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Effect of thromboxane A_2 antagonists on bronchial hyperresponsiveness induced immediately after interleukin-8 inhalation in guinea-pigs

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1 Although repeated intranasal administration of interleukin-8 (IL-8) causes bronchial hyperresponsiveness (BHR) mediated via thromboxane A_2 (TXA_2) and airway neutrophil accumulation in guinea-pigs, the acute effect of inhaled IL-8 is unclear. We performed this study to clarify the acute effect of IL-8 on bronchial responsiveness and the role of TXA_2.

2 The effects of inhaled IL-8 on bronchial responsiveness and of the TXA_2 antagonists, S-1452 (0.01 and 0.1 mg kg^{-1}) and ONO-NT-126 (1.0 or 10 μg kg^{-1}), on IL-8-induced BHR were examined by use of a modified Konzett-Rössler method in guinea-pigs.

3 Inhaled IL-8 at 100 ng ml^{-1}, which failed to induce significant changes in Pao (pressure at the airway opening), enhanced an increase in Pao induced by subsequent inhalations of ascending doses (50–200 μg ml^{-1}) of methacholine and histamine, suggesting the potentiating effect of IL-8 on bronchial responsiveness. No significant leukocyte infiltration was observed histologically sixteen minutes after the IL-8 inhalation. Both S-1452 and ONO-NT-126 reduced the IL-8-induced BHR.

4 In conclusion, IL-8 rapidly causes BHR via TXA_2 release in guinea-pigs.

Keywords: Interleukin-8; bronchial responsiveness; thromboxane A_2

Introduction

Various cytokines are presumed to be involved in the signalling between cells and to contribute to the pathophysiology of bronchial asthma (Kelly, 1990; Robinson *et al*., 1993). A novel chemotactic and activating cytokine for neutrophils, interleukin-8 (IL-8), purified initially from lipopolysaccharide (LPS)-stimulated human peripheral blood mononuclear cell culture supernatants (Matsushima *et al*., 1988), can be produced by a number of cells present in lung tissue, such as bronchial epithelial cells (Marini *et al*., 1992), macrophages (Sylvester *et al*., 1990), mast cells (Möller *et al*., 1993), endothelial cells (Strieter *et al*., 1989) and fibroblasts (Rolfe *et al*., 1991). Several lines of evidence indicate that IL-8 may also act as a potent eosinophil chemoattractant (Smith *et al*., 1991; Collins *et al*., 1993; Sehmi *et al*., 1993; Lagente *et al*., 1995). Several studies have shown a correlation between eosinophil (Kirby *et al*., 1987) or neutrophil (Kelly *et al*., 1988) accumulation in the airway and the degree of bronchial hyperresponsiveness (BHR).

It has been demonstrated that the concentration of IL-8 in nasal lavage fluid (Noah *et al*., 1995) or sputum (Kurashima *et al*., 1996) and its production in bronchial epithelium (Marini *et al*., 1992) are increased in asthmatic subjects compared with non asthmatics. In addition, development of BHR and eosinophil accumulation, both of which are main features of bronchial asthma, have been observed several hours after exposure to IL-8 in guinea-pigs (Smith *et al*., 1991). We (Xiu *et al*., 1995) also demonstrated that BHR was induced by intranasal administration of 5 μg kg^{-1} day^{-1} of IL-8 twice a week for 3 weeks accompanied by an increased number of neutrophils both in BALF and bronchial tissues in guinea-pigs. Medhurst *et al*. (1991) showed that BHR is induced 24 and 48 h after an intraperitoneal injection of IL-8, 2 μg kg^{-1}, in guinea-pig perfused lungs. As these two studies did not examine the airway responsiveness immediately after the administration of high doses of IL-8, there is a possibility that inhaled IL-8 rapidly enhances the bronchial responsiveness. Indeed, Burrows *et al*. (1991) demonstrated that IL-8 is a spasmogen for airway smooth muscle *in vitro*. Therefore, this study was conducted to clarify the acute effect of IL-8 on bronchial responsiveness. We examined bronchial responsiveness to inhaled methacholine and histological findings after IL-8 inhalation. In addition, we also assessed the effect of specific thromboxane receptor antagonists, S-1452 and ONO-NT-126, on the development of BHR in this animal model, since it has been shown that IL-8 can induce the release of several chemical mediators (Dahinden *et al*., 1989; White *et al*., 1989; Krieger *et al*., 1992), and that thromboxane A_2 (TXA_2) is important in the development of BHR induced by repeated administration of IL-8 (Xiu *et al*., 1995).

Installation of artificial respiration

Male, albino, Hartley strain guinea-pigs weighing 300 to 350 g were obtained from Sankyou Laboratory Service (Toyama, Japan). They were quarantined in the Animal Research Centre of Kanazawa University for 1 week before the study. All the animal procedures in this study complied with the standards set out in the Guideline for the Care and Use of Laboratory Animals in Takara-machi Campus of Kanazawa University.

Guinea-pigs were anaesthetized by an intraperitoneal injection of 75 mg kg^{-1} sodium pentobarbitone and were placed in a supine position. After the trachea had been cannulated with a polyethylene tube (outside diameter, 2.5 mm; inside diameter, 2.1 mm) and the left jugular vein cannulated for the administration of drugs, the animals were artificially ventilated by use of a small animal respirator (Model 1680, Harvard Apparatus Co., Inc., South Natick, MA) adjusted to a tidal volume of 10 ml kg^{-1} at a rate of 60 strokes min^{-1}. The changes in lung resistance to inflation, the lateral pressure of

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the tracheal tube (pressure at the airway opening; Pao, cmH_2O), were measured with a pressure transducer (TP-603T, Nihon Koden Kogyo Co., Ltd., Tokyo, Japan) according to the modified method of Konzett and Roessler (1940) described by Jones *et al*. (1982). Since we confirmed that the change in Pao following inhalation of leukotriene C_4 represented the average of the changes in pulmonary resistance (R_L) and reciprocal dynamic lung compliance ($1/C_{dyn}$) (Fujimura, 1983), Pao was used as an overall index of bronchial response to bronchoactive agents (Songur *et al*., 1994; Mizuguchi *et al*., 1996). After completion of all surgical procedures, the animals were overinflated by twice the tidal volume for two breaths by closing the outlet port of the respirator to unify the volume history of the lung.

Experimental protocols

Study 1 — IL-8-induced BHR to methacholine and effects of terfenadine The guinea-pigs were divided arbitrarily into 3 groups receiving either oral vehicle and inhaled phosphate buffered saline (PBS) (negative control (NC) group), vehicle and inhaled IL-8 (positive control (PC) group) or a potent histamine H_1-receptor antagonist terfenadine and IL-8 (terfenadine (T) group) ($n=10$ in each group).

Terfenadine at a dose of 2.0 mg kg^{-1} or vehicle was administered orally 1 h before the anaesthesia. Fifteen minutes after installation of artificial respiration when the Pao had stabilized, either PBS (vehicle for IL-8) or 100 ng ml^{-1} IL-8 was given intrabronchially for 20 s under continuous ventilation with an ultrasonic nebulizer. The nebulizer generated the aerosol at a rate of 15.2 μl min^{-1} during the 20 s period and 46.4% of the generated aerosol was deposited in the lung, as measured by the radioaerosol technique (Minami *et al*., 1982). To evaluate the bronchial responsiveness, sixteen seconds after the termination of IL-8 or PBS treatment, ascending concentrations of methacholine (50, 100, and 200 μg ml^{-1}) were given for 20 s at intervals of 5 min with an ultrasonic nebulizer.

Study 2 — effects of TXA_2 antagonists on IL-8-induced BHR to methacholine Fifteen minutes after the intravenous administration of S-1452 (0.01 or 0.1 mg kg^{-1}, dissolved in saline), ONO-NT-126 (1.0 or 10 μg kg^{-1}, dissolved with 0.5 ml of ethanol and 0.5 ml of Tween 80 and then diluted with saline), or appropriate vehicle ($n=10$ in each group), guinea-pigs received IL-8 and subsequently methacholine by inhalation over the same time schedule as the experiments described in Study 1.

Study 3 — IL-8-induced BHR to histamine and effects of S-1452 The guinea-pigs were divided arbitrarily into 4 groups ($n=10$ in each group) receiving either vehicle for S-1452 and inhaled PBS (negative control (NC) group), vehicle for S-1452 and inhaled IL-8 (positive control (PC) group), S-1452 at a dose of 0.01 mg kg^{-1} (S 0.01 group) and S-1452 at a dose of 0.1 mg kg^{-1} (S 0.1 group). Fifteen minutes after drug treatment, guinea-pigs received IL-8 and subsequently histamine by inhalation over the same time schedule as the experiments described in Study 1.

Study 4 — effects of TXA_2 antagonists on methacholine- or histamine-induced bronchoconstriction Fifteen minutes after the intravenous administration of S-1452 (0.1 mg kg^{-1}), ONO-NT-126 (10 μg kg^{-1}) or each vehicle ($n=7$ in each group), guinea-pigs received methacholine by inhalation over the same time schedule as the experiments described in Study 1. In addition, we examined the effect of S-1452 on histamine-induced bronchoconstriction by the same methods ($n=7$ in each group).

Histological examination

After Study 1, the heart and lungs were removed and inflated with 10% formalin solution. Tissues were taken from adjacent sites at three places along the axial airway, running from the right main bronchus to distal right lower lobe: the first block containing extrapulmonary bronchus at the hilus and the second intrapulmonary bronchus (150 to 300 μm in a diameter). Tissues were stained with haematoxylin-eosin stain.

Quantitative light microscopic techniques were used to identify the location and severity of inflammatory cell infiltration. All slides were examined in a 'blind' fashion. Initially, the number of inflammatory cells in the epithelium or the subepithelium (the layer of connective tissue immediately beneath the epithelium and superficial to a dense layer of elastin) were counted at each location. We averaged the numbers of inflammatory cells infiltrated in 150 μm length at 3 different sites of each airway. For estimating the damage of bronchial epithelium, we used the 'injury score'; the total of 10 locations' points. Each point was defined according to the degree of the desquamation in a 150 μm length of airway epithelium; 0 point for intact airways epithelium, 1 point for less than 50% desquamation, 2 points for greater than 50% desquamation, and 3 points for complete desquamation.

Chemicals

The following chemicals were used: sodium pentobarbitone (Abbot Laboratories, North Chicago, U.S.A.), methacholine (Wako Pure Chemical Ind., Osaka, Japan), ONO-NT-126 (5(Z)-6-[(1**R**,2**R**,3**R**,4**S**)-3-(N-4-bromobenzensulphonylaminomethyl)bicyclo [2,2,1] heptane-2-yl]hex-5-enoic acid) (Ono Pharmaceutical Co., Ltd., Osaka, Japan), and S-1452 (calcium 5(z)-1**R**, 2**S**, 3**S**, 4**S**-7-[3-phenylsylphonyl-aminobicyclo [2.2.1] hept-2-yl]-5-heptenoate hydrate) (Shionogi Pharmaceutical Ind., Osaka, Japan). Human recombinant IL-8 was a kind gift of Dainippon Pharmaceutical Co. (Osaka, Japan).

Statistical analysis

Bronchoconstriction was determined as percentage increase in Pao compared with the value before the first inhalation of PBS or IL-8. Statistical differences were determined by unpaired Student's t test. Significance was based on a 95% confidence level ($P<0.05$) and all measurements are expressed as mean $\pm$ s.e.mean.

Results

There were no significant differences in baseline Pao. While IL-8 at 100 ng ml^{-1} caused no significant percentage changes in Pao, subsequent inhalation of methacholine induced a significantly greater increase in Pao in animals pretreated with IL-8 compared with those with PBS (Figure 1), indicating the potentiating effect of IL-8 on bronchial responsiveness. Terfenadine failed to reduce the IL-8-induced BHR. Although S-1452 and ONO-NT-126 did not alter the baseline Pao or the increase in Pao induced by methacholine in the animals without IL-8 treatment (Figures 2 and 3), both compounds significantly lessened the increase in Pao induced by methacholine in the IL-8-treated animals (Figures 4 and 5), indicating that TXA_2 is involved in IL-8-induced BHR. No change in neutrophil or eosinophil number was observed in bronchial tissues, nor was there any evidence of epithelial damage in animals given IL-8 by inhalation (Table 1).

S-1452 had no effect on histamine-induced bronchoconstriction (Figure 6). However, the IL-8-induced BHR was significantly attenuated by S-1452 in a dose-dependent manner (Figure 7).

Discussion

In this study, we investigated that acute effect of inhaled IL-8 on bronchial responsiveness and a role of TXA_2 in the IL-8-induced BHR in guinea-pigs. The results of the present study

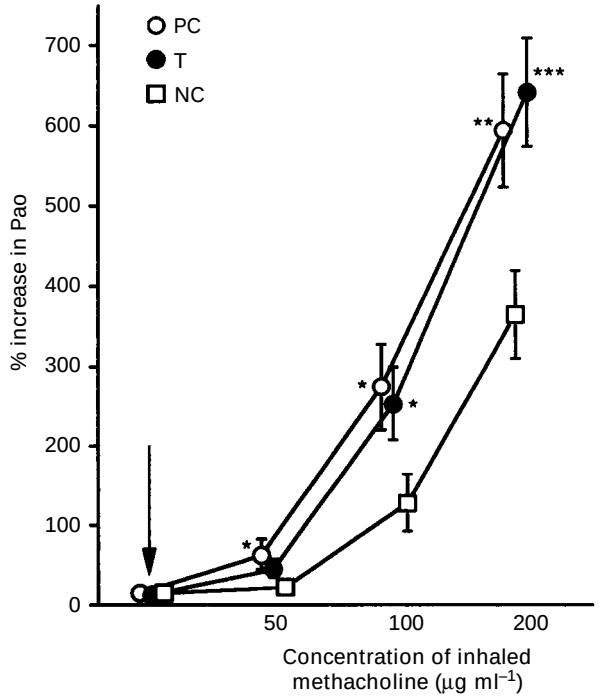

Figure 1 Effects of interleukin-8 (IL-8, 100 ng ml^{-1}) and terfenadine (T) (given at arrow) on bronchial responsiveness (pressure at airway opening; Pao) to methacholine. Each point represents the mean and vertical lines show s.e.mean ($n=10$ in each group). Statistical significance is indicated as follows; $^{*}P<0.05$, $^{**}P<0.02$ and $^{***}P<0.01$ compared with the normal control (NC) group. PC-positive control, vehicle + inhaled IL-8 group; T-terfenadine + Il-8 group.

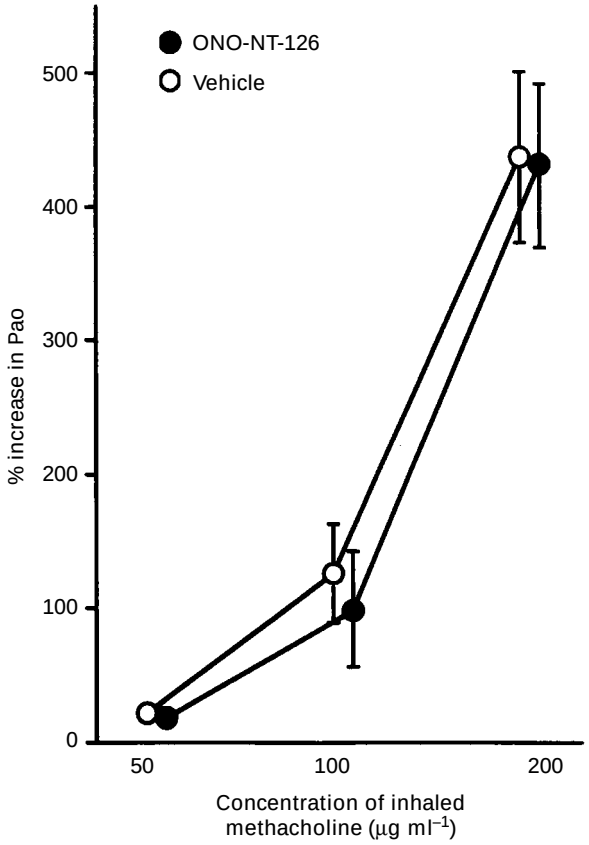

Figure 3 Effect of ONO-NT-126 (10 $\mu g\ kg^{-1}$) on methacholine-induced bronchoconstriction. Each point represents the mean and vertical lines show s.e.mean ($n=7$ in each group).

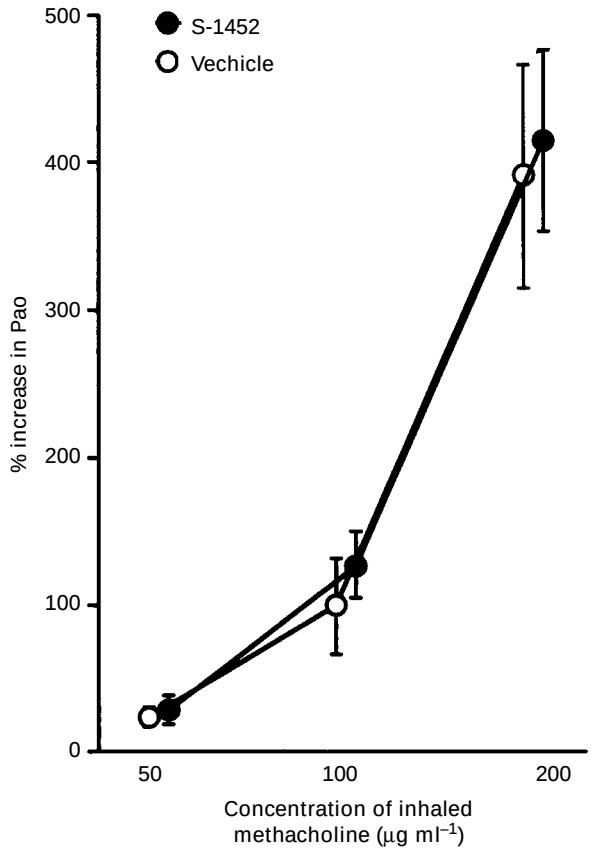

Figure 2 Effect of S-1452 (1 mg kg^{-1}) on methacholine-induced bronchoconstriction. Each point represents the mean and vertical lines show s.e.mean ($n=7$ in each group).

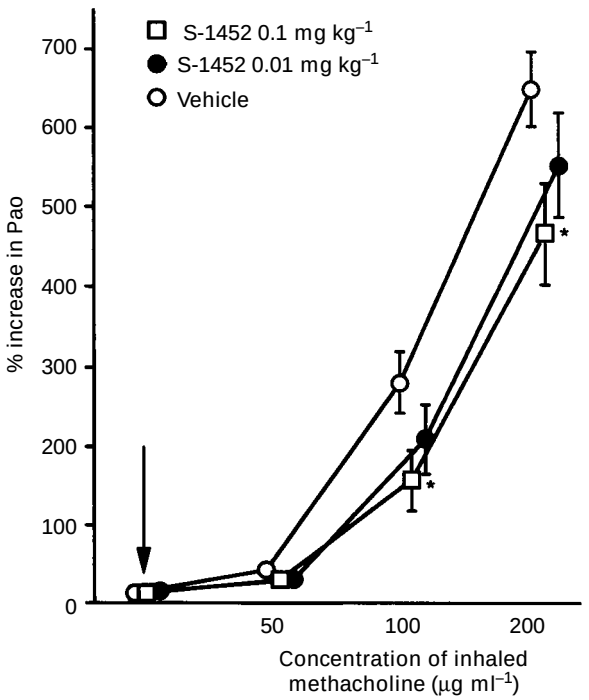

Figure 4 Effect of S-1452 on interleukin-8 (IL-8, 100 ng ml^{-1} given at arrow)-induced bronchial hyperresponsiveness. Each point represents the mean and vertical lines show s.e.mean ($n=10$ in each group). Statistical significance is indicated as follows: $^{*}P<0.05$ compared with the vehicle group.

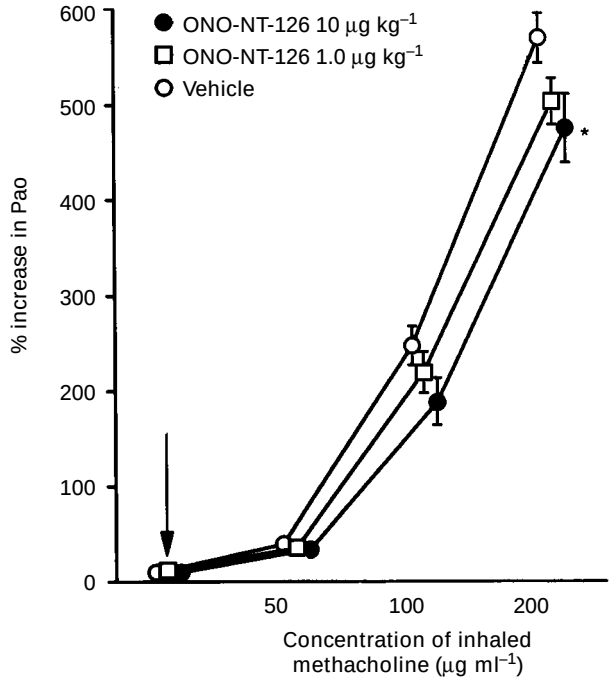

Figure 5 Effect of ONO-NT-126 on interleukin-8 (IL-8, 100 ng ml^{-1} given at arrow)-induced bronchial hyperresponsiveness. Each point represents the mean and vertical lines show s.e.mean ($n=10$ in each group). Statistical significance is indicated as follows: $*P<0.05$ compared with the vehicle group.

Table 1 Histological findings in guinea-pigs given an inhalation of interleukin-8 or PBS after studying the effect of terfenadine on interleukin-8-induced bronchial hyperresponsiveness

	PBS	*IL-8*
Neutrophil infiltration		
Extrapulmonary bronchus		
Epithelium (cells)#	0.00±0.00	0.00±0.00
Subepithelium (cells)	0.07±0.05	0.11±0.07
Intrapulmonary bronchus		
Epithelium (cells)	0.00±0.00	0.00±0.00
Subepithelium (cells)	0.14±0.07	0.11±0.05
Eosinophil infiltration		
Extrapulmonary bronchus		
Epithelium (cells)	0.04±0.04	0.07±0.07
Subepithelium (cells)	1.00±0.28	1.36±0.24
Intrapulmonary bronchus		
Epithelium (cells)	0.11±0.07	0.07±0.05
Subepithelium (cells)	1.54±0.31	1.29±0.29
Injury score		
Extrapulmonary bronchus	1.86±0.51	2.00±0.58
Intrapulmonary bronchus	1.57±0.69	1.71±0.57

Data shown are means±s.e.mean ($n=7$). #The average number of cells counted in 150 μm length at three different sites. No significant difference was observed between the two groups.

show that inhalation of IL-8 enhanced bronchial responsiveness to methacholine and histamine, and that specific TXA_2 antagonists, S-1452 and ONO-NT-126, inhibited this effect.

S-1452 is a highly potent and specific antagonist for TXA_2 receptors (Hanasaki & Arita, 1988). This agent has been shown to antagonize the binding of [^{3}H]-U46619 in washed rat platelets with stereospecificity and high potency (K_i: 2.5 nM), but to have no inhibitory effect on the binding of [^{3}H]-prostaglandin E_1 (PGE_1), [^{3}H]-PGD_2 and [^{3}H]-$PGF_{2\alpha}$ to rat platelet membranes (Hanasaki & Arita, 1988). It has also been shown that S-1452 (100 nM) completely suppresses both

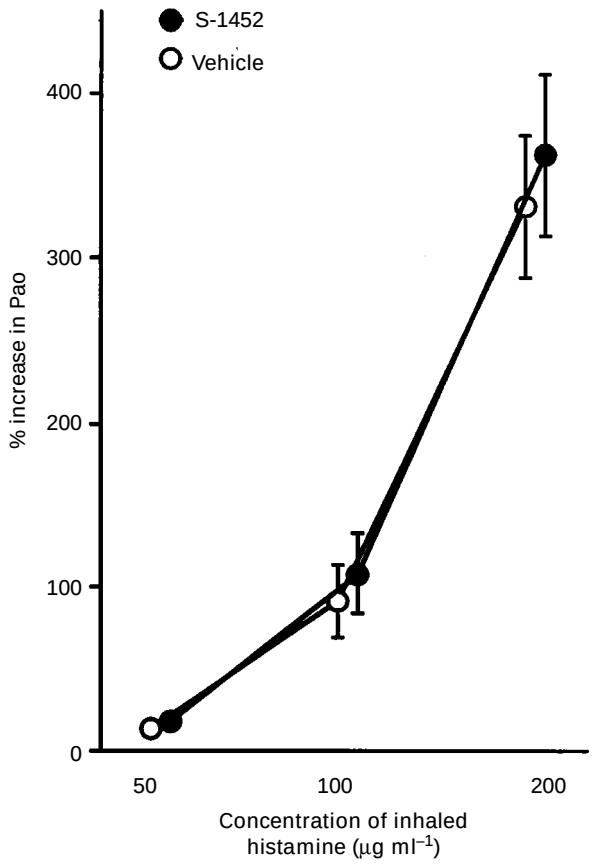

Figure 6 Effect of S-1452 (0.1 mg kg^{-1}) on histamine-induced bronchoconstriction. Each point represents the mean and vertical lines show s.e.mean ($n=7$ in each group).

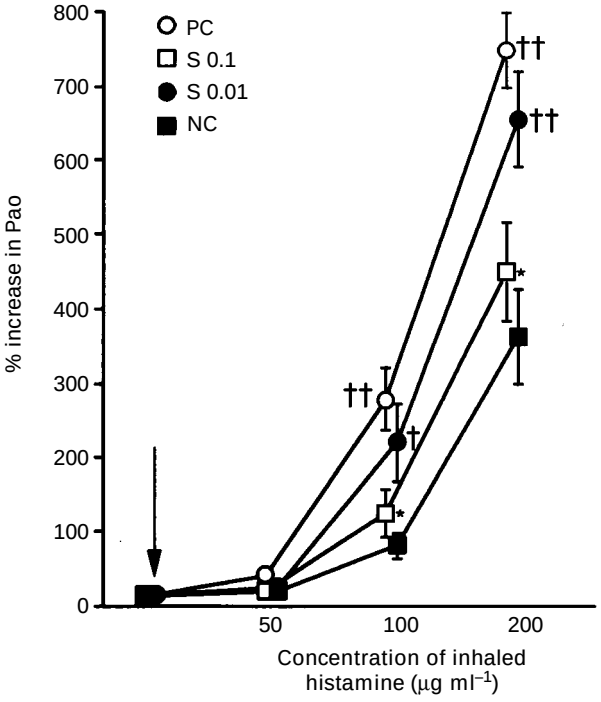

Figure 7 Effects of interleukin-8 (IL-8, 100 ng ml^{-1} given at arrow) and S-1452 (S, 0.1 or 0.01 mg kg^{-1}) on bronchial responsiveness to histamine. Each point represents the mean and vertical lines show s.e.mean ($n=10$ in each group). Statistical significance is indicated as follows: $*P<0.01$ compared with the PC group; $\dagger P<0.05$ and $\dagger\dagger P<0.01$ compared with the NC group.

U46619-induced shape change and collagen-induced shape change and aggregation of rat platelets (Hanasaki & Arita, 1988). With regard to ONO-NT-126, it has been shown that [^{3}H]-SQ29548 binding to membranes of human astrocytoma cells was inhibited by ONO-NT-126 and the other TXA_2 antagonists, with K_i values of 0.09, 2.08, 8.35 and 25.9 nM for ONO-NT-126, S-1452, SQ29548 and ONO3078, respectively (Nakahata *et al*., 1990). Since there was no antagonism by ONO-NT-126 or S-1452 of the direct effect of methacholine and histamine, TXA_2 is presumed to be involved in IL-8-induced BHR, but it is generated at a subthreshold concentration that has no direct bronchoconstrictor effect. Indeed, it has been shown in asthmatic subjects that inhalation of a subthreshold concentration of the thromboxane mimetic, U46619, causes airway hyperresponsiveness to methacholine (Jones *et al*., 1992), and that a selective TXA_2 synthetase inhibitor OKY-046 (Fujimura *et al*., 1986) and specific thromboxane receptor antagonists, AA-2414 (Fujimura *et al*., 1991), S-1452 and BayU 3405 (Fujimura *et al*., 1995) antagonize BHR.

Since several independent groups have shown that IL-8 causes concentration-dependent histamine release from human basophils *in vitro* (White *et al*., 1989), and that IL-8 induces histamine release in IL-3-pretreated human blood basophils (Krieger *et al*., 1992), we examined a role for histamine in IL-8-induced BHR. Although terfenadine was administered at doses sufficient to inhibit histamine-induced bronchoconstriction (Cheng & Woodward, 1982; Mauser *et al*., 1990), terfenadine failed to reduce the BHR induced by IL-8, making it unlikely that histamine-mediated mechanisms play an important role in the IL-8-induced BHR.

While IL-8 at 10^{-8} M and 10^{-7} M activates human basophils by a receptor-mediated mechanism similar to that operating in neutrophils (Krieger *et al*., 1992), at a higher concentration (10^{-6} M) of IL-8, histamine release is presumed to be induced by cationic interactions that do not involve the IL-8 receptor (Krieger *et al*., 1992). Since the molecular weight of IL-8 is about 8 kDa, 100 ng ml^{-1} IL-8 is about 1.25×10^{-11} M. Thus, it is likely that receptor-mediated mechanisms rather than cationic interactions may be responsible for the IL-8-induced BHR which was mediated via thromboxane generation in our experiments. Furthermore, since we observed that sputum IL-8 levels increase and reach more than 50 ng ml^{-1} during an asthmatic attack (Kurashima *et al*., 1996), the concentration of inhaled IL-8 used in this study is thought to be appropriate.

In the present study, the number of leukocytes, particularly neutrophils and eosinophils, in the airway tissue did not change significantly after treatment with IL-8 and methacholine. These results suggest that IL-8 induces bronchial hyperresponsiveness in guinea-pigs in the absence of leukocyte infiltration. Collins *et al*. (1993) demonstrated that an intradermal injection of IL-8 induces local neutrophil infiltration in guinea-pigs *in vivo* and that eosinophil infiltration also occurred 1 and 2 h after the injection of IL-8. In addition, Smith *et al*. (1991) showed that the absolute number and the percentage of eosinophils in bronchoalveolar lavage fluid (BAL) elevated only at 24 and 48 h after administration of IL-8 as an aerosol in guinea-pigs. It is possible that a significant increase in eosinophils might be observed even in our model if examined at 24 h.

In conclusion, inhaled IL-8 rapidly produced BHR partly through the release of TXA_2 in guinea-pigs, in the absence of cellular infiltration into the airway.

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