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Effects of TAK-427 on acute nasal symptoms and nasal obstruction in guinea pig model of experimental allergic rhinitis

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Abstract

TAK-427 {2-[6-[[3-[4-(diphenylmethoxy)piperidino]propyl]amino]imidazo[1,2-*b*]pyridazin-2-yl]-2-methylpropionic acid dihydrate} is a novel anti-allergic agent that has both histamine H_1-receptor antagonist and anti-inflammatory activities. In this study, we evaluated the efficacy of TAK-427 on acute nasal responses and nasal obstruction using various guinea pig models of allergic rhinitis. TAK-427 inhibited the histamine-induced nasal reactions with an ID_{50} value of 0.633 mg/kg, p.o. TAK-427 (0.1–10 mg/kg, p.o.) and most histamine H_1-receptor antagonists tested inhibited the increase in intranasal pressure, nasal hypersecretion, sneezing and nasal itching caused by a single antigen challenge in sensitized guinea pigs. In addition, TAK-427 (0.3, 30 mg/kg, p.o.) significantly inhibited the development of nasal obstruction when sensitized guinea pigs were repeatedly challenged via inhalation with Japanese cedar pollen, whereas the histamine H_1-receptor antagonist, azelastine (1 mg/kg, p.o.), and ketotifen (1 mg/kg, p.o.) were without effect. These results suggest that TAK-427 might not only suppress acute nasal symptoms but also ameliorate nasal obstruction via the effects other than those as a histamine H_1-receptor antagonist.

Keywords: TAK-427 {2-[6-[[3-[4-(diphenylmethoxy) piperidino]propyl]amino]imidazo[1,2-*b*]pyridazin-2-yl]-2-methylpropionic acid dihydrate}; Nasal acute symptom; Allergic rhinitis; Histamine H_1-receptor antagonist; Cedar pollen; Nasal obstruction

1. Introduction

Allergic rhinitis is a typical immunoglobulin (Ig) E-mediated disease associated with nasal responses such as sneezing, itching, nasal hypersecretion and nasal obstruction (Naclerio, 1991). Over 90% of patients manifest these symptoms (acute phase reaction) immediately when specific allergens are applied to the nasal cavities, approximately 50% further develop a late phase reaction with the predominant symptom being nasal obstruction 4 to 12 h after allergen challenge (Pelikan, 1978; Dvoracek et al., 1984; Iliopoulos et al., 1990).

Contact with allergens stimulates the nasal sensory nerve via histamine, which is released from mast cells by an IgE-mediated mechanism. This triggers early-phase nasal responses such as sneezing, itching, and nasal hypersecretion via a sensory nerve reflex and an increase in nasal mucosal microvascular permeability, which histamine H_1-receptor antagonists are widely used to treat (Simons, 1989). Late-phase nasal responses, mainly nasal obstruction, are caused by a complex network involving activation and infiltration of inflammatory cells and release of a number of neurotransmitters, mediators and cytokines. These mediators induce plasma leakage and vasodilation of the nasal mucosal sinusoids resulting in nasal mucosal oedema, and nasal mucosal congestion. Nasal obstruction is considered to be a serious problem for patients with these symptoms, since most drugs other than glucocorticoids, decongestants and disodium cromoglycate are largely ineffective against nasal obstruction (Naclerio, 1991; Pipkorn et al., 1987; Pelikan, 1982).

It has been shown that TAK-427 {2-[6-[[3-[4-(diphenylmethoxy)piperidino]propyl]amino]imidazo[1,2-*b*]pyridazin-2-yl]-2-methylpropionic acid dihydrate} has anti-inflamma-

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tory effects in the guinea pig model of eczema (Fukuda et al., 2002), as well as inhibitory effects on acute phase allergic reaction based on its antihistaminic activity (Fukuda et al., 2003).

In the present study, in order to assess the potential value of TAK-427 as a therapeutic agent for allergic rhinitis, the effects of TAK-427 on various antigen-induced nasal responses were investigated using several guinea pig models of allergic rhinitis. However, it was difficult to estimate the efficacy of test compounds with antihistaminic activities on nasal obstruction in allergic rhinitis patients from results of animal studies. In most animal studies, nasal obstruction was induced by single antigen challenge in sensitized animals, and was inhibited by pretreatment with histamine H_1-receptor antagonists, which is not necessarily the case in the patient with allergic rhinitis. Recently, we reported that repetitive inhalation challenge with Japanese cedar pollen in sensitized guinea pigs caused sustained nasal obstruction, refractory to antihistamine treatment (Nabe et al., 1998; Yamasaki et al., 2001). Therefore, we evaluated the effect of TAK-427 on nasal obstruction using this guinea pig model. Herein, we demonstrate that TAK-427 ameliorates not only acute phase nasal symptoms but also nasal obstruction refractory to antihistamine treatment.

2. Materials and methods

2.1. Animals

Male std: Hartley guinea pigs (3–5 weeks of age) were purchased from Japan SLC (Hamamatsu, Japan). The animals were housed under controlled temperature (24 ± 1 °C) and humidity (55 ± 5%) and given access to food and water ad libitum. The care and use of the animals and experimental protocol used in this study were approved by the Experimental Animal Care and Use Committee of Takeda Chemical Industries (Osaka, Japan).

2.2. Drugs and materials

Histamine dihydrochloride was purchased from Wako (Osaka, Japan). Ketotifen fumarate, terfenadine, ovalbumin (Grade III) and dexamethasone were purchased from Sigma-Aldrich (St. Louis, MO). Azelastine hydrochloride was extracted from tablets of AZEPTIN® (Eisai, Tokyo, Japan). TAK-427 {2-[6-[[3-[4-(diphenylmethoxy)piperidino]propyl]amino]imidazo[1,2-*b*]pyridazin-2-yl]-2-methylpropionic acid dihydrate}, loratadine and cetirizine were synthesized at Takeda Chemical Industries. Japanese cedar pollen was obtained from the 1998 Japanese cedar crop.

TAK-427 and the other drugs tested were suspended in a 0.5% methylcellulose solution. TAK-427 and histamine H_1-receptor antagonist were administered orally 1 h before challenge with antigen or histamine, and dexamethasone was administered orally 3 h before the challenge.

2.3. Histamine-induced nasal reactions in guinea pigs

Nasal reactions were measured as previously described (Yamasaki et al., 1997). Briefly, the animals (weighing 500–650 g) were anesthetized with an intraperitoneal (i.p.) injection of 30 mg/kg of pentobarbital sodium. A cannula was inserted into the trachea, and the animals were allowed to breathe spontaneously through the cannula. A polyethylene cannula was inserted into the nasopharynx from the side of the larynx, and the other end of the cannula was connected to an artificial respirator set at a flow volume of 4 ml and a frequency of 70 strokes/min. The two duct pores, which are situated in the upper oral cavity wall and lead to the nasal cavity, were closed with Aron alpha A®. Intranasal pressure was measured by a transducer (Model DP45-22; Validyne Engineering, Northridge, CA) connected to a lateral port at the proximal end of the endonasopharyngeal cannula. A saline or 0.1% histamine aerosol, generated by an ultrasonic nebulizer (Model 5000D; Delvilbiss Health Care, Somerset, PA) placed be-

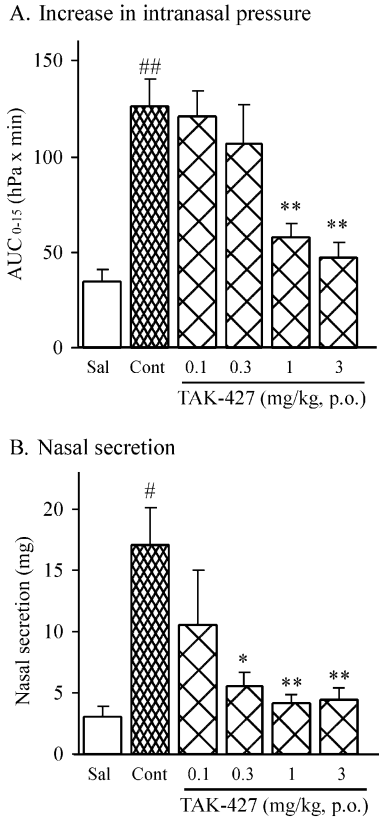

Fig. 1. Effect of TAK-427 on the histamine-induced increase in intranasal pressure (A) and nasal secretion (B) in guinea pigs. Animals were challenged by inhalation of saline or histamine aerosol for 3 min. Intranasal pressure was measured for 15 min after the cessation of inhalation. Nasal secretions were then collected and weighed. The increase in intranasal pressure was expressed as the AUC value from 0 to 15 min after inhalation. TAK-427 was administered 1 h before inhalation. Data represent the means ± S.E.M. for 10 guinea pigs. Sal: Saline inhalation group; Cont: Control group. $^{\#}P<0.05$, $^{\#\#}P<0.01$ vs. saline inhalation group (*t*-test), $^{*}P<0.05$, $^{**}P<0.01$ vs. Control (Dunnett's test).

Table 1
Inhibitory effects of TAK-427, several histamine H_1-receptor antagonists and dexamethasone on the histamine-induced increase in intranasal pressure in normal guinea pigs

Drug	ID_{50} (mg/kg, p.o.)	95% confidence interval
TAK-427	0.633	(0.365–1.162)
Azelastine	0.037	(0.007–0.096)
Ketotifen	0.010	(0.006–0.020)
Terfenadine	0.443	(0.207–0.835)
Cetirizine	0.105	(0.012–0.237)
Dexamethasone	>10	

Animals were challenged by inhalation of saline or histamine aerosol for 3 min. Intranasal pressure was measured for 15 min after the cessation of inhalation. Nasal secretions were then collected and weighed. Histamine H_1-receptor antagonist and dexamethasone were administered 1 or 3 h before inhalation, respectively. ED_{50} values were calculated using the AUC value from 0 to 15 min after inhalation.

tween the nasopharynx and the artificial respirator, was then insufflated into the nasal cavity for 3 min. The increase in intranasal pressure was obtained by subtracting the pre-challenge baseline pressure. To evaluate the effects of test compounds, the area under the curve (AUC) was calculated for the increase in intranasal pressure from 0 to 15 min after the end of histamine insufflation. After 15-min measurement of intranasal pressure, nasal secretions were absorbed with cottonwool, and the secretions were weighed.

2.4. Ovalbumin-induced increases in intranasal pressure and nasal secretion in actively sensitized guinea pigs

The animals (6-week-old) were sensitized with an i.p. injection of 20 μg of ovalbumin mixed with 5 mg of $Al(OH)_3$ in 1 ml of saline four times at 2-week intervals. One week after the last sensitization, experiments were conducted as described above. A saline or 3% ovalbumin aerosol, generated by an ultrasonic nebulizer (Model 5000D; Delvilbiss Health Care) placed between the nasopharynx and the artificial respirator, was then insufflated into the nasal cavity for 3 min. To evaluate the effects of test compounds, the area under the curve (AUC) was calculated for the increase in intranasal pressure from 0 to 30 min after the end of ovalbumin inhalation. After 30-min measurement of intranasal pressure, nasal secretions were absorbed with cottonwool, and the secretions were weighed.

2.5. Ovalbumin-induced sneezing and nasal itching in actively sensitized guinea pigs

The animals (6-week-old) were exposed to an ovalbumin aerosol (1%) for 10 min on two occasions, 7 days apart. After the last antigen exposure, the animals were randomly allocated to cages and housed in groups of two per cage. They were acclimatized to the environmental conditions for at least 7 days before the experiment. One week after the last antigen exposure, they were subjected to daily topical application of an ovalbumin solution (10 mg/ml, 20 μl × 2) via a nasal drip for three consecutive days as a booster. Five to seven days later, nasal challenge was carried out by topical application of an ovalbumin solution (20 mg/ml, 20 μl × 2) via a nasal drip. The numbers of sneezes and nose rubbing movements were counted for the first 20 min after the antigen challenge. The nose rubbing movements were assumed to be an index of nasal itching.

2.6. Pollen-induced allergic rhinitis in guinea pigs

The animals (3–4 weeks old) were sensitized to Japanese cedar pollen according to the method described by Nabe et al. (1998). Briefly, the animals were sensitized by intranasal instillation of cedar pollen extracts adsorbed onto $Al(OH)_3$ gel at a concentration of 0.3 μg protein/0.3 mg $Al(OH)_3$/3 μl/nostril twice daily for 7 days. Prior to

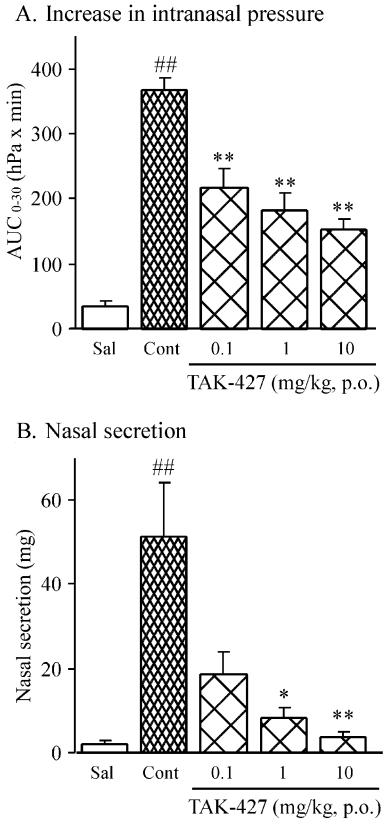

Fig. 2. Effect of TAK-427 on ovalbumin-induced increases in intranasal pressure (A) and nasal secretion (B) in guinea pigs. Animals were sensitized by i.p. injection of ovalbumin (20 μg) + $Al(OH)_3$ (5 mg) four times at 2-week intervals. The sensitized animals were challenged by inhalation of saline or 3% ovalbumin aerosol for 3 min. Intranasal pressure was measured for 30 min after the cessation of inhalation. Nasal secretions were then collected and weighed. The increase in intranasal pressure was expressed as the AUC value from 0 to 30 min after inhalation. TAK-427 was administered 1 h before inhalation. Data represent the means ± S.E.M. for 10 animals. Sal: Saline inhalation group; Cont: Control group. $^{\#\#}P<0.01$ vs. Saline inhalation group (*t*-test), $^{*}P<0.05$, $^{**}P<0.01$ vs. Control (Dunnett's test).

each sensitization, the upper airway mucosal surface was anesthetized by 5-min insufflation of a 4% lidocaine hydrochloride solution mist, generated with an ultrasonic nebulizer (NE-U12, Omron, Osaka, Japan), to prevent rapid elimination of the antigen by ciliary movement. The sensitized animals were then intranasally challenged once a week by inhalation of cedar pollen using a hand-made inhalation apparatus (Nabe et al., 1997), which allows quantitative inhalation of pollen. The apparatus, into which 3 mg of pollen had been poured, was positioned in the left nostril of conscious guinea pigs for 1 min so that the animals would inhale the pollen while breathing spontaneously. During the inhalation period, the right nostril was plugged with a finger. Thereafter, the apparatus was removed and inserted into the right nostril, and the procedure was repeated.

Specific airway resistance (sRaw) was measured using a two-chambered, double-flow plethysmograph system according to the method of Pennock et al. (1979). Briefly, the animal was placed with its neck extending through the partition of a two-chambered box, and sRaw was measured with the data analyzer Pulmos-I (MIPS, Osaka, Japan) and a PC 9821 Xe or PC 9821 Xs (NEC, Tokyo, Japan) after the detection of airflow by sensors installed in both the front and rear chambers.

sRaw was measured before the fifth antigen challenge, and 1 and 4 h after the challenge. The animals were then assigned to six groups, each consisting of 17 animals, based on the increase in sRaw such that there was little difference among the groups.

Experiments were conducted at the sixth antigen challenge, and sRaw was measured in conscious guinea pigs before and 10 min to 10 h after the antigen inhalation challenge. Sneezing frequencies were determined for the first 8 min after the antigen challenge.

2.7. Statistical analysis

Statistical analyses were performed using the *t*-test when two groups were compared, and Dunnett's test, when more than two groups were compared. Values of $P<0.05$ were considered statistically significant. All statistical calcula-

Table 2
Effects of several histamine H_1-receptor antagonists and dexamethasone on ovalbumin-induced increases in intranasal pressure and secretion in sensitized guinea pigs

Drug	Dose (mg/kg, p.o.)	*N*	AUC $_{0-30\ min}$[a] (hPa min)	% Inhibition	Nasal secretion (mg)	% Inhibition
Saline[b]		10	37.62 ± 6.56		2.5 ± 0.6	
Control	–	10	306.46 ± 33.67[c]		47.2 ± 19.5[d]	
Azelastine	0.03	10	179.52 ± 23.99[e]	47	25.9 ± 11.6	48
Azelastine	0.3	10	187.42 ± 43.26	44	17.7 ± 6.1	66
Azelastine	3.0	10	147.79 ± 38.16[f]	59	16.7 ± 11.5	68
Saline[b]		10	29.18 ± 6.12		2.4 ± 0.7	
Control	–	10	269.48 ± 28.34[c]		31.0 ± 7.2[c]	
Terfenadine	0.1	10	333.05 ± 41.20	− 26	41.7 ± 14.2	− 37
Terfenadine	1.0	10	240.54 ± 30.72	12	16.6 ± 4.4	50
Terfenadine	10	10	175.55 ± 24.76	39	13.1 ± 4.9	63
Saline[b]		10	37.18 ± 7.43		2.5 ± 0.6	
Control	–	10	362.23 ± 33.08[c]		49.1 ± 8.2[c]	
Ketotifen	0.01	10	217.73 ± 34.88[f]	44	18.4 ± 5.8[e]	66
Ketotifen	0.1	10	218.03 ± 28.65[f]	44	22.1 ± 9.0[e]	58
Ketotifen	1.0	10	138.08 ± 29.22[f]	69	9.8 ± 4.9[f]	84
Saline[b]		10	47.04 ± 9.87		1.2 ± 0.4	
Control	–	10	329.81 ± 42.90[c]		67.7 ± 21.3[d]	
Cetirizine	0.03	9	247.54 ± 37.20	29	21.0 ± 6.4	70
Cetirizine	0.3	10	222.64 ± 28.60	38	33.8 ± 11.9	51
Cetirizine	3.0	10	179.72 ± 38.06[e]	53	14.2 ± 7.6[f]	80
Saline[b]		10	51.94 ± 12.82		1.7 ± 0.6	
Control	–	9	279.05 ± 31.62[c]		45.1 ± 12.9[c]	
Dexamethasone	1.0	10	255.80 ± 32.63	10	49.3 ± 16.6	− 10
Dexamethasone	3.0	10	176.92 ± 26.91[e]	45	17.8 ± 6.9	63
Dexamethasone	10	10	129.20 ± 23.78[f]	66	9.3 ± 3.2[f]	83

Animals were sensitized by i.p. injection of ovalbumin (20 μg) + $Al(OH)_3$ (5 mg) four times at 2-week intervals. The sensitized animals were challenged by inhalation of a saline or 3% ovalbumin aerosol for 3 min. Intranasal pressure was measured for 30 min after the cessation of inhalation. Histamine H_1-receptor antagonist and dexamethasone were administered 1 or 3 h before the inhalation, respectively.
Data represent the means ± S.E.M.

[a] Area under the curve of increase in intranasal pressure from 0 to 30 min after ovalbumin inhalation.
[b] Saline inhalation group.
[c] $P<0.01$ vs. Saline (*t*-test).
[d] $P<0.05$ vs. Saline (*t*-test).
[e] $P<0.05$ vs. Control (Dunnett's test).
[f] $P<0.01$ vs. Control (Dunnett's test).

tions were performed with the SAS statistical package (SAS Institutes, Cary, NC) in our laboratory.

3. Results

3.1. Histamine-induced nasal reactions in guinea pigs

Intranasal pressure increased rapidly with inhalation of histamine and peaked 10 to 15 min after the cessation of histamine inhalation. The AUC for the increase in intranasal pressure from 0 to 15 min (AUC_{0-15}) was 126.45 ± 14.08 hPa·min in the control group (Fig. 1A). TAK-427 at 0.1–3 mg/kg, p.o. suppressed the histamine-induced increase in intranasal pressure at every time point and decreased the AUC_{0-15} dose-dependently (Fig. 1A), with an ID_{50} value of 0.633 mg/kg, p.o. (Table 1). Statistical significance was observed with 1 and 3 mg/kg (Fig. 1A). Azelastine (0.01–0.3 mg/kg), ketotifen (0.001–0.03 mg/kg), terfenadine (0.1–3 mg/kg) and cetirizine (0.1–3 mg/kg) also showed dose-dependent inhibition of the histamine-induced increase in intranasal pressure; the ID_{50} values were 0.037, 0.010, 0.443, and 0.105 mg/kg, respectively (Table 1). On the other hand, dexamethasone (1–10 mg/kg) did not significantly prevent the histamine-induced increase in intranasal pressure (Table 1).

Nasal secretion was significantly increased by histamine inhalation as compared with saline inhalation. The weight of the nasal secretions collected over the first 15 min after the cessation of histamine inhalation was 17.0 ± 3.0 mg in the control group (Fig. 1B). TAK-427 inhibited histamine-induced nasal hypersecretion, and statistical significance was observed with 0.3 mg/kg and above (Fig. 1B). Among the histamine H_1-receptor antagonists tested, terfenadine (0.1–3 mg/kg) and cetirizine (0.1–3 mg/kg) significantly inhibited the histamine-induced nasal hypersecretion. Azelastine at 0.1 mg/kg and ketotifen at 0.01 mg/kg strongly inhibited the increase in nasal secretion by 82% and 83%, respectively, but these effects were not statistically significant due to a large variation in the control group of this experiment. Dexamethasone (1–10 mg/kg) showed partial inhibition of the histamine-induced nasal hypersecretion with a 63% reduction at the highest dose, although the effect was not statistically significant (data not shown).

3.2. Ovalbumin-induced increases in intranasal pressure and nasal secretion in actively sensitized guinea pigs

The intranasal pressure gradually increased with time and reached a plateau 25 min after the cessation of ovalbumin inhalation. The AUC for the increase in intranasal pressure from 0 to 30 min after antigen inhalation was 367.73 ± 17.46 hPa·min in the control group. TAK-427 (0.1–10 mg/kg) significantly inhibited the antigen-induced increase in intranasal pressure (Fig. 2). However, the inhibition was partial, and the inhibition obtained with the highest dose of TAK-427 was 65% (Fig. 2). Azelastine (0.03–3 mg/kg), ketotifen (0.01–1 mg/kg), cetirizine (0.03–3 mg/kg) and dexamethasone (1–10 mg/kg) also showed partial but significant inhibition of the antigen-induced increase in intranasal pressure (Table 2). Terfenadine at 10 mg/kg inhibited the increase in intranasal pressure by 39%, but the effect was not statistically significant (Table 2).

Nasal secretion was greatly increased by antigen inhalation. The weight of the nasal secretions collected over the first 30 min after the cessation of antigen inhalation was 51.2 ± 13.0 mg in the control group. TAK-427 (0.1–10 mg/kg) inhibited the antigen-induced nasal hypersecretion, and statistical significance was observed with 1 and 10 mg/kg (Fig. 2). Among the other histamine H_1-receptor antagonists tested, ketotifen (0.01–1 mg/kg) and cetirizine (0.03–3 mg/kg) inhibited the antigen-induced increase in nasal secretion significantly (Table 2). Azelastine (0.03–3 mg/kg) and terfenadine (0.1–10 mg/kg) inhibited the increase in nasal

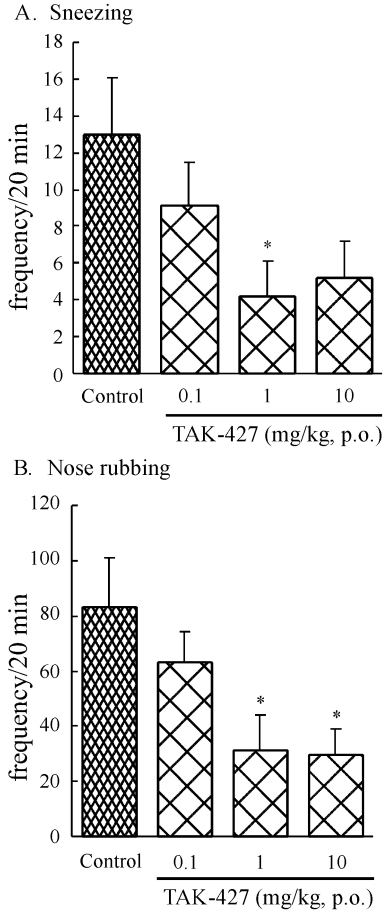

Fig. 3. Effects of TAK-427 on the ovalbumin-induced sneezing (A) and nose rubbing (B) in sensitized guinea pigs. The animals were sensitized as described in Materials and methods. Five to seven days after the last sensitization, nasal challenge was carried out by topical application of an ovalbumin solution (20 mg/ml, 20 μl × 2) via a nasal drip. The numbers of sneezes and nose rubbing movements were counted for the first 20 min after the antigen challenge. TAK-427 was administered 1 h before challenge. Data represent the means ± S.E.M. for 10 animals. *$P < 0.05$, vs. Control (Dunnett's test).

Table 3
Effects of several histamine H_1-receptor antagonists and dexamethasone on the sneezing and nose rubbing caused by ovalbumin challenge in sensitized guinea pigs

Drug	Dose (mg/kg, p.o.)	*N*	Number of sneezes (times/20 min)	Inhibition (%)	Number of nose rubbing movements (times/20 min)	Inhibition (%)
Control		10	8.5 ± 3.4		51.4 ± 16.3	
Azelastine	0.003	10	5.5 ± 1.1	35	52.8 ± 9.8	− 3
	0.03	10	4.4 ± 0.9	48	49.0 ± 8.5	5
	0.3	10	2.8 ± 0.9	67	16.4 ± 5.6[a]	68
Control		10	9.6 ± 2.4		57.7 ± 11.1	
Terfenadine	0.1	10	6.4 ± 1.6	33	42.4 ± 9.5	27
	1	10	5.9 ± 1.6	39	32.9 ± 7.9	43
	10	10	4.3 ± 1.2	55	18.4 ± 4.1[b]	68
Control		10	6.9 ± 1.5		56.0 ± 17.5	
Ketotifen	0.003	10	5.2 ± 1.1	25	36.1 ± 11.0	36
	0.03	10	3.0 ± 0.8	57	15.4 ± 5.2	73
	0.3	10	6.5 ± 1.0	6	36.5 ± 9.6	35
Control		10	9.0 ± 1.7		60.2 ± 12.8	
Cetirizine	0.03	10	6.4 ± 1.1	29	42.7 ± 8.3	29
	0.3	10	2.9 ± 0.5[b]	68	17.2 ± 6.4[b]	71
	3	10	2.5 ± 0.8[b]	72	14.3 ± 5.4[b]	76
Control		10	6.2 ± 1.4		44.9 ± 13.9	
Dexamethasone	1	10	9.2 ± 1.9	− 48	80.6 ± 16.4	− 80
	3	10	5.9 ± 2.1	5	47.1 ± 10.5	− 5
	10	10	3.3 ± 1.0	47	44.1 ± 11.0	2

The animals were sensitized as described in Materials and methods. Five to seven days after the last sensitization, nasal challenge was carried out by topical application of an ovalbumin solution (20 mg/ml, 20 μl × 2) via a nasal drip. The numbers of sneezes and nose rubbing movements were counted for the first 20 min after the antigen challenge. Histamine H_1-receptor antagonist and dexamethasone were administered 1 or 3 h before the challenge, respectively. Data represent the means ± S.E.M.

[a] $P<0.05$ vs. Control (Dunnett's test).

[b] $P<0.01$ vs. Control (Dunnett's test).

secretion with inhibition of 68% and 63% observed at the highest doses, respectively, although these effects were not statistically significant (Table 2). On the other hand, dexamethasone at 10 mg/kg significantly inhibited nasal hypersecretion (Table 2).

3.3. Ovalbumin-induced sneezing and nasal itching in actively sensitized guinea pigs

The application of ovalbumin inside the nostrils induced sneezing and nose rubbing in sensitized guinea pigs. Normal animals hardly responded to ovalbumin solution. In the control group, the numbers of sneezes and nose rubbing movements were 13.0 ± 3.1 and 83.2 ± 18.0 times, respectively, when counted for the first 20 min after antigen challenge. TAK-427 (0.1, 1 and 10 mg/kg) inhibited the antigen-induced sneezing by 30%, 68% and 60%, respectively, and statistical significance was observed with 1 mg/kg (Fig. 3). TAK-427 also inhibited the antigen-induced nose rubbing by 25%, 62% and 64%, respectively, and statistical significance was observed with 1 and 10 mg/kg (Fig. 3).

Cetirizine (0.03–3 mg/kg) also inhibited both the antigen-induced sneezing and nose rubbing, and significant inhibition was observed with doses of 0.3 mg/kg and above (Table 3). Azelastine (0.003–0.3 mg/kg) and terfenadine (0.1–10 mg/kg) apparently inhibited sneezing, although not to a statistically significant degree, and significantly inhibited nose rubbing (Table 3). Ketotifen, though only at 0.03 mg/kg, showed a strong tendency to inhibit the sneezing and nose rubbing (57% and 73% inhibition,

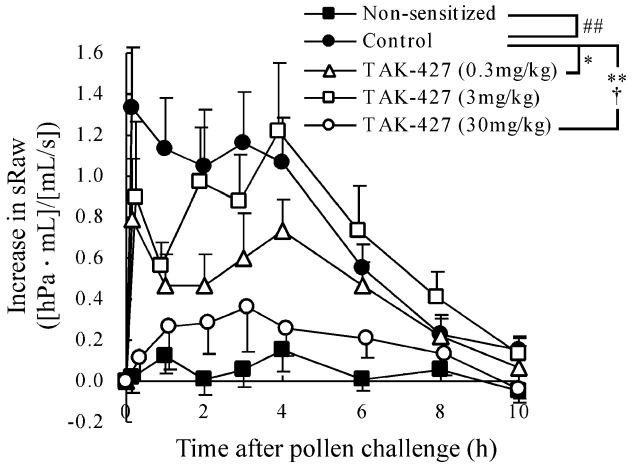

Fig. 4. Effects of TAK-427, azelastine and ketotifen on the increase in specific airway resistance (sRaw) after the sixth challenge with cedar pollen in sensitized guinea pigs. The sensitized animals were intranasally challenged once a week. At the sixth antigen challenge, the specific airway resistance (sRaw) was measured before and 10 min to 10 h after antigen inhalation in conscious guinea pigs. TAK-427 was orally administered 1 h before antigen challenge. Data represent the means ± S.E.M. for 17 guinea pigs. Statistical analyses were performed using AUC data. $^{\#\#}P<0.01$ vs. Non-sensitized (*t*-test); $^{*}P<0.05$, $^{**}P<0.01$ vs. Control (AUC_{0-2}: Dunnett's test); $^{\dagger}P<0.05$ vs. Control (AUC_{2-10}: Dunnett's test).

Table 4
Effects of TAK-427, azelastine and ketotifen on increases in specific airway resistance (sRaw) and sneezing after the sixth challenge with cedar pollen in sensitized guinea pigs

Drug	Dose (mg/kg, p.o.)	*N*	Early-phase AUC_{0-2}	Inhibition (%)	Late-phase AUC_{2-10}	Inhibition (%)	Sneezing (frequency/8 min)	Inhibition (%)
Non-sens	–	17	0.130 ± 0.120	(100)	0.383 ± 0.456	(100)	5.6 ± 1.0	(100)
Control	–	17	2.232 ± 0.464[a]	–	5.002 ± 0.905[a]	–	12.2 ± 1.7[a]	–
TAK-427	0.3	17	1.059 ± 0.290[b]	56	3.356 ± 0.897	36	6.8 ± 1.5[b]	82
	3	17	1.451 ± 0.274	37	5.611 ± 1.120	− 13	7.9 ± 1.5	65
	30	17	0.439 ± 0.292[c]	85	1.549 ± 0.696[b]	75	7.1 ± 1.4	77
Azelastine	1	17	1.725 ± 0.502	24	3.847 ± 0.746	25	10.0 ± 1.7	34
Ketotifen	1	17	1.931 ± 0.455	14	4.695 ± 0.896	7	9.2 ± 1.6	46

The sensitized animals were intranasally challenged once a week. At the sixth antigen challenge, the specific airway resistance (sRaw) was measured before and 10 min to 10 h after antigen inhalation in conscious guinea pigs. TAK-427 and other drugs were orally administered 1 h before the antigen challenge. Data represent the means ± S.E.M. for 17 guinea pigs. Non-sens: non-sensitized group.

[a] $P < 0.01$ vs. Non-sensitized group (*t*-test).

[b] $P < 0.05$ vs. Control (Dunnett's test).

[c] $P < 0.01$ vs. Control (Dunnett's test).

respectively), but neither effect was statistically significant (Table 3). Dexamethasone (1–10 mg/kg) did not significantly inhibit the sneezing or nose rubbing. The highest dose of dexamethasone tended to decrease the number of sneezes but barely affected the nose rubbing (Table 3).

3.4. Cedar pollen-induced allergic rhinitis in guinea pigs

Inhalation of cedar pollen induced a biphasic increase in sRaw in the sensitized guinea pigs, whereas no obvious changes were observed in non-sensitized guinea pigs (Fig. 4). As shown in Table 4, the early and late responses were evaluated from the AUC for the increase in sRaw from 0 to 2 h (AUC_{0-2}) and from 2 to 10 h (AUC_{2-10}) after the antigen challenge, respectively. The AUC_{0-2} and AUC_{2-10} in the sensitized guinea pigs were 2.232 ± 0.464 and 5.002 ± 0.905 ([hPa·ml]/[ml/s])h, respectively, while those for the non-sensitized guinea pigs were 0.130 ± 0.120 and 0.383 ± 0.456 ([hPa·ml]/[ml/s])h, respectively (Table 4). TAK-427 at 0.3 to 30 mg/kg, p.o. decreased the AUC_{0-2} by 56%, 37% and 85%, respectively, and statistical significance was observed with 0.3 and 30 mg/kg. TAK-427 at 0.3 and 30 mg/kg decreased the AUC_{2-10} by 36% and 75%, respectively, and significant inhibition was observed with 30 mg/kg, while TAK-427 at 3 mg/kg did not inhibit the late response (Table 4 and Fig. 4). On the other hand, ketotifen and azelastine had no significant effects on either the early or the late increase in sRaw after the antigen challenge (Table 4).

The inhalation of cedar pollen induced sneezing in both the sensitized and non-sensitized guinea pigs; the numbers of sneezes over the first 8 min after antigen challenge were 12.2 ± 1.7 and 5.6 ± 1.0, respectively (Table 4). Sneezing frequency in the sensitized group was significantly higher than that in the non-sensitized group, and the difference was thought to be related to the allergic reaction following inhalation of the pollen. TAK-427 at 0.3, 3 and 30 mg/kg inhibited the sneezing by 82%, 65% and 77%, respectively. Statistical significance was observed with 0.3 mg/kg of TAK-427 (Table 4). On the other hand, the histamine H_1-receptor antagonist ketotifen and azelastine inhibited the sneezing by 46% and 34%, respectively, but neither effect was statistically significant (Table 4).

4. Discussion

Our results show that TAK-427 suppresses histamine- and ovalbumin-induced increases in intranasal pressure, sneezing, nasal itching and nasal hypersecretion. TAK-427 also ameliorates the nasal obstruction induced by repeated exposure of sensitized guinea pigs to Japanese cedar pollen, which is resistant to histamine H_1-receptor antagonists.

Allergic rhinitis is a typical IgE-mediated disease associated with nasal symptoms such as sneezing, itching, nasal hypersecretion and nasal obstruction, and histamine H_1-receptor antagonists are effective for acute phase symptoms, but often ineffective for nasal obstruction due to inflammation. TAK-427 is a novel anti-allergic agent that has long-lasting antihistaminic activity without sedative side effects (Fukuda et al., 2003). TAK-427 has also been shown to have anti-inflammatory activity in a guinea pig model of eczema (Fukuda et al., 2002). In this study evaluating the potential of TAK-427 as a therapeutic agent for allergic rhinitis, the effects of TAK-427 on acute phase nasal responses and nasal obstruction were investigated using several guinea pig models of allergic rhinitis.

TAK-427 and most histamine H_1-receptor antagonists tested inhibited histamine-induced symptoms such as increased intranasal pressure and nasal hypersecretion, and suppressed ovalbumin-induced acute phase nasal responses including sneezing, nasal itching, increased intranasal pressure and nasal hypersecretion. The effective doses of TAK-427 for ovalbumin-induced nasal responses were approximately equal to those required for inhibition of histamine-induced nasal responses (Table 1) and skin reactions (Fukuda et al., 2003). These results suggested that the suppressive effects of TAK-427, at least those on

these ovalbumin-induced acute phase nasal responses, may be based on its antihistaminic effect. We anticipate that, like other histamine H_1-receptor antagonists, TAK-427 may be effective in allergic rhinitis patients suffering mainly from acute phase symptoms.

Nasal obstruction is experimentally induced by a single challenge with antigens in sensitized animals, and is suppressed by histamine H_1-receptor antagonists (Demoly et al., 1999). However, the situation seems to be very different in patients who are exposed daily to airborne antigens, because clinically, classical histamine H_1-receptor antagonists and some second-generation histamine H_1-receptor antagonists including terfenadine have proven to be ineffective for the nasal obstruction frequently experienced by patients suffering from allergic rhinitis (Naclerio, 1991; Simons, 1989; Rökenes et al., 1988). Recent studies suggest that thromboxane A_2 and peptide leukotrienes participate in nasal obstruction (Yamasaki et al., 1997; Terada et al., 1998; Fujita et al., 1999; Donnelly et al., 1995), and antagonists were thus developed as therapeutic agents for allergic rhinitis because of their ameliorating effect on nasal obstruction. In order to evaluate the effect of TAK-427 on nasal obstruction, we used the guinea pig rhinitis model in which biphasic nasal obstruction was caused by repetitive challenge with cedar pollen. In this experimental model, seratrodast (thromboxane A_2 antagonist), pranlukast (peptide leukotriene antagonist), naphazoline (α_1-stimulant) and dexamethasone were effective for the nasal obstruction (Yamasaki et al., 2001; Imai et al., 2001) but histamine H_1-receptor antagonists such as terfenadine and mepyramine were not (Yamasaki et al., 2001; Mizutani et al., 2001; Nabe et al., 2001). Our present study also indicated that azelastine and ketotifen were ineffective for the nasal obstruction in this model even when the two drugs were given in doses 30 to 100 times higher than those required for 50% inhibition of histamine-induced responses (Table 1). In terms of pharmacological properties, this experimental model has many similarities to patients with allergic rhinitis (Naclerio, 1991; Pipkorn et al., 1987; Terada et al., 1998; Donnelly et al., 1995).

In the present study, TAK-427 showed potent and significant inhibition of the pollen-induced nasal obstruction, and this inhibitory effect of TAK-427 may not have been due to its antihistaminic activity because none of the histamine H_1-receptor antagonists tested had a significant effect. These results suggest that TAK-427 may be useful for treatment of nasal obstruction refractory to antihistamine treatment in allergic rhinitis patients.

As we have already reported, TAK-427 has an anti-inflammatory effect in addition to its antihistaminic effect; TAK-427 suppressed mRNA expression of proinflammatory cytokines and dermal inflammation leading to eczema formation in a guinea pig model of eczema that is also resistant to histamine H_1-receptor antagonists (Fukuda et al., 2002). TAK-427 does not have an antagonistic effect against thromboxane A_2 and peptide leukotriene receptors (unpublished data). The anti-inflammatory effect of TAK-427 may contribute to the inhibition of nasal obstruction; however, the precise mechanisms responsible for amelioration of the pollen-induced nasal obstruction by TAK-427 remain to be clarified.

From these results, we concluded that TAK-427 might be useful in the treatment of allergic rhinitis because it directly ameliorates nasal obstruction in addition to inhibiting acute phase nasal symptoms such as sneezing, nasal itching and nasal hypersecretion.

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