S-1452 inhalation. Although aerosolized S-1452 itself caused bronchoconstriction, airway responsiveness to inhaled histamine did not change 10 min after S-1452-induced bronchoconstriction. $n = 7$ in control and 0.033 mg/ml of S-1452 groups, and $n = 8$ in 0.01 mg/ml of S-1452 group.

Effect of S-1452 on antigen-induced bronchoconstriction (Fig. 2)

Pretreatment with aerosolized S-1452 inhibited antigen-induced bronchoconstriction in a dose-dependent manner. Percent increases in Pao were 189.5 ± 26.5 , 107.4 ± 37.1 , 88.9 ± 9.2 , and $58.8 \pm 9.5\%$ in controls and each concentration (0.01, 0.033, and 0.1 mg/ml) of the S-1452-inhaled group, respectively. The value was significantly different between each concentration of the S-1452-administered group and the control group.

Effect of S-1452 on histamine-induced bronchoconstriction (Fig. 3)

Pretreatment with S-1452 (0.01 and 0.033 mg/ml) did not alter percent increase in Pao induced by inhalation of each concentration of histamine in nonsensitized, normal guinea pigs.

Discussion

Many physical or chemical factors are implicated in the pathogenesis of bronchial asthma. Among them, arachidonate metabolites, such as prostaglandins or leukotrienes, are thought to be essential. TXA_2 is one of the arachidonate metabolites and has been reported to be a potent bronchoconstrictor (18). It seems to play a role in bronchial asthma, because TXA_2 is reported to be produced in the allergen-induced immediate and late asthmatic responses (10). We previously reported that a TXA_2 synthetase inhibitor (OKY-046) and a receptor antagonist (AA-2414) of TXA_2 reduced bronchial hyperresponsiveness in bronchial asthma (5, 7, 8). On the other hand, Iwamoto et al. have shown that OKY-046 inhibited both immediate and late asthmatic responses with decreased plasma TXB_2 levels after allergen challenge in asthmatic subjects (10). Therefore, TXA_2 is thought to be one of the major chemical mediators in the pathogenesis of bronchial hyperresponsiveness and bronchial asthma.

S-1452 is a highly potent and selective antagonist for TXA_2 receptors (9). This agent has been shown to antagonize the bindings of 3H-U-46619 in rat washed platelets with stereospecificity and high potency (K_i : 2.5 nM), but it does not inhibit the binding of 3H-PGE₁, 3H-PGD₂, and 3H-PGF_{2 α} to rat platelet membranes (9). It has also been reported that S-1452 (100 nM) completely suppresses both U-46619-induced and collagen-induced shape change and aggregation of rat platelets (9). Contractions of the trachea and lung parenchyma of the guinea pig induced by U-46619 were concentration-dependently inhibited by S-1452, but those induced by histamine and leukotriene D₄ were not affected

(16). In addition, S-1452 had no discernible effect on TXA₂ synthetase or cyclooxygenase of platelet microsomes, or on 5-lipoxygenase up to 1 μ M concentrations (19). Thus, we investigated the effect of this new chemical in our experimental model for asthma. Some experimental studies have shown the effectiveness of S-1452 as an antiasthmatic drug, but they were *in vitro* experiments or *in vivo* studies in which S-1452 was administered systemically (1, 19). We administered the agent by aerosol inhalation because it is well known that aerosolized administration of antiasthmatic drugs is more effective and less dangerous than systemic administration. Accordingly, this method must be more valuable to confirm the effectiveness of aerosolized S-1452.

Our first experiment shows that aerosolized S-1452 can inhibit antigen-induced bronchoconstriction (immediate-type asthmatic reaction), and that it can be a potent antiasthmatic drug when administered locally. But, at the same time, inhaled S-1452 itself causes weak bronchoconstriction by its partial agonist effect. It is possible that at the time of bronchoconstriction by aerosolized S-1452, some chemical mediators are released secondarily and consequently promote bronchial hyperresponsiveness, because we have shown that the bronchoconstrictive property of TXA₂ is partially due to secondary activation of thromboxane synthetase (6), and that subthreshold concentration of the TXA₂ analog, STA₂, heightens bronchial responsiveness to histamine when histamine is inhaled 90 s after inhalation of STA₂ (6). However, inhaled S-1452 did not alter bronchial responsiveness to histamine at each concentration in this study. In the previous experiment, we observed that bronchial responsiveness to histamine was not increased when histamine was inhaled 5–10 min after STA₂ inhalation (unpublished data). In a human study, Jones et al. reported that bronchial responsiveness to methacholine was heightened 1 min after inhalation of the TXA₂ mimetic U-46619 in the subthreshold concentration, but it did not change 1 h after U-46619 inhalation in asthmatic subjects (12). Therefore, the effect of mediators that might be released by the partial agonist effect of S-1452 is thought to have disappeared 10 min after its inhalation and may be negligible, as compared with its antiasthmatic effect.

These results suggest that aerosolized S-1452 administration can be useful in treatment of bronchial asthma.

Summary

Inhaled S-1452 inhibited antigen-induced bronchoconstriction in a dose-dependent manner in passively sensitized guinea pigs pretreated with diphenhydramine, but it did not alter bronchial responsive-

ness to histamine in nonsensitized guinea pigs. This means that S-1452 inhalation is a useful treatment of bronchial asthma.

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