

Effects of Fexofenadine Hydrochloride in a Guinea Pig Model of Antigen-Induced Rhinitis

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Key Words

Rhinitis · Guinea pig · Fexofenadine · Histamine · Thromboxane

Abstract

Allergic rhinitis is an inflammatory disease of the nasal mucosa, induced by histamine, leukotrienes, and other substances released from mast cells. Fexofenadine hydrochloride, the active metabolite of terfenadine, is a novel, nonsedating antiallergic drug having H₁ receptor antagonistic activity. Fexofenadine is effective for the treatment of allergic rhinitis. However, its mechanism of action in attenuating nasal congestion has not yet been elucidated. Therefore, we first examined the effects of fexofenadine on a guinea pig model of antigen-induced rhinitis. We also evaluated the effects of mepyramine, zafirlukast and ramatroban in this model; these drugs are an H₁ receptor antagonist, a selective leukotriene antagonist and a selective thromboxane antagonist, respectively. Rhinitis was induced by ovalbumin (OVA) instillation into the nasal cavity of animals that had been sensitized by two earlier OVA injections (s.c. and i.p.). The nasal airway resistance was measured for 45 min after the challenge. Fexofenadine hydrochloride (20 mg/kg) and terfenadine (20 mg/kg) administered orally 70 min prior to the challenge significantly inhibited

(fexofenadine, $p < 0.001$, terfenadine, $p < 0.05$) the increase in nasal airway resistance. Ramatroban (30 mg/kg) administered orally 60 min prior to the challenge also significantly inhibited ($p < 0.05$) the increase in nasal airway resistance. In contrast, mepyramine (3 mg/kg i.v.) and zafirlukast (3 mg/kg p.o.) failed to reduce the increase in nasal airway resistance. These results suggest that thromboxane may be involved in the increase in the nasal airway resistance in this model. Accordingly, fexofenadine may reduce the increase in nasal airway resistance by inhibiting the release of chemical mediators, including thromboxane, that are involved in the increase in nasal airway resistance in this model.

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Introduction

Allergic rhinitis is a disease characterized by manifestation of symptoms such as sneezing, increased nasal secretion and nasal obstruction, which are induced by exposure to a specific antigen, such as house dust, mites, or cedar pollen. These symptoms are caused by an antigen-antibody reaction due to a type I allergic reaction, and it is thought that the induction mechanism depends on histamine, leukotrienes, or other substances that are released from mast cells or basophils [1–5].

Guinea pig models of antigen-induced allergic rhinitis induce symptoms of allergic rhinitis similar to those seen in humans. That is, the experimental animals also exhibit sneezing [6–10], nasal secretion [9] and nasal obstruction [6–17]. It has been demonstrated that histamine [10, 12, 14, 16], leukotrienes [9, 10, 12, 13, 16], and thromboxane [6, 10, 12, 14, 15, 17, 18] are involved in the mechanism of development of the pathology in these models. Accordingly, these guinea pig models have come to be widely used for evaluation of the pharmacological effects of drugs being developed for the treatment of allergic rhinitis.

Fexofenadine hydrochloride ((±)-2-[4-[1-hydroxy-4-[4-(hydroxydiphenylmethyl) piperidino]butyl] phenyl]-2-methylpropanoic acid monohydrochloride), shown in figure 1, is the active metabolite of terfenadine. Fexofenadine hydrochloride expresses H₁ receptor antagonistic activity, is widely used for treatment of allergic rhinitis, urticaria and atopic dermatitis, and causes little sedation or cardiac toxicity [19, 20]. In addition, fexofenadine hydrochloride has been shown to attenuate the release or expression of various chemical mediators or molecules, including cytokines and adhesion molecules, from various cell types (e.g. epithelial cells, fibroblasts, basophils and mast cells) *in vitro* [21–24]. Only a few *in vivo* studies using an asthma model have evaluated the antiallergic effects of fexofenadine hydrochloride [25–27]. It has been reported that fexofenadine hydrochloride inhibits the antigen-induced increase in airway resistance in guinea pigs [25, 26] and the development of airway hyperresponsiveness in antigen-sensitized mice [27]. Furthermore, fexofenadine hydrochloride showed inhibitory effects on the increase in eosinophils and release of leukotrienes or Th2 cytokines in the bronchoalveolar lavage fluid [26, 27]. However, there have been no reports of studies of the effects of fexofenadine hydrochloride on the antigen-induced allergic rhinitis model in guinea pigs.

The mechanism of action of fexofenadine hydrochloride in attenuating sneezing, and increased nasal secretion is considered to be the antagonization of the effects of histamine. It is well known that histamine is a main mediator of induction of sneezing and increased nasal secretion. In clinical studies, fexofenadine hydrochloride relieves nasal congestion as well as sneezing and rhinorrhea [28–33]. However, antihistamine drugs have little effect on nasal congestion in allergic rhinitis patients [34, 35]. Thus, it is surmised that fexofenadine hydrochloride inhibits nasal congestion in humans by inhibiting the release of some chemical mediator(s) other than histamine.

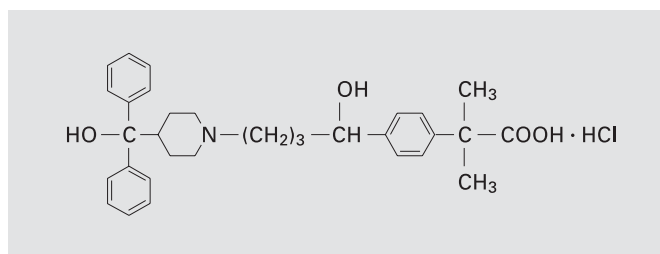


Fig. 1. Chemical structure of fexofenadine hydrochloride.

First, we investigated the effect of fexofenadine hydrochloride on the increase in nasal airway resistance in OVA-sensitized guinea pigs. In addition, we examined the effects of a selective histamine antagonist, mepyramine, a selective leukotriene (LT) antagonist, zafirlukast, and a selective thromboxane (TX) antagonist, ramatroban, on the increase in nasal airway resistance in the same model to elucidate the mechanisms by which fexofenadine hydrochloride reduces nasal airway resistance.

Materials and Methods

Chemicals

Fexofenadine hydrochloride was imported from Aventis Pharma Inc. (Bridgewater, N.J., USA). Other drugs and compounds were commercially obtained from the following sources: mepyramine and ovalbumin (OVA) (Sigma Aldrich Japan K.K., Tokyo, Japan), zafirlukast (AstraZeneca K.K., Osaka, Japan), ramatroban (Bayer Yakuhin, Ltd., Osaka, Japan), NaCl (Wako Pure Chemical Industries, Ltd., Osaka, Japan), carboxymethyl cellulose (CMC) sodium, NaOH and Al₂(SO₄)₃ (Kanto Chemical Co., Inc., Tokyo, Japan), and pentobarbital sodium (Dainippon Pharmaceutical, Osaka Co., Ltd., Japan). OVA and mepyramine were dissolved in physiological saline. CMC sodium was dissolved in water for injection. Fexofenadine hydrochloride, zafirlukast and ramatroban were suspended in 0.5% CMC sodium solution.

Animals

This experiment was conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals and all experimental procedures were approved by the Committee for Animal Experiments of Aventis Pharma Ltd.

Male Hartley guinea pigs (Charles River Japan Inc., Yokohama, Japan), 5–6 weeks old, were subjected to the first sensitization. The animals were housed in an air conditioned room at 23 ± 3°C and 55 ± 20% humidity with a 12-hour light/dark cycle. The ventilation in the animal room ranged between 12 and 20 air changes/h. Food and water were provided *ad libitum*.

Preparation of Aluminum Hydroxide Gel

Aluminum hydroxide gel was prepared from Al₂(SO₄)₃ and NaOH. NaOH was added to Al₂(SO₄)₃, and the mixture was reacted at room temperature for 30 min and then the supernatant

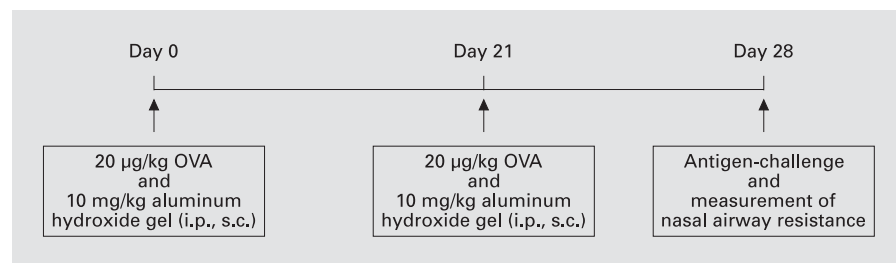


Fig. 2. Active sensitization and antigen-challenge schedule to establish a guinea pig model of antigen-induced rhinitis. The antigen solution consisted of 8 ml of physiological saline containing 20 µg of OVA and 10 mg of aluminum hydroxide gel. Each animal was administered this antigen solution in a volume dosage of 8 ml/kg to sensitize them. Three weeks after this initial sensitization, booster immunization was performed by administering the same antigen solution in the same dosage, by the same method. The rhinitis reaction was induced by administration of 5% OVA solution into the nasal cavity.

was collected and discarded, and distilled water was added. The washing operation was continued until the pH of the supernatant became neutral. Finally, distilled water was added to the above residue so that the concentration of aluminum hydroxide gel was adjusted to 40 mg/ml. NaCl was added to a concentration of 0.9%, and this mixture was stored at 4°C.

Active Sensitization Procedure of Animals

Figure 2 shows the active sensitization procedure used for the animals in this study. The antigen solution consisted of 8 ml of physiological saline containing 20 µg of OVA and 10 mg of aluminum hydroxide gel. Each animal was administered this antigen solution in a volume dosage of 8 ml/kg, with 1 ml injected subcutaneously and the remaining volume administered intraperitoneally to sensitize the animals. Three weeks after this initial sensitization, booster immunization was performed by administering the same antigen solution in the same dosage, using the same method described above. On the day before measurement of nasal airway resistance, we divided these animals into 4 groups (table 1).

Measurement of Nasal Airway Resistance Induced by Antigen Challenge

The measurement of nasal airway resistance was basically that reported by Noguchi et al. [11], with slight modification. Each guinea pig was anesthetized with pentobarbital (30 mg/kg i.p.), and an incision was made in the neck region. A catheter for monitoring blood pressure was inserted into the carotid artery. Next, the esophagus was ligated to prevent leakage of air. The trachea was then incised, and a catheter for measuring the nasal airway resistance was inserted from the larynx in the direction of the nasal cavity. In addition, a catheter for securing respiration was inserted in the direction of the lung. A respirator (Model SN-480-7; Shinano Manufacturing Co., Tokyo, Japan or Catalog No. 7025, Ugo Basile, Co., Comerio, Italy) was used to blow air heated to 38°C into the nasal cavity from the end of the catheter; the flow volume was 8 ml/beat at 70 beats/min. The nasal airway resistance caused by the delivered air was recorded on a polygraph (Model RM-6000, carrier amplifier: AP-601G; thermographic recorder: WT 685G; Nihon

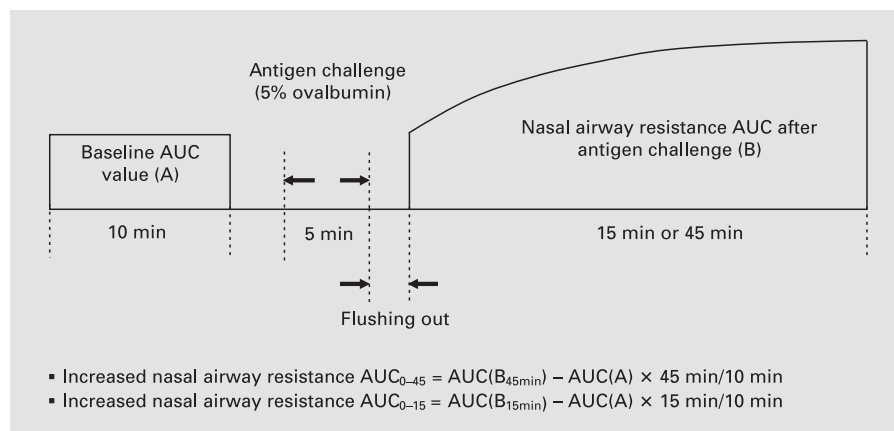
Table 1. Summary of the grouping in the experiments

Group	Animals, n
<i>Experiment 1</i>	
Saline-challenged, 0.5% CMC-treated group	20
OVA-challenged, 0.5% CMC-treated group	20
OVA-challenged, fexofenadine (20 mg/kg p.o.)-treated group	19
<i>Experiment 2</i>	
Saline-challenged, 0.5% CMC-treated group	20
OVA-challenged, 0.5% CMC-treated group	19
OVA-challenged, terfenadine (20 mg/kg p.o.)-treated group	19
<i>Experiment 3</i>	
Saline-challenged, saline-treated group	9
OVA-challenged, saline-treated group	8
OVA-challenged, mepyramine (3 mg/kg i.v.)-treated group	9
<i>Experiment 4</i>	
Saline-challenged, 0.5% CMC-treated group	10
OVA-challenged, 0.5% CMC-treated group	11
OVA-challenged, zafirlukast (3 mg/kg p.o.)-treated group	11
OVA-challenged, ramatroban (30 mg/kg p.o.)-treated group	11

Kohden Corp., Tokyo, Japan) via a transducer (SPB104 [DT-XX]; NEC Co., Ltd., Tokyo, Japan).

Prior to the antigen challenge, a 10-min monitoring period of the nasal airway resistance was used to define the baseline nasal airway resistance, calculated from the area under the curve (AUC). After the baseline assessment, the delivery of air was temporarily stopped, and 1 ml of the antigen solution (5% OVA solution) was

Fig. 3. Method for calculation of the AUC of the nasal resistance. The baseline nasal airway resistance was measured for a 10-min period. The antigen solution (5% OVA solution) was instilled into the nasal cavity for 5 min as a challenge. After the challenge, the antigen solution was completely eliminated from the nasal cavity. The nasal airway resistance was then measured for 45 min. The increase in the AUC (AUC_{0-45} and AUC_{0-15}) of the nasal airway resistance was calculated by subtracting the converted baseline AUC from the AUC values for the 45-min/15-min period after antigen challenge.



administered through the catheter. This was followed by a 5-min waiting period. After the waiting period, warm air was again blown into the nasal cavity and the antigen solution was eliminated from the nasal cavity. The nasal airway resistance was measured for 45 min after the antigen challenge, because (1) usually it has been determined for about 30 min in studies in the acute allergic rhinitis model [11, 12, 14, 15]; (2) such studies suggest that the resistance is still increased at 45 min, and (3) it is difficult to anesthetize guinea pigs with pentobarbital for a longer time. At the end of the assessment of the nasal airway resistance, the animal was euthanized by injection of an excessive amount of pentobarbital.

The chart recordings of the nasal airway resistance measured during the baseline (pre-challenge) period and during the 45-min period after antigen challenge were subjected to image analysis using MacScope software (Version 2.53, Mitani Corp., Tokyo, Japan), and the AUC values were calculated. The increase in the AUC value for the nasal airway resistance induced by the antigen challenge was calculated by subtracting the converted baseline AUC value from the AUC value for the 45-min period after antigen challenge (fig. 3). Similarly, the AUC was determined for the initial 15-min period after antigen challenge. In addition, the nasal airway resistance was measured immediately before, immediately after, and at 2, 5, 10, 15, 20, 25, 30, 35, 40 and 45 min after antigen challenge. The difference was calculated between the nasal airway resistance value determined at each time point after the antigen challenge and the value determined immediately before the antigen challenge.

Drug Administration

Fexofenadine hydrochloride (20 mg/kg) and terfenadine (20 mg/kg) were administered orally 70 min prior to the antigen challenge. The administration time of fexofenadine hydrochloride was the same as used in a study in the guinea pig model of immediate-type asthma in which the time was determined by the response surface model [26]. Terfenadine was administered at 70 min because it was the parent compound of fexofenadine hydrochloride. Mepyramine (3 mg/kg) was given intravenously 5 min prior to the antigen challenge, because under this condition it caused nearly complete inhibition of histamine-induced wheal in guinea pigs (data not shown). Zafirlukast (3 mg/kg) and ramatroban (30 mg/kg) were administered orally 60 min prior to the antigen challenge.

Zafirlukast was chosen because (1) it is the same Cys LT_1 antagonist as pranlukast, and (2) we could obtain the bulk powder commercially and (3) zafirlukast has recently been reported to attenuate nasal congestion in allergic rhinitis patients [36]. The dosage of 3 mg/kg was expected to express a sufficient antagonistic effect on the LTD_4 receptor and is about twice the dose (1.7 mg/kg) that significantly inhibited LTD_4 -induced pulmonary mechanical aggravation in anesthetized guinea pigs [37]. The reasons for administration of zafirlukast at 60 min were as follows: (1) it is administered 90 min before LTD_4 -induced pulmonary mechanical aggravation in anesthetized guinea pigs [37]; (2) 1 h is the T_{max} of zafirlukast orally treated in the guinea pig [38]; (3) 45 min was the period of the measurement of nasal airway resistance in our study. The dose of and time of treatment with ramatroban were selected because the TXA_2 antagonist sufficiently inhibited, under this condition, the increase in nasal airway resistance in sensitized guinea pigs [17].

Statistical Analysis

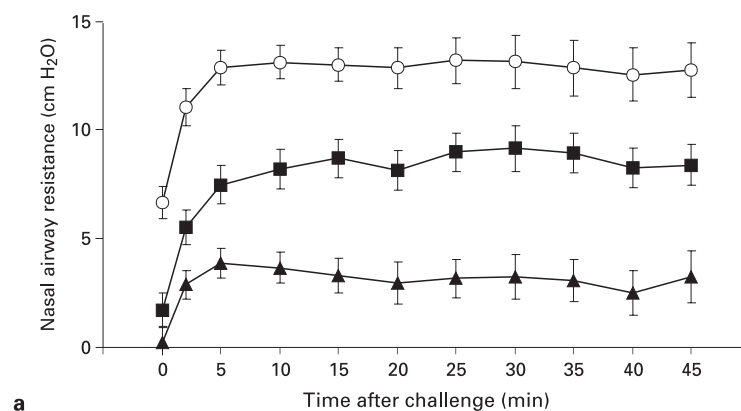
Results are expressed as means $\pm$ SE. All analyses of the experimental data were carried out using JMP statistical analysis software (Release 3.2.2, SAS Institute Inc., USA).

The data between two groups were compared by means of the two-tailed t test, and statistical significance was set at $p < 0.05$.

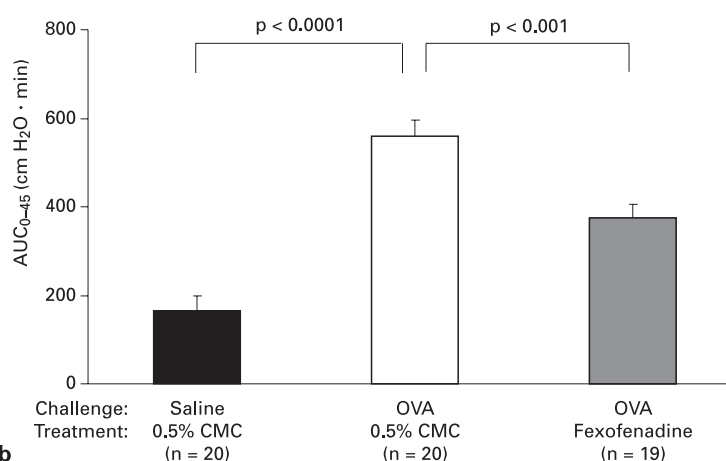
Results

Effects of Fexofenadine Hydrochloride on the Increase in Nasal Airway Resistance in the Antigen-Induced Rhinitis Model

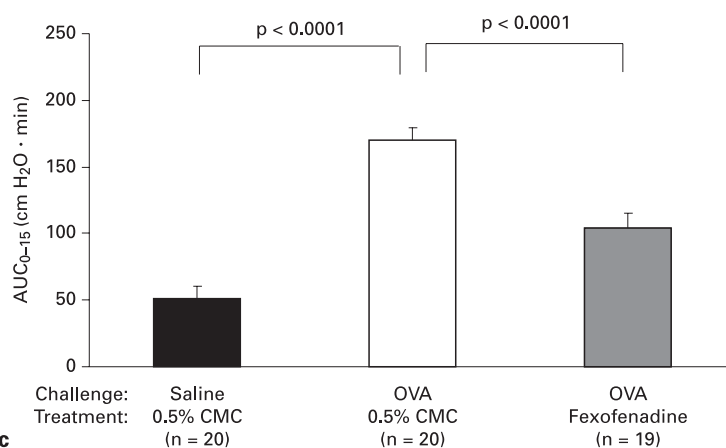
Figure 4a shows the time-course of the antigen-induced increase in the nasal airway resistance (i.e. the difference from the value recorded immediately before antigen challenge) in the saline-challenged group, OVA-challenged group and fexofenadine hydrochloride-treated group. The nasal airway resistance increased rapidly within 5 min after the antigen challenge and continued



a



b



c

Fig. 4. Effects of fexofenadine hydrochloride (20 mg/kg) on the increase in nasal airway resistance in OVA-sensitized guinea pigs. Each value represents the mean $\pm$ SE. **a** Time-course changes in the increase in nasal airway resistance in OVA-sensitized guinea pigs. $\blacktriangle$ = Saline-challenged, 0.5% CMC sodium-treated group; $\circ$ = OVA-challenged, 0.5% CMC sodium-treated group; $\blacksquare$ = OVA-challenged, fexofenadine hydrochloride (20 mg/kg)-treated group. **b** Effects of fexofenadine hydrochloride (20 mg/kg) on the increase in nasal airway resistance in OVA-sensitized guinea pigs (AUC_{0-45}). **c** Effects of fexofenadine hydrochloride (20 mg/kg) on the increase in nasal airway resistance in OVA-sensitized guinea pigs (AUC_{0-15}).

Table 2. Effects of terfenadine, mepyramine, zafirlukast and ramatroban on the increase in nasal airway resistance in OVA-sensitized guinea pigs

Group	Animals n	AUC	
		0–45 min	0–15 min
<i>Terfenadine</i>			
Saline-challenged, 0.5% CMC-treated group	20	251.06 ± 27.16	68.88 ± 6.57
OVA-challenged, 0.5% CMC-treated group	19	859.83 ± 154.86 ^a	188.84 ± 16.62 ^a
OVA-challenged, terfenadine-treated group (20 mg/kg p.o.)	19	485.55 ± 58.60 ^b	124.24 ± 14.26 ^c
<i>Mepyramine</i>			
Saline-challenged, saline-treated group	9	254.07 ± 67.97	85.80 ± 21.13
OVA-challenged, saline-treated group	8	943.33 ± 165.93 ^a	241.33 ± 32.45 ^a
OVA-challenged, mepyramine-treated group (3 mg/kg i.v.)	9	739.69 ± 70.67	168.18 ± 20.17
<i>Zafirlukast, Ramatroban</i>			
Saline-challenged, 0.5% CMC-treated group	10	164.08 ± 39.67	48.64 ± 13.20
OVA-challenged, 0.5% CMC-treated group	11	640.67 ± 50.11 ^a	176.60 ± 13.20 ^a
OVA-challenged, zafirlukast-treated group (3 mg/kg p.o.)	11	649.91 ± 70.54	168.89 ± 20.09
OVA-challenged, ramatroban-treated group (30 mg/kg p.o.)	11	493.35 ± 26.03 ^b	150.95 ± 12.68

Each value represents the mean ± SE. ^a $p < 0.01$ compared to the saline-challenged, 0.5% CMC-treated group or saline-challenged, saline-treated group. ^b $p < 0.05$, ^c $p < 0.01$ compared to OVA-challenged, 0.5% CMC-treated group or OVA-challenged, saline-treated group.

to increase gradually thereafter in the OVA-challenged group. On the other hand, fexofenadine hydrochloride reduced the increase in nasal airway resistance during 45 min after antigen challenge. As shown in figure 4b, the AUC of nasal airway resistance for 45 min (AUC_{0-45}) in the OVA-challenged, 0.5% CMC sodium-treated group was significantly ($p < 0.01$) increased compared with the saline-challenged group. In contrast, administration of fexofenadine hydrochloride (20 mg/kg) reduced the AUC_{0-45} significantly ($p < 0.01$) compared with the OVA-challenged, 0.5% CMC sodium-treated group. To investigate the effects of the test compounds on the early stage of induction of increased nasal airway resistance, the AUC was determined for the first 15 min after the antigen challenge. Figure 4c shows the AUC of the nasal airway resistance for 15 min (AUC_{0-15}) after the antigen challenge in the three treatment groups. The AUC_{0-15} in the OVA-challenged, 0.5% CMC sodium-treated group was significantly ($p < 0.01$) increased compared with the saline-challenged group. Moreover, the data show that after administration of fexofenadine hydrochloride the AUC_{0-15} was significantly inhibited ($p < 0.01$) in comparison with the OVA-challenged, 0.5% CMC sodium-treated group.

Effects of Terfenadine on the Increase in Nasal Airway Resistance in the Antigen-Induced Rhinitis Model

There were statistically significant differences between the OVA-challenged, 0.5% CMC sodium-treated group and the terfenadine (20 mg/kg) treated group for the AUC_{0-45} data and the AUC_{0-15} data (table 2). Accordingly, the administration of terfenadine inhibited the increase in the nasal airway resistance induced by antigen challenge. Fexofenadine hydrochloride and terfenadine exerted the same inhibitory effect on the increase in the nasal airway resistance.

Effects of Mepyramine, Ramatroban and Zafirlukast on the Increase in Nasal Airway Resistance in the Antigen-Induced Rhinitis Model

To elucidate the chemical mediators involved in the increase in nasal airway resistance in this rhinitis model, we investigated the effects of a selective H_1 antagonist, mepyramine, a selective thromboxane antagonist, ramatroban, and a selective leukotriene antagonist, zafirlukast. As shown in figure 5a, mepyramine did not attenuate the increase in nasal airway resistance during 45 min after antigen challenge. In fact, administration of mepyramine (3 mg/kg) failed to reduce either the AUC_{0-45} ($p = 0.2572$) or the AUC_{0-15} ($p = 0.0685$) (table 2).

Fig. 5. Time-course changes in the increase in nasal airway resistance in OVA-sensitized guinea pigs. Mean $\pm$ SE are reported. **a** Effects of mepyramine (3 mg/kg) on the increase in nasal airway resistance in OVA-sensitized guinea pigs. $\blacktriangle$ = Saline-challenged, saline (i.v.)-treated group; $\circ$ = OVA-challenged, saline (i.v.)-treated group; $\blacksquare$ = OVA-challenged, mepyramine (3 mg/kg i.v.)-treated group. **b** Effects of zafirlukast (3 mg/kg) or ramatroban (30 mg/kg) on the increase in nasal airway resistance in OVA-sensitized guinea pigs. $\blacktriangle$ = Saline-challenged, 0.5% CMC sodium-treated group; $\circ$ = OVA-challenged, 0.5% CMC sodium-treated group; $\blacksquare$ = OVA-challenged, zafirlukast (3 mg/kg)-treated group; $\square$ = OVA-challenged, ramatroban (30 mg/kg)-treated group.

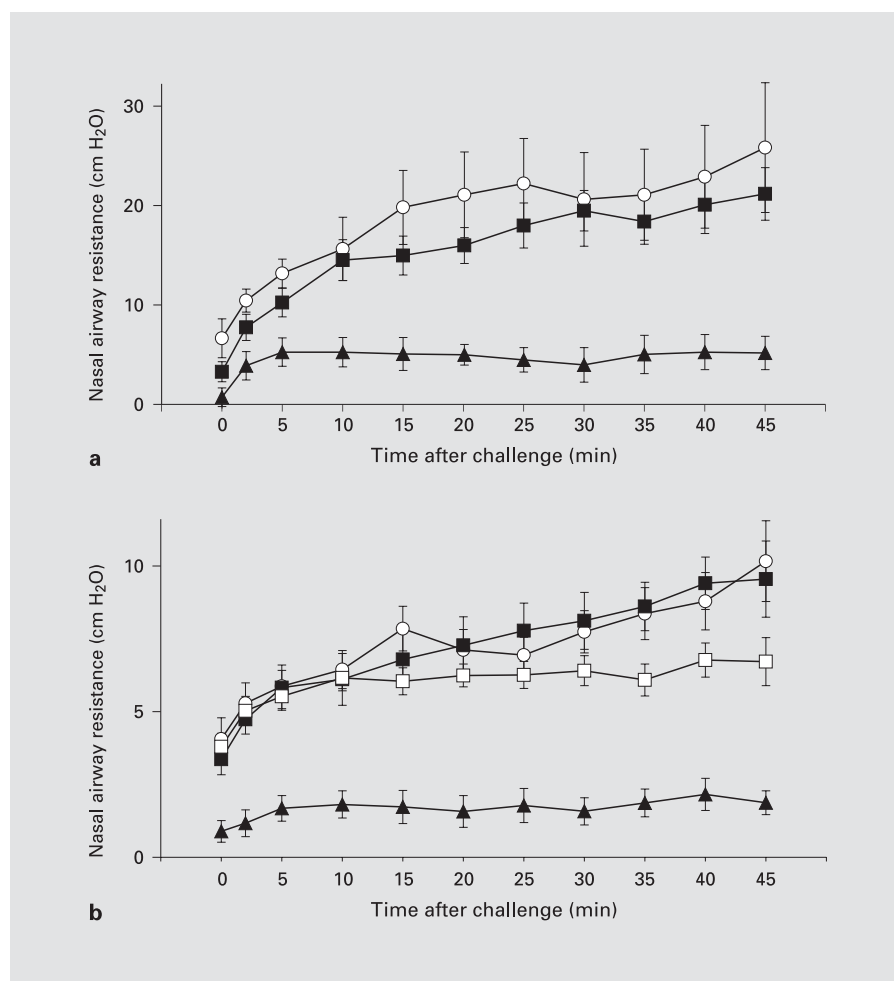
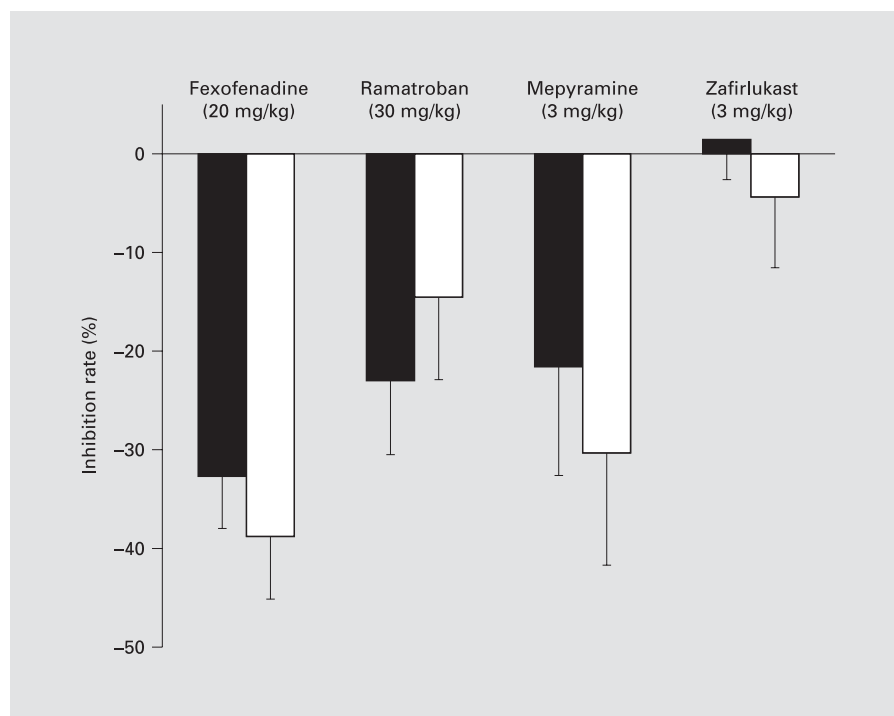


Figure 5b shows the time-course of changes in the increase in nasal airway resistance in OVA-sensitized guinea pigs as a result of administration of ramatroban or zafirlukast. Administration of ramatroban reduced the late stage of the increase in nasal airway resistance, but the zafirlukast-treated group showed no difference in comparison with the OVA-challenged, 0.5% CMC sodium-treated group. Regarding the AUC_{0-15} and AUC_{0-45} data, no significant differences were observed between the OVA-challenged, 0.5% CMC sodium-treated group and the zafirlukast-treated group (table 2). On the other hand, the administration of ramatroban reduced the AUC_{0-45} data significantly compared to the OVA-challenged, 0.5% CMC sodium-treated group, whereas the administration of ramatroban failed to reduce the AUC_{0-15} data significantly (table 2).

Inhibition Rates (%) of the Administrations of Fexofenadine Hydrochloride, Mepyramine, Ramatroban and Zafirlukast on the AUC

Figure 6 shows the inhibition (%) of the AUC_{0-45} and AUC_{0-15} with administration of fexofenadine hydrochloride, mepyramine, zafirlukast and ramatroban in comparison with each control. In the fexofenadine hydrochloride-treated group, the highest inhibition rates were observed for the AUC_{0-45} and AUC_{0-15} data. Administration of ramatroban showed a slightly higher inhibition rate than that of mepyramine for the AUC_{0-45} . Regarding the AUC_{0-15} , administration of mepyramine inhibited nasal airway resistance more strongly than ramatroban. On the other hand, the group treated with zafirlukast showed no inhibition of the increase in the nasal airway resistance in terms of either the AUC_{0-45} or the AUC_{0-15} .

Fig. 6. Inhibition rates (%) of the administrations of fexofenadine, hydrochloride, mepyramine, zafirlukast and ramatroban on the AUC of nasal resistance increase in comparison with control. ■ = Inhibition rates (%) on the AUC_{0–45}; □ = inhibition rates (%) on the AUC_{0–15}.



Discussion

Our findings show that fexofenadine hydrochloride, an anti-allergic drug, and terfenadine inhibited the increase in nasal airway resistance in this model. A selective thromboxane antagonist, ramatroban, also inhibited the increase in the nasal airway resistance. On the other hand, the selective histamine H₁ receptor antagonist, mepyramine, and the selective leukotriene antagonist, zafirlukast, both failed to inhibit significantly the increase in the nasal airway resistance.

It has been reported that antigen-induced allergic rhinitis models in guinea pigs exhibit symptoms similar to those seen in human allergic rhinitis, including sneezing, nasal secretion and nasal obstruction [6–17]. It has also been reported that histamine, leukotrienes, and thromboxane, are involved in the mechanism of development of allergic rhinitis in guinea pigs [6, 9, 10, 12–18].

Histamine is one of the major chemical mediators triggering the symptoms of rhinitis, especially sneezing and rhinorrhea, in allergic rhinitis patients. On the other hand, it is thought that histamine is not extensively involved in the increase in nasal airway resistance in allergic rhinitis patients because antihistamines have little effect on nasal congestion [34, 35]. In the present study, the selective H₁ receptor antagonist, mepyramine (3 mg/

kg), failed to reduce significantly the increase in nasal airway resistance (AUC_{0–15} [$p = 0.0685$] and AUC_{0–45} [$p = 0.2572$]). This result corresponds to the previously reported results seen in allergic rhinitis patients [34, 35]. Accordingly, the results indicate that histamine is not extensively involved in the increase in nasal resistance in this model. However, some reports have suggested that histamine is involved in the increase in nasal airway resistance during the early stage of the rhinitis reaction in guinea pigs. Yamasaki et al. [14] reported that the antihistamine, azelastine (1 mg/kg), reduced the increase in nasal resistance during the first 5 min of antigen-induced allergic rhinitis in guinea pigs. Yasui et al. [15] reported similar findings with a selective H₁ receptor antagonist, diphenhydramine. In the mepyramine-treated group, the degree of inhibition of the AUC_{0–15} (30.3% inhibition) was larger than that of the AUC_{0–45} (21.6% inhibition). These results suggest the possibility that histamine is involved in the increase in nasal airway resistance during the early phase of the rhinitis reaction in this model.

Leukotrienes are also considered to be major chemical mediators that induce allergic rhinitis reactions. Administration of a leukotriene into the nasal cavity caused increases in nasal vascular permeability and intranasal pressure in sensitized guinea pigs and allergic rhinitis pa-

tients [39–41]. In addition, it was reported that a selective leukotriene antagonist attenuated the nasal response after antigen challenge in sensitized guinea pigs [9, 13].

Pranlukast, a selective peptide leukotriene antagonist, is already available in Japan and is indicated for the treatment of allergic rhinitis. We administered a selective leukotriene antagonist, zafirlukast, to examine whether leukotrienes are involved in the increase in nasal airway resistance in this model. The results showed that zafirlukast failed to reduce significantly the nasal airway resistance (AUC_{0-45} and AUC_{0-15}), thereby suggesting that leukotrienes are not critically involved in the increase in nasal airway resistance in this model. However, in earlier reports, a selective peptide leukotriene antagonist, pranlukast, attenuated the increase in the nasal airway resistance [9, 13]. Although the reason for this difference has not yet been elucidated, leukotrienes may not be strongly involved in the increase in nasal airway resistance in acute models such as ours, whereas those earlier findings of inhibitory effects of leukotriene antagonists [9, 13] were observed in a chronic allergic rhinitis model in guinea pigs. In one study [9], pranlukast inhibited the increase in nasal airway resistance to a lesser degree in the early phase of rhinitis reaction than in the late although the effect was significant in both phases. In the other study [13], pranlukast did not significantly inhibit the increased resistance in the early phase while its inhibitory effect was significant in the late phase. Yamasaki et al. [14] reported results similar to ours. They reported that the selective peptide leukotrienes antagonist, pranlukast (3 and 30 mg/kg) failed to inhibit the OVA-induced increase in nasal airway resistance in their acute rhinitis model in guinea pigs. Fujita et al. [12] also reported that the release of leukotrienes into the nasal cavity induced by antigen challenge was low in the acute allergic rhinitis model. The results of these studies are in accordance with the failure of zafirlukast to produce significant reduction of the nasal airway resistance in our present study.

Thromboxane (TX) has also recently been thought to be an important mediator in the pathophysiology of rhinitis. TXB_2 is found in the nasal lavage fluid from patients with allergic rhinitis [42, 43] as well as the sensitized guinea pigs [10, 14, 15] after antigen provocation. In addition, it has been reported that U-46619, a TXA_2 mimetic, increased nasal vascular permeability [14, 18] and nasal airway resistance [14, 15] in guinea pigs, and that a TXA_2 antagonist attenuated the increase in nasal airway resistance in sensitized guinea pigs [6, 10, 14, 15, 17]. In allergic rhinitis patients, it was reported that a se-

lective TXA_2 antagonist, ramatroban approved for the treatment of allergic rhinitis in Japan in 2000, attenuated the increase in nasal airway resistance [44, 45].

The AUC_{0-45} was significantly reduced by ramatroban (inhibition rate, 23.0%), but the AUC_{0-15} was not reduced. The results of the present study suggest that thromboxane may be involved in the late stage rather than early stage of increased nasal airway resistance in our acute model. This result is consistent with the results of studies in antigen-induced rhinitis model in guinea pigs [6, 10, 14, 15, 17].

Various chemical mediators (e.g. PGD_2 , PAF) other than thromboxane have also been reported to be involved in the increase in nasal airway resistance in the antigen-induced rhinitis model in guinea pigs [46, 47]. In addition, it was recently reported that NO is also involved in the increase in nasal airway resistance [48]. Accordingly, some chemical mediators other than thromboxane may be involved in the increase in the nasal airway resistance because administration of a selective thromboxane antagonist, ramatroban, did not completely reduce the nasal airway resistance in this study. Further studies are warranted to clearly identify the chemicals involved in the development of nasal obstruction in this model and more precisely elucidate the mechanisms of action by which fexofenadine hydrochloride inhibits the increase in nasal airway resistance.

Clinical studies found that fexofenadine hydrochloride relieves nasal congestion as well as sneezing and rhinorrhea [28–33]. It is reported that antihistamines have little effect on nasal congestion in allergic rhinitis patients [34, 35]. The above findings with fexofenadine hydrochloride and antihistamines suggest that fexofenadine hydrochloride inhibits the release and/or generation of some chemical mediators other than histamine during the allergic reaction and thereby reduces the increase in nasal airway resistance in allergic rhinitis patients. In allergic rhinitis patients, it is thought that leukotrienes are main chemical mediators inducing nasal obstruction in late phase [49]. We have demonstrated that fexofenadine hydrochloride inhibits the release of leukotrienes in BALF from an asthma model in guinea pigs [26]. Therefore, there is a possibility that fexofenadine hydrochloride inhibits the release and/or generation of leukotrienes and thus exerts an inhibitory effect on nasal congestion particularly in the late phase in allergic rhinitis patients. On the other hand, it is controversial whether or not thromboxane is involved in the nasal congestion in allergic rhinitis patients. In a recent study, it was reported that clinical efficacy of a selective thromboxane antagonist, ra-

matroban, may be in part achieved by antagonistic effect of one of the PGD₂ receptors, CRTH2 (a chemoattractant receptor-homologous molecule expressed on Th2 cells) which mediates eosinophil migration into the inflammation site in the late phase [50]. There could be a possibility that fexofenadine hydrochloride may antagonize CRTH2 and improve the nasal congestion in allergic rhinitis patients, although further studies are needed to clarify chemical mediators involved in the pathogenesis of allergic rhinitis patients.

In conclusion, we demonstrated that fexofenadine hydrochloride and its parent compound, terfenadine, reduced the increase in nasal airway resistance after antigen challenge in this model. In addition, the selective thromboxane antagonist, ramatroban, also attenuated the increased nasal airway resistance. On the other hand, the selective H₁ receptor antagonist, mepyramine, and the selective leukotriene antagonist, zafirlukast, both failed to reduce significantly the increase in nasal airway resistance. In the mepyramine-treated group, the degree of inhibition of the AUC₀₋₁₅ was larger than that of the

AUC₀₋₄₅. On the basis of these findings, it can be surmised that (1) thromboxane may be involved in the increase in nasal airway resistance; (2) there is a possibility that histamine is involved in the increase in nasal airway resistance in the early phase of rhinitis reaction, and (3) leukotrienes do not play an important role in this model. Accordingly, the results of our present study suggest that fexofenadine hydrochloride may inhibit the release and/or generation of various chemical mediators, including thromboxane, after antigen challenge and reduce the increase in nasal airway resistance in this model. However, further investigations are necessary to clarify the chemical mediators involved in the development of nasal obstruction in this model.

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