

Protective effects of ketotifen on allergen-induced bronchoconstriction and skin weal

G. TAMURA, S. MUE and T. TAKISHIMA

The First Department of Internal Medicine, Tohoku University School of Medicine, Sendai, Japan

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Summary

A single-blind cross-over method was used to investigate the effects of ketotifen on the total respiratory resistance response curve to inhalation of a high concentration of allergen (1:5 w/v house dust or 1:10 w/v Japanese cedar pollen) and the weal response to intradermal injection of serially diluted allergen (1:10³–1:10⁶ w/v). Five subjects received 2 mg of ketotifen every 12 hr for the first week and an inactive placebo in the same manner for the second week. The remaining five subjects received the placebo for the first week and ketotifen for the second week. Ketotifen did not alter base-line pulmonary function, but significantly delayed the time from the start of allergen exposure to the beginning of elevation of total respiratory resistance ($P < 0.02$), and the time from the start of allergen exposure to the point at which total respiratory conductance decreased by 35% from the base-line value ($P < 0.01$). Ketotifen also decreased skin test sensitivity significantly ($P < 0.01$).

Introduction

This was a routinely performed allergen inhalation challenge with the single exposure method [1]. A previous report [1] demonstrated that the single exposure method can be used to detect specific bronchial response to house dust allergen, with very little discomfort on the part of the patient. Using this method, allergen-induced bronchoconstriction can be continuously and graphically observed as the change in total respiratory resistance (Rrs), using the forced 3 Hz oscillation technique. Consequently, appearance time (the time from allergen exposure to the beginning of elevation of Rrs) has been introduced as a new index for allergen inhalation challenge.

Correspondence: Dr T. Takishima, The First Department of Internal Medicine, Tohoku University School of Medicine, 1-1, Seiryomachi, Sendai, 980, Japan.

Abbreviation used: Rrs: total respiratory resistance, Rrs base: mean value of Rrs during inhalation of physiologic saline, Ta: time from the start of allergen exposure to the beginning of elevation of Rrs, Grs: total respiratory conductance, PT₃₅–Grs: time from the start of allergen exposure to the point at which Grs decrease by 35% from the base-line value, Δ Grs/ Δ t: decrease in Grs per min from Ta to PT₃₅–Grs.

Ketotifen is a tricyclic derivative of the benzocycloheptathiophene group. In Japan it has been used in the treatment of asthma, especially extrinsic asthma, since 1981. *In vitro* studies have demonstrated that ketotifen can inhibit antigen-induced release of chemical mediators from mast cells [2] and also has both antihistaminic and antianaphylactic properties [3]. Clinical studies [4–6] have demonstrated that ketotifen can inhibit histamine-, allergen- and exercise-induced bronchoconstriction.

In this study, the effects of ketotifen on the continuous change in Rrs to inhaled allergen and skin weal response to injected allergen were examined.

Subjects and methods

Subjects

Ten asthmatic subjects (three males and seven females) were studied. Anthropomorphic data and airway reactivity to metacholine [7] are summarized in Table 1. Eight subjects had positive bronchial responses to house dust allergen and two subjects had positive bronchial responses to Japanese cedar pollen allergen. None of the subjects had experienced upper respiratory infection or exacerbation of asthma for at least 4 weeks prior to the study.

Protocol

The study was carried out in a single-blind cross-over fashion. Subjects 1–5 received ketotifen for the first week and an inactive placebo for the second week. The remaining five patients received the placebo and the drug in the reverse order. Each allergen inhalation challenge was carried out at the same time of day on a different day, 2 hr after administration of the last dose.

Ketotifen and placebo, in 1 mg capsule form, were administered orally, two

Table 1. Patient characteristics

Subject	Age		B.H. (cm)	B.W. (kg)	Airway reactivity to methacholine			
	(years)	Sex			Rrs base (cmH ₂ O/l sec)	*Dmin (Unit)	†SGrs/Gr _s base (l min)	‡Medication
1	40	F	149	56	5.5	0.069	0.025	T,S
2	48	F	154	60		not done		T
3§	30	F	153	50	7.4	0.114	0.039	T
4	33	M	170	83	5.0	3.391	0.136	none
5	64	F	144	48	6.3	0.016	0.143	none
6	58	M	167	70	8.4	0.049	0.033	M
7§	30	F	158	45	3.0	0.265	0.078	none
8	54	M	168	70	5.2	0.003	0.034	T,P
9	27	F	153	48	5.6	0.082	0.009	T,Tc
10	30	F	153	48	6.0	0.342	0.042	T,P
Mean	41.4		156.9	57.8	5.82	0.481	0.060	
s.d.	13.6		8.7	12.7	1.52	1.097	0.049	

*Dmin is the cumulative dose of inhaled methacholine from the start to the point at which Rrs begins to increase. (One unit is 1-min inhalation of 1 mg/ml methacholine).

†SGrs/Gr_s cont is the ratio of the decrease in Grs per min to the base-line value of Grs.

§Subjects sensitive to Japanese cedar pollen allergen.

‡T:theophylline, S:salbutamol, M:metaproterenol, P:procaterol, Tc:terbutaline.

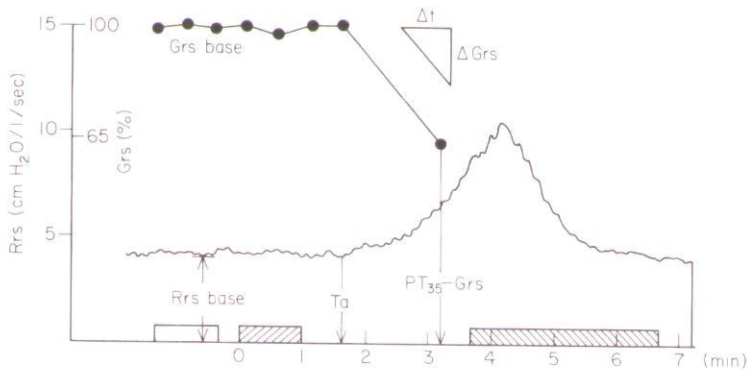


Fig. 1. A typical positive respiratory resistance (Rrs) response curve to house dust allergen. After a 1-min inhalation of physiologic saline ($\square$), aerosolized 1:5 w/v house dust allergen ($\blacksquare$) was inhaled for 1 min, and, finally, aerosolized 2% metaprotefenol ($\blacksquare$) was inhaled for 3 min immediately after Rrs had reached twice baseline value. Rrs base: the mean value of Rrs during inhalation of physiologic saline, Ta: the time from the start of allergen exposure to the beginning of elevation of Rrs, PT₃₅-Grs: the time from start of allergen exposure to the point at which total respiratory conductance (Grs) decreased by 35% from the base-line value, and $\Delta\text{Grs}/\Delta t$ —the decrease in Grs per min from Ta to PT₃₅-Grs.

capsules every 12 hr. However, subject 2 took one capsule each of ketotifen and placebo every 12 hr, because two capsules of the drug produced daytime sedation. Additionally, β_2 -adrenergic stimulating agents and phosphodiesterase inhibitory agents were added when necessary, but they were withheld for at least 12 hr before each allergen inhalation challenge. No subject received DSCG, antihistamine or glucocorticosteroid agents during the period of study.

Allergen inhalation challenge

Allergen inhalation challenge was performed using the single exposure method [1]. We estimated Rrs with a device (Astograph; TCK 6100H, CHEST) which utilizes the forced 3 Hz oscillation technique. Briefly, 3 Hz sine wave oscillations were generated from a large speaker (30 cm woofer) mounted in a speaker box. The airflow at the mouthpiece was measured with a heated pneumotachometer (Fleisch no. 2) and a differential pressure transducer (Validyne MP45-1). The mouth pressure was measured with a differential pressure transducer (Validyne MP45-1). Nebulizers were connected to the main tube near the mouthpiece. A constant bias flow of 0.4 l/sec was extracted between the nebulizer and the flowmeter to avoid dead space ventilation. Rrs calculation was performed with an analog computer. Rrs was continuously plotted on an X-Y recorder (Watanabe WX-4420).

Aerosol was generated by a Bird nebulizer connected near the mouthpiece and driven at a constant air flow of 6 l/min. Subjects, wearing a noseclip, sat on a chair and performed tidal breathing through the mouthpiece. Subjects inhaled an aerosol of 1:5 w/v house dust allergen or 1:10 w/v Japanese cedar pollen allergen for 1 min, after it had been confirmed that 1-min inhalation of physiologic saline did not change their base-line Rrs. Rrs was observed until it had reached twice the base-line value, or for 10 min after the start of allergen exposure, and then an aerosol of 2% metaprotefenol was inhaled for 3 min.

In order to evaluate the Rrs response curve to inhaled allergen, as shown in Fig. 1, four indices were defined (1) the base-line value of Rrs (Rrs base); the mean value of

Rrs during inhalation of physiologic saline; (2) appearance time (Ta): the time from the start of allergen exposure to the beginning of elevation of Rrs; (3) provocation time (PT₃₅-Grs): the time from the start of allergen exposure to the point at which total respiratory conductance (Grs) decreased by 35% from the base-line value, and (4) the rate of decrease of Grs ($\Delta\text{Grs}/\Delta t$): the decrease in Grs per minute from Ta to PT₃₅-Grs. The response was considered to be positive when Grs decreased by more than 35% from its base-line value within 10 min of the start of allergen exposure.

Intradermal skin tests with allergen

A 27-gauge 3/4-inch needle was used for an intradermal injection of 0.02 ml of allergen to the forearm just below the elbow. This produced a weal approximately 4 × 4 mm. Prior to allergen inhalation challenge, allergens serially diluted from 10⁻³ to 10⁻⁶ w/v were given, and the largest and smallest dimensions of weals were measured 10 min after injection. We expressed the results of the intradermal skin tests as the highest dilution required for a positive response (i.e., a weal of more than 9 × 9 mm). The responses was considered to be negative when a 10⁻³ dilution induced a weal of less than 9 × 9 mm.

Statistical analysis

Paired Student's *t*-test was used to examine all paired data.

Results

Table 2 summarizes base-line pulmonary function prior to each allergen inhalation challenge. Mean $\pm$ s.d. of the FEV₁ values (% predicted) was 77.9 $\pm$ 25.8% after placebo treatment and 82.0 $\pm$ 26.2% after ketotifen treatment and that of the Rrs values was 5.97 $\pm$ 1.62 and 5.92 $\pm$ 1.70 cmH₂O/l/sec, respectively. There was no statistical difference in base-line pulmonary function between the placebo-treated and ketotifen-treated groups.

As shown in Fig. 2, the values of Ta (Mean $\pm$ s.d.) were 1'50" $\pm$ 1'03" after placebo treatment and 4'09" $\pm$ 3'17" after ketotifen treatment ($P < 0.02$). The PT₃₅-Grs values

Table 2. Pulmonary function prior to each allergen inhalation challenge

Subject	Post-placebo		Post-ketotifen	
	FEV ₁ (%predicted)	Rrs (cmH ₂ O/l/sec)	FEV ₁ (%predicted)	Rrs (cmH ₂ O/l/sec)
1	79	7.8	78	7.8
2	76	7.0	89	6.0
3	58	7.8	77	7.5
4	104	3.5	105	3.0
5	87	6.0	90	6.0
6	62	5.4	60	5.5
7	127	3.5	130	3.3
8	37	6.2	37	7.2
9	90	5.0	95	5.4
10	59	7.5	59	7.5
Mean	77.9	5.97	82.0	5.92
s.d.	25.8	1.62	26.2	1.70

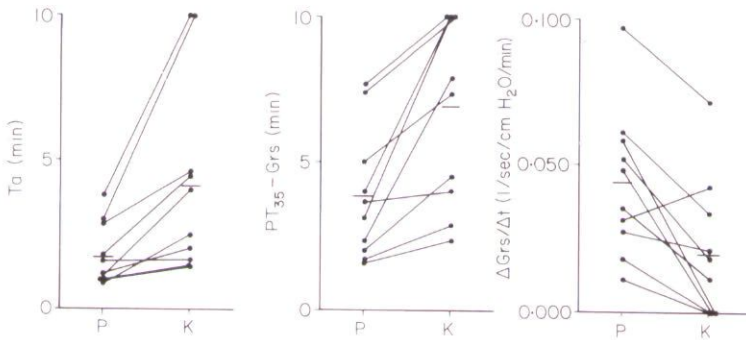


Fig. 2. Results obtained with the single exposure method after placebo treatment (P) and ketotifen treatment (K). The horizontal bars indicate mean values. The values of T_a (Mean $\pm$ s.d) are $1'50'' \pm 1'03''$ after placebo treatment and $4'09'' \pm 3'17''$ after ketotifen treatment ($P < 0.02$), those of $PT_{35}\text{-GrS}$ are $3'52'' \pm 2'12''$ and $6'54'' \pm 3'11''$ ($P < 0.01$), and those of $\Delta\text{GrS}/\Delta t$ are 0.044 ± 0.025 and 0.020 ± 0.023 l/sec/cmH₂O/min ($P < 0.01$), respectively

were $3'52'' \pm 2'12''$ with placebo and $6'54'' \pm 3'11''$ with ketotifen ($P < 0.01$), and those of $\Delta\text{GrS}/\Delta t$ were 0.044 ± 0.025 l/sec/cmH₂O/min with placebo and 0.020 ± 0.023 l/sec/cmH₂O/min with ketotifen ($P < 0.01$). Four subjects had negative responses to inhaled allergen after ketotifen treatment and, in two of these subjects, Rrs 10 min after allergen exposure did not change from Rrs base at all. Thus, ketotifen significantly reduced bronchial response to inhaled allergen.

Figure 3 shows the results of weal response after both ketotifen treatment and placebo treatment. Ketotifen significantly reduced skin test sensitivity ($P < 0.01$).

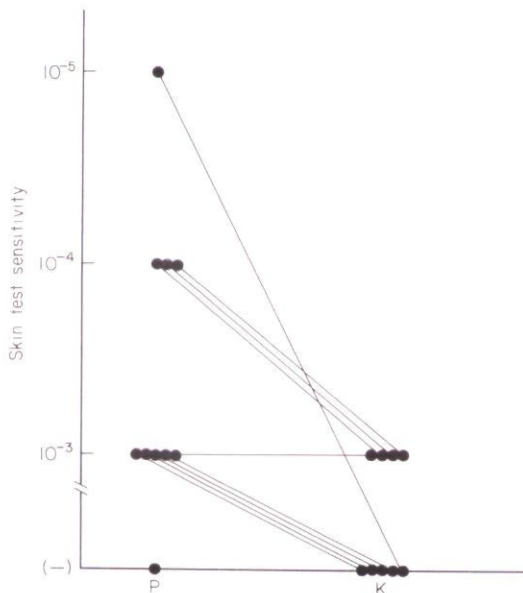


Fig. 3. Skin test sensitivity after placebo treatment (P) and ketotifen treatment (K). Skin test sensitivity is expressed as the highest dilution required for a positive response (i.e., a weal of more than 9×9 mm). (—) Indicates that a 10^{-3} dilution induced a weal of less than 9×9 mm. Ketotifen significantly reduced skin test sensitivity ($P < 0.01$).

Discussion

In this study, 1 week treatment with ketotifen at a dosage of 2 mg every 12 hr reduced the bronchial response to inhaled allergen. Ketotifen, in the routine treatment of asthma, was administered orally at a dosage of 1 mg every 12 hr. Generally, the maximal clinical effect of ketotifen is obtained after 4–5 weeks of treatment [8]. Other investigators [4–6] have demonstrated that long-term treatment with ketotifen at this dosage protects against allergen-, histamine-, and exercise-induced bronchoconstriction. However, Esav *et al.* [9] showed that 3 weeks treatment with ketotifen at this dosage did not reduce allergen-induced bronchoconstriction. Therefore, in this study ketotifen was administered at a dosage of 2 mg every 12 hr for 1 week.

It was previously reported that the single exposure method with house dust allergen is highly reproducible, when the difference in Rrs base between the first test and the second test is within $\pm 15\%$. As shown in Results, the difference in Rrs base between ketotifen-treated and placebo-treated groups was within $\pm 15\%$ and ketotifen significantly delayed PT₃₅-Grs ($P < 0.01$). Thus, these results showed that ketotifen significantly reduces bronchial responses to inhaled allergen. Beta-adrenergic stimulating agent and theophylline also reduce antigen-induced bronchospasm directly by relaxation of bronchial smooth muscle and/or indirectly by inhibition of allergen-induced mediator release [10]. To the authors' knowledge, however, it has not been previously reported that ketotifen has the property of direct relaxation of bronchial smooth muscle. Therefore, it is concluded that ketotifen significantly reduces allergen-induced bronchoconstriction via pathways other than dilatation of bronchial smooth muscle.

In vitro studies have demonstrated that histamine is released within 1–2 min after allergen challenge [11–13]. In allergen-induced bronchoconstriction, it has also been demonstrated that plasma histamine increases within a few minutes after inhalation of allergen [14,15]. Thus, it is possible that histamine is the mediator first released by allergen challenge. Martin & Roemer [2] showed that ketotifen can inhibit the histamine effect on H₁ receptors as well as the antigen-induced release of histamine from mast cells. As stated in Results, ketotifen significantly delayed Ta from $1'50'' \pm 1'03''$ to $4'09'' \pm 3'17''$. Therefore, Ta may reflect the time when histamine released by inhaled allergen begins to induce bronchoconstriction.

In vivo studies [9,16,17] of the effect of H₁ receptor blockers on allergen-induced bronchoconstriction have suggested that mediators other than histamine are released 10 min after allergen exposure. In this study, it was only possible in four subjects to observe continuously the change in Rrs to inhaled allergen for 10 min after allergen exposure following ketotifen treatment, because the single exposure method for allergen inhalation challenge was used. As stated in Results, Rrs 10 min after allergen exposure did not change from the base-line value at all in two of these subjects. Therefore, this data suggest two possibilities; one is that ketotifen inhibits allergen-induced release of histamine as well as other mediators and the other is that it directly inhibits the action of these mediators. However, this remains inconclusive until further studies are conducted.

The results also demonstrated that ketotifen can decrease allergen-induced skin weal. There is evidence [18–21] that the H₁ blockers, isoproterenol and dibutyryl cyclic-AMP inhibit weal response to intradermal injection of allergen. Thus, weal response to

injected allergen is inhibited not only by inhibiting the histamine effect on H_1 receptors but also by inhibiting histamine release from mast cells. *In vitro* study [2] previously showed that ketotifen has both properties. However, in this study, it is not possible to conclude which property of ketotifen is mainly responsible for the decreased allergen-induced skin weal.

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