Antiallergic Effects of ZCR-2060: Effect on Allergic Cutaneous Reactions and Rhinitis Models in Mice and Rats

Tooru Abe^{1,2}, Takeshi Omata², Kenji Yoshida², Yoshihide Segawa², Kazuo Matsuda² and Hiroichi Nagai¹

¹Department of Pharmacology, Gifu Pharmaceutical University, 5-6-1 Mitahora-higashi, Gifu 502, Japan

²Department of Pharmacology, Central Research Laboratories, Zeria Pharmaceutical Co., Ltd., Saitama 360-01, Japan

Received February 28, 1994 Accepted June 16, 1994

ABSTRACT—The antiallergic action of 2-[2-[4-(diphenylmethyl)-1-piperadiny]ethoxy] benzoic acid maleate (ZCR-2060) was investigated on allergic cutaneous reactions and nasal vascular permeability in mice and rats. ZCR-2060 markedly inhibited immediate allergic cutaneous reactions, including passive cutaneous anaphylaxis (PCA) in rats and mice; histamine-, compound 48/80- and calcium ionophore A 23187-induced cutaneous reactions in rats; and biphasic skin reactions mediated by monoclonal IgE antibody and epicutaneous challenge with antigen in mice, but did not affect 5-hydroxytryptamine-induced cutaneous reaction in rats. The antigen-induced nasal vascular permeability increase in actively and passively sensitized rats and histamine-induced nasal vascular permeability increase in rats (allergic rhinitis model) were clearly inhibited in a dose-dependent fashion by ZCR-2060. Moreover, ZCR-2060 significantly inhibited antigen-induced anaphylactic histamine release from rat peritoneal mast cells and carrageenin-induced paw edema in rats. These results suggest that ZCR-2060 has antiallergic effects on allergic cutaneous reactions and experimental rhinitis, probably due to histamine H₁-receptor blockage and the inhibition of histamine release.

Keywords: Antiallergy, Allergic skin reaction, Allergic rhinitis, Histamine H₁-receptor antagonist, ZCR-2060

2-[2-[4-(Diphenylmethyl)-1-piperadiny]ethoxy] benzoic acid maleate (ZCR-2060) is a newly synthesized anti-allergic agent. In our previous report (1), we showed that this compound antagonized the specific binding of ³H-mepyramine to the membranes of guinea pig lung and brain and histamine-induced contraction of isolated guinea pig ileum and trachea, without affecting ileal contractions induced by carbachol, 5-hydroxytryptamine (5-HT) and barium chloride. In addition, the histamine-induced cutaneous reaction in rats was markedly inhibited by orally administered ZCR-2060. However, ZCR-2060, even at high doses, did not affect thiopental-induced sleep and spontaneous ambulatory activity in mice. These results suggest that ZCR-2060 selectively binds to the histamine H₁-receptor, without affecting the CNS.

In general, immediate hypersensitivity, which involves urticaria, allergic rhinitis and asthma, is mediated by various chemical mediators released from mast cells or basophils (2). It is well known that various anaphylactic chemical mediators are divided into preformed mediators, which include histamine and 5-HT, and newly syn-

thesized mediators, which include leukotrienes and PAF (3). Various organs show distinct forms of immediate hypersensitivity involving different mediators and target cell types (4). Consequently, antihistamines have been useful in the management of allergic disorders caused by histamine (5). Recently developed histamine H₁-receptor antagonists such as terfenadine, cetirizine and astemizole have been shown to give better clinical results without the CNS side effects seen with classical antihistamines (6). Furthermore, recent findings suggest that the newer non-sedating antihistamines not only block the histamine H₁-receptor but also inhibit chemical mediator release, inflammatory cell activation and infiltration (7).

In the present study, the antiallergic effects of ZCR-2060 on experimental allergic cutaneous reaction and rhinitis in mice and rats were investigated. The antiinflammatory effect of the compound on carrageenin-induced paw edema in rats was also investigated.

MATERIALS AND METHODS

Animals

The following animals were used: female and male SD rats (weighing 120–270 g; Charles River Japan, Atsugi), male Wistar rats (weighing 150–200 g; Japan SLC, Hamamatsu), male and female BALB/c mice (5 weeks of age, Charles River Japan or 10 weeks of age, Japan SLC).

Drugs

ZCR-2060, ketotifen fumarate (ketotifen) and cetirizine dihydrochloride (cetirizine) were synthesized, and terfenadine was extracted from Triludane® (Shionogi, Osaka) in the Central Research Laboratory, Zeria Pharmaceutical Co., Ltd. (Saitama). Prednisolone (acetate, aqueous suspension; Takeda, Osaka) and indomethacin (Sigma, St. Louis, MO, USA) were purchased commercially.

For the *in vivo* studies, these drugs were suspended in 0.5% methyl cellulose saline solution. For the *in vitro* study, these drugs were dissolved in dimethylsulfoxide and diluted in phosphate-buffered saline (pH 7.4).

Antigens

Ascaris extract coupled with the 2,4-dinitrophenyl group (DNP-As) was prepared by the method of Tada and Okumura (8). Bovine serum albumin (BSA, Sigma) was coupled with the DNP group according to the method of Lee and Sehon (9). Dinitrofluorobenzene (DNFB; Nacalai Tesque, Kyoto) and ovalbumin (OA, Sigma) were also used.

Antisera and monoclonal antibody

Rat anti-DNP and anti-OA IgE serum were prepared according to the method of Tada and Okumura (8). Briefly, female SD rats were immunized *s.c.* with 1 mg protein of DNP-As or OA with killed organisms (2×10^{10}) of *Bordetella pertussis* (Wako, Osaka) by injection into the four food pads and then boosted by injecting *i.m.* 0.5 mg protein of DNP-As or OA alone 5 days later. They were then bled 3 days after the booster injection. The anti-DNP and anti-OA IgE antibody titers of each antiserum were 1 : 128–256 as estimated by 48-hr homologous PCA in rats. Monoclonal anti-DNP IgE antibody prepared in ascites fluid of BALB/c $\times$ C57BL/6 F₁ mice was purchased from Serotec, Ltd. (Bicester, UK). The 48-hr PCA in the mouse ear titer of the antibody was 1 : 512–1024.

Forty-eight-hour homologous PCA in rats

Male SD rats were injected intradermally with 0.1 ml of homologous anti-DNP IgE serum at 1/60 dilution into the shaved back. After 48 hr, PCA was elicited by an *i.v.* injection of 2.5 mg of DNP-BSA dissolved in 0.5% Evans

blue saline solution in a volume of 1 ml. The animals were sacrificed 30 min after antigen challenge, and the skin samples were removed for the colorimetric measurement of the bluing spot. The amount of the dye extravasated due to PCA was measured by the method of Katayama et al. (10). ZCR-2060 was administered orally 1 hr or at varying times before the antigen challenge. The other drugs were administered orally 1 hr before the antigen challenge.

Mediator- or mediator releaser-induced cutaneous reactions in rats

This experiment was carried out according to the previously described method (11, 12). Briefly, male Wistar rats were injected intradermally into the shaved back with 0.1 ml of histamine (dihydrochloride, 20 μ g/ml; Nacalai Tesque), 5-HT (creatinine sulfate, 0.5 μ g/ml; Sigma), compound 48/80 (0.5 μ g/ml, Sigma) or calcium ionophore A23187 (A23187, 50 μ g/ml; Sigma). Immediately after the injection, the animals received an intravenous injection of 1 ml of 0.5% Evans blue saline solution. Thirty minutes later, they were sacrificed and the reaction sites were excised for colorimetric measurement of extravasated dye. ZCR-2060 was administered orally 1 hr before the injection of each inducer.

IgE antibody-mediated mouse ear PCA

PCA was elicited in the ear of male BALB/c mice as previously reported (13, 14). Briefly, monoclonal anti-DNP IgE antibody at 1/20 dilution was injected in a volume of 10 μ l into each side of the ears under ether anesthesia. After 2 hr, mice were challenged with an intravenous injection of 0.25 ml of 0.5% Evans blue saline solution containing 0.25 mg of DNP-BSA. Thirty minutes later, the mice were sacrificed, and their ears were removed for determination of extravasated dye. Each drug was administered orally 1 hr before the antigen challenge.

IgE antibody-mediated biphasic skin reactions in mice

This experiment was carried out according to the method reported previously (15). Briefly, female BALB/c mice were passively sensitized by an *i.v.* injection of monoclonal anti-DNP IgE antibody (1 ml/body). After 24 hr, biphasic skin reactions were induced by spreading the ears with 25 μ l of DNFB at a concentration of 0.5% in acetone-olive oil (3 : 1). The ear thickness was measured with an engineer's micrometer (Ozaki, Tokyo) at 0, 1 and 24 hr after the challenge. Each drug was given intraperitoneally 1 hr before the antigen challenge.

Nasal vascular permeability induced by antigen and histamine

These experiments were performed according to the

method of Kojima et al. (16) using actively sensitized, passively sensitized and non-sensitized male SD rats. For active sensitization, rats were immunized with OA and killed *Bordetella pertussis* as previously described. The animals were operated on 13 or 14 days after the first immunization. Passive sensitization was carried out by an injection of 5 ml/kg of homologous anti-OA IgE serum at 1/4 dilution intravenously. After 48 hr, they were operated on. Non-sensitized rats were used to determine histamine induced nasal vascular permeability.

The sensitized and non-sensitized rats were anesthetized by an i.p.-injection of pentobarbital sodium (30–40 mg/kg; Dinabot, Osaka). The trachea was cannulated with a polyethylene tubing (PE-260; Clay Adams, Parsippany, NJ, USA) for spontaneous respiration. The nasal cannula was inserted through the cephalic end of the trachea into the nasal cavity. The nasopalatine was blocked with glycerinated cotton. Physiological saline, antigen or histamine solution, which had been warmed at 37°C, was perfused from the cannula through the nasal cavity at a rate of 0.25 ml/min with a peristaltic pump (Atto, Tokyo), and the perfusate was collected. Following the operation, saline was perfused for 10 min before (Period; P-0) and after (P-1) an i.v.-injection of 4% Pontamine sky blue (5 ml/kg, Sigma). Next, OA (1 mg/ml) or histamine (40 µg/ml) was perfused for 10 min at P-2. Furthermore, saline alone was perfused, and the perfusate collected 4 times for 10 min at P-3–6. Each pooled perfusate was centrifuged at 3,000 rpm for 10 min. The amount of dye in the soup was estimated colorimetrically at 620 nm. Each drug was administered orally 1 hr before the antigen or histamine perfusion.

Antigen-induced histamine release from rat peritoneal mast cells

This experiment was performed according to the method reported previously (17). Briefly, male SD rats received an i.p. injection of 1 ml of homologous anti-DNP IgE serum. After 48 hr, they were bled and injected intraperitoneally with 10 ml/animal of Tyrode's solution (pH 7.4) containing 0.3% BSA and 5 units/ml heparin (Green Cross, Osaka). After gentle massage of the abdomen for 2 to 3 min, peritoneal cells were obtained and washed 3 times with Tyrode's solution. After counting the number of mast cells in the cell suspension by staining with 0.05% toluidine blue (Chroma, Kongen, FRG), they were resuspended in Tyrode's solution containing 0.1% BSA and 10 mM HEPES buffer (Gibco, Gaithersburg, MD, USA), and adjusted to a concentration of 1×10^5 cells/ml. The cell suspension was prewarmed for 5 min at 37°C, challenged with DNP-BSA at a final concentration of 10 µg/ml in the presence of 10 µg/ml phosphatidyl serine (Tokyo Kasei, Tokyo), and further incubated for 10

min. Histamine concentration in the supernatant was determined by the method of Shore et al. (18). The percentage of released histamine was calculated from the total cellular content of histamine in each experiment.

Carrageenin-induced paw edema

This experiment was performed according to the method reported previously (19). Briefly, male SD rats were injected intradermally with 0.1 ml of 1% carrageenin (Iwai, Tokyo) into the planter region of the right paw. The paw volume was measured with a plethysmometer (MK-550; Muromachi-Kikai, Tokyo) at 0, 1, 2, 3 and 4 hr after the carrageenin injection. Paw edema was expressed as the increase in paw volume as a percentage of the initial paw volume. Each drug was administered orally 1 hr before the carrageenin injection.

Statistics

The results are expressed as a mean $\pm$ S.E. Either Student's or Welch's *t*-test was used after the *F*-test between the control and other groups. $P < 0.05$ was considered to be significantly different.

RESULTS

Effect on 48-hr homologous PCA in rats

The efficacy of ZCR-2060 was compared with those of ketotifen, terfenadine and cetirizine when administered orally 1 hr before the antigen challenge. As shown in Table 1, 48-hr homologous PCA was inhibited in a dose-dependent fashion by both ZCR-2060 and ketotifen at doses of 0.1–1.0 mg/kg. Terfenadine and cetirizine inhibited this PCA in a dose-dependent fashion at doses of 0.3–10 mg/kg. The ID_{50} values of ZCR-2060, ketotifen, terfenadine and cetirizine were 0.3, 0.3, 2.7 and 0.8 mg/kg, respectively. Note that ZCR-2060 was more potent than either terfenadine or cetirizine. As illustrated in Fig. 1, a significant degree of inhibition by 1 mg/kg of ZCR-2060 was still observed 8 hr before the antigen challenge.

Effect on mediator- or mediator releaser-induced cutaneous reactions in rats

As shown in Fig. 2, the histamine-induced cutaneous reaction was markedly inhibited by 1 mg/kg of ZCR-2060 administered orally 1 hr before histamine injection. In addition, ZCR-2060 inhibited significantly both the compound 48/80- and A23187-induced cutaneous reactions. In contrast, the 5-HT-induced cutaneous reaction was not inhibited by ZCR-2060 at the same dose.

Effect of IgE antibody-mediated mouse ear PCA

As shown in Table 2, ZCR-2060 at doses of 0.3–10 mg/kg inhibited IgE antibody-mediated mouse ear PCA

Table 1. Effects of ZCR-2060, ketotifen, terfenadine and cetirizine on 48-hr homologous PCA in rats

Drugs	Dose (mg/kg)	Amount of dye ($\mu\text{g}/\text{site}$)	Inhibition (%)	ID ₅₀ (mg/kg) (95% C.I.)
Control	—	11.9 $\pm$ 1.5	—	
ZCR-2060	0.1	8.6 $\pm$ 2.8	27.7	0.3
	0.3	5.9 $\pm$ 2.8	50.4	(0.09–0.8)
	1.0	2.2 $\pm$ 0.8*	81.5	
Control	—	18.5 $\pm$ 2.9	—	
Ketotifen	0.1	13.6 $\pm$ 1.7	26.5	0.3
	0.3	7.8 $\pm$ 4.4	57.8	(0.06–1.1)
	1.0	4.0 $\pm$ 1.6**	78.4	
Control	—	7.6 $\pm$ 0.8	—	
Terfenadine	0.3	7.6 $\pm$ 3.4	0	2.7
	1.0	3.7 $\pm$ 1.8	51.3	(0.3–26.4)
	3.0	2.9 $\pm$ 0.8**	61.8	
	10.0	1.9 $\pm$ 0.8**	75.0	
Control	—	11.1 $\pm$ 2.6	—	
Cetirizine	0.3	6.7 $\pm$ 1.1	39.6	0.8
	1.0	5.8 $\pm$ 1.2	47.7	(0.03–2.3)
	3.0	3.3 $\pm$ 0.7*	70.3	
	10.0	1.3 $\pm$ 0.5*	88.3	

Each drug was given orally 1 hr before the antigen challenge. Each data value indicates the mean $\pm$ S.E. of 4 animals. * or **: Significant difference from the control at $P < 0.05$ or $P < 0.01$, respectively.

Fig. 1. Time course of inhibitory effect of ZCR-2060 on histamine-induced cutaneous reaction in rats. Amount of dye in the control was $6.7 \pm 0.7 \mu\text{g}/\text{site}$. ZCR-2060 at a dose of 1 mg/kg was given orally each time before the antigen challenge. Each point indicates the mean $\pm$ S.E. of 5 animals. **: Significant difference from the control at $P < 0.01$. **Fig. 2.** Effect of ZCR-2060 on the cutaneous reaction induced by histamine, 5-HT, compound 48/80 (48/80) and calcium ionophore A23187 (A23187) in rats. ZCR-2060 at a dose of 1 mg/kg was given orally 1 hr before the inducer-injection. Each column indicates the mean $\pm$ S.E. of 6 animals. * or **: Significant difference from the control at $P < 0.05$ or $P < 0.01$, respectively.**Table 2.** Effects of ZCR-2060, ketotifen, terfenadine and cetirizine on 2-hr PCA in the mouse ear

Drugs	Dose (mg/kg)	N	Amount of dye ($\mu\text{g}/\text{ears}$)	Inhibition (%)
Non-sensitization	—	7	2.8 $\pm$ 0.2	—
Control	—	11	10.4 $\pm$ 1.2	—
ZCR-2060	0.3	7	10.1 $\pm$ 1.0	3.9
	1	7	7.5 $\pm$ 2.2	38.2
	3	7	4.5 $\pm$ 0.8**	77.6
	10	7	3.5 $\pm$ 0.6**	90.8
Ketotifen	3	6	7.4 $\pm$ 0.8	39.5
	10	7	4.5 $\pm$ 0.6**	77.6
Terfenadine	10	7	8.3 $\pm$ 1.1	27.6
	30	7	7.4 $\pm$ 1.1	39.5
Cetirizine	10	7	8.3 $\pm$ 1.1	27.6
	30	7	5.5 $\pm$ 0.7**	64.5

Mice were passively sensitized with monoclonal IgE antibody. After 2 hr, mice were challenged with antigen. Each drug was given orally 1 hr before the antigen challenge. Each data value indicates the mean $\pm$ S.E. **: Significant difference from the control at $P < 0.01$.

in a dose-dependent fashion. Ketotifen (10 mg/kg) and cetirizine (30 mg/kg) significantly inhibited the mouse ear PCA. Terfenadine (10 and 30 mg/kg) caused slight inhibition of mouse ear PCA. Note that the efficacy of ZCR-2060 was greater than those of the other tested drugs.

Effect on IgE antibody-mediated biphasic skin reactions in mice

Monoclonal IgE antibody-mediated contact hypersen-

Fig. 3. Effect of ZCR-2060 and prednisolone on IgE antibody-mediated immediate (□) and late (■) phase skin reactions in mice. ZCR-2060 was given orally 1 hr before the antigen challenge. Prednisolone was given intraperitoneally 2 hr before the antigen challenge. Each column indicates the mean $\pm$ S.E. of 6 animals. * or **: Significant difference from the control at $P < 0.05$ or $P < 0.01$, respectively.

sitivity produced biphasic skin reactions with two peak responses, one at 1 (immediate phase) and one at 24 hr (late phase) after the antigen challenge. As shown in Fig. 3, ZCR-2060 (10 and 30 mg/kg), when administered intraperitoneally 1 hr before the antigen challenge, significantly inhibited both immediate and late phase skin reactions. Prednisolone (10 mg/kg), when administered intraperitoneally 2 hr before the antigen challenge, also inhibited both reactions.

Effect on antigen-induced nasal vascular permeability in sensitized rats

Antigen-induced nasal vascular permeabilities in actively and passively sensitized rats were increased by extending the perfusion time after the antigen challenge, and both reached the maximum at P-5 (Figs. 4 and 5). These extravasations were clearly inhibited by ZCR-2060 between P2–6. The amounts of dye leakage between P2–6 in these models are indicated in Tables 3 and 4. ZCR-2060 (0.1–1.0 mg/kg) decreased the amount of dye leakage induced by antigen in actively and passively sensitized rats in a dose-dependent fashion. Ketotifen (0.3 or 1.0 mg/kg), terfenadine (3 mg/kg) and cetirizine (1 mg/kg) also inhibited dye leakage. Note that the inhibitory effect of ZCR-2060 and other drugs were stronger in actively sensitized rats than in passively sensitized rats.

Effect on histamine-induced nasal vascular permeability

As shown in Fig. 6, the extravasated dye in the nasal cavity increased by extending the perfusion time after the histamine treatment, and this reached the maximum at P-

Fig. 4. Effect of ZCR-2060 on antigen-induced nasal vascular permeability in actively sensitized rats. ZCR-2060 was given orally 1 hr before the antigen challenge. Each point indicates the mean $\pm$ S.E. of 5 or 6 animals. * or **: Significant difference from the control at $P < 0.05$ or $P < 0.01$, respectively. ○: Control, ●: 0.1 mg/kg, △: 0.3 mg/kg, ▲: 1.0 mg/kg.

Fig. 5. Effect of ZCR-2060 on antigen-induced nasal vascular permeability in passively sensitized rats. ZCR-2060 was given orally 1 hr before the antigen challenge. Each point indicates the mean $\pm$ S.E. of 6 or 7 animals. *: Significant difference from the control at $P < 0.05$. ○: Control, △: 0.3 mg/kg, ▲: 1.0 mg/kg, □: 3.0 mg/kg.

5. ZCR-2060 (0.3–3 mg/kg) significantly inhibited the histamine-induced increase in dye leakage in nasal cavities at each point. As summarized in Table 5, doses of 0.3–3 mg/kg of either ZCR-2060 or ketotifen inhibited the dye leakage in a dose-dependent fashion. Terfenadine (1, 3 mg/kg) and cetirizine (0.3, 1 mg/kg) also clearly inhibited

Table 3. Effects of ZCR-2060, ketotifen, terfenadine and cetirizine on antigen-induced nasal vascular permeability in actively sensitized rats

Drugs	Dose (mg/kg)	N	Amount of dye ($\mu\text{g/ml}$) Σ (Period 2–6)	Inhibition (%)
Control	—	6	12.3 $\pm$ 2.5	—
ZCR-2060	0.1	6	9.3 $\pm$ 2.0	24.4
	0.3	6	2.7 $\pm$ 1.2**	78.0
	1	5	1.5 $\pm$ 0.8**	87.8
Control	—	6	15.9 $\pm$ 3.9	—
Ketotifen	0.3	6	2.7 $\pm$ 2.1*	83.0
Terfenadine	3	6	2.1 $\pm$ 1.3*	86.6
Cetirizine	1	6	3.4 $\pm$ 1.2*	78.6

Each drug was given orally 1 hr before the antigen challenge. Each data value indicates the mean $\pm$ S.E. * or **: Significant difference from the control at $P < 0.05$ or $P < 0.01$, respectively.

Table 4. Effects of ZCR-2060, ketotifen, terfenadine and cetirizine on antigen-induced nasal vascular permeability in passively sensitized rats

Drugs	Dose (mg/kg)	N	Amount of dye ($\mu\text{g/ml}$) Σ (Period 2–6)	Inhibition (%)
Control	—	7	25.2 $\pm$ 5.8	—
ZCR-2060	0.3	6	18.1 $\pm$ 6.3	28.2
	1	7	8.2 $\pm$ 1.9**	67.5
	3	7	9.6 $\pm$ 2.5**	61.9
Ketotifen	0.3	7	12.3 $\pm$ 5.2	51.2
	1	6	8.0 $\pm$ 1.9*	68.3
Terfenadine	3	7	11.3 $\pm$ 2.8	55.2
Cetirizine	1	6	18.8 $\pm$ 8.6	25.4

Each drug was given orally 1 hr before the antigen challenge. Each data value indicates the mean $\pm$ S.E. * or **: Significant difference from the control at $P < 0.05$ or $P < 0.01$, respectively.

this model. Note that the efficacy of ZCR-2060 was higher than those of terfenadine and cetirizine, and it was almost the same as that of ketotifen.

Effect on antigen-induced histamine release from rat peritoneal mast cells

As illustrated in Fig. 7, anaphylactic histamine release was clearly inhibited in a dose-dependent fashion by both ZCR-2060 and cetirizine at concentrations of 10^{-6} – 10^{-4} M. Ketotifen markedly inhibited the anaphylactic histamine release. In contrast, terfenadine at 10^{-5} M did not affect the anaphylactic histamine release (data not shown).

Effect on carrageenin-induced paw edema in rats

As shown in Table 6, the paw edema caused by carrageenin was significantly inhibited by 10 mg/kg of ZCR-

Fig. 6. Effect of ZCR-2060 on antigen-induced nasal vascular permeability in rats. ZCR-2060 was given orally 1 hr before histamine perfusion. Each point indicates the mean $\pm$ S.E. of 6 or 8 animals. *: Significant difference from the control at $P < 0.05$. ○: Control, △: 0.3 mg/kg, ▲: 1.0 mg/kg, □: 3.0 mg/kg.**Table 5.** Effects of ZCR-2060, ketotifen, terfenadine and cetirizine on histamine-induced nasal vascular permeability in rats

Drugs	Dose (mg/kg)	N	Amount of dye ($\mu\text{g/ml}$) Σ (Period 2–6)	Inhibition (%)
Control	—	8	16.3 $\pm$ 4.8	—
ZCR-2060	0.3	6	13.2 $\pm$ 3.6	19.0
	1	7	7.8 $\pm$ 1.8	52.1
	3	8	5.3 $\pm$ 1.4*	67.5
Control	—	7	22.5 $\pm$ 3.9	—
Ketotifen	0.3	7	15.1 $\pm$ 2.6	32.9
	1	8	11.9 $\pm$ 5.2	47.1
	3	7	5.5 $\pm$ 1.6**	75.6
Control	—	7	26.0 $\pm$ 5.4	—
Terfenadine	1	7	20.8 $\pm$ 5.4	20.0
	3	6	10.2 $\pm$ 2.6*	60.8
Cetirizine	0.3	6	23.2 $\pm$ 3.6	10.8
	1	6	12.0 $\pm$ 4.2	53.8

Each drug was given orally 1 hr before the histamine perfusion. Each data value indicates the mean $\pm$ S.E. * or **: Significant difference from the control at $P < 0.05$ or $P < 0.01$, respectively.

2060 1–4 hr after the carrageenin injection. Note that the maximum inhibitory effect of ZCR-2060 was observed during the early phase (1 hr). Ketotifen (10 mg/kg), terfenadine (100 mg/kg) and cetirizine (30 mg/kg) did not affect the paw edema. In contrast, indomethacin in a dose of 3 mg/kg markedly inhibited the response 1–4 hr after the carrageenin injection.

Fig. 7. Effects of ZCR-2060, ketotifen and cetirizine on antigen-induced anaphylactic histamine release from passively sensitized rat PEC. Percentage of net histamine release in the control for ZCR-2060 and cetirizine or ketotifen were 29.9 ± 2.5 or 7.2 ± 1.2 , respectively. Each point indicates the mean $\pm$ S.E. of 4 to 6 experiments. * or **: Significant difference from the control at $P < 0.05$ or $P < 0.01$, respectively. ○: ZCR-2060, ●: Ketotifen, △: Cetirizine.

DISCUSSION

Immediate hypersensitivity reactions of the skin, nose and airway of experimental animals sensitized with IgE antibody have been used as models for allergic skin reactions such as urticaria or atopic eczema, allergic rhinitis and bronchial asthma in humans (14). PCA is one of the most important *in vivo* models of immediate hypersensitivity in allergic cutaneous reactions. Rat or guinea pig dorsal skin and mouse ear are useful sites for studying PCA (11, 13). Experimental allergic rhinitis has usually

been induced by intranasal application of antigen in sensitized animals, resulting in an increased nasal vascular permeability and intranasal resistance (16, 20, 21). In spite of the increasing evidence of the role of several other mediators (22, 23), histamine is still regarded as the principal mediator of antigen-induced allergic skin reactions and rhinitis. In addition, intradermal and intranasal application of chemical mediators and chemical mediator releasers increase vascular permeability in a manner similar to that of allergic models (12, 14).

In the present study, the antiallergic effects of ZCR-2060 in experimental allergic skin reactions and rhinitis in rats and mice were investigated, and they were compared with those of ketotifen, terfenadine and cetirizine. ZCR-2060 clearly inhibited immediate allergic skin reaction and rhinitis models in mice and rats. The efficacy of ZCR-2060 was greater than those of terfenadine and cetirizine, and it was almost the same as that of ketotifen. Note that the inhibitory activity of ZCR-2060 and other tested drugs on allergic rhinitis models was stronger in actively sensitized animals than in passively sensitized animals. Kojima et al. (16) reported that tranilast also had a stronger effect on actively sensitized animals. In contrast, the classical histamine H_1 -receptor antagonist diphenhydramine did not affect the allergic rhinitis model in passively sensitized animals. Although these findings are not completely understood, it is certain that ZCR-2060 is effective against immediate allergic skin reactions and rhinitis. Furthermore, ZCR-2060 inhibited the histamine-induced cutaneous reaction and nasal vascular permeability, compound 48/80- and A23187-induced cutaneous reactions, but did not affect the 5-HT-induced cutaneous reaction in rats. In addition, ZCR-2060 inhibited the antigen-induced histamine release from rat peritoneal mast cells. It

Table 6. Effects of ZCR-2060, ketotifen, terfenadine, cetirizine and indomethacin on carrageenin-induced paw edema in rats

Drugs	Dose (mg/kg)	N	% Swelling (% Inhibition)			
			1 hr	2 hr	3 hr	4 hr
Control	—	26	30.8 ± 1.4	63.7 ± 1.9	73.4 ± 1.6	73.8 ± 1.6
ZCR-2060	10	14	$22.9 \pm 1.6^{**}$ (25.8)	$55.9 \pm 2.1^*$ (12.2)	$61.7 \pm 2.2^{**}$ (15.9)	$60.7 \pm 1.9^{**}$ (17.7)
Ketotifen	10	14	27.3 ± 3.0 (11.3)	58.2 ± 3.5 (8.6)	66.2 ± 3.6 (9.8)	$65.3 \pm 3.2^*$ (11.5)
Control	—	18	26.9 ± 1.5	60.8 ± 2.3	73.3 ± 2.6	74.0 ± 2.5
Terfenadine	100	7	27.6 ± 3.8 (-2.8)	55.2 ± 6.3 (9.2)	67.1 ± 6.0 (8.4)	64.4 ± 5.4 (13.0)
Control	—	26	30.6 ± 1.5	63.4 ± 1.9	72.4 ± 2.0	72.8 ± 2.0
Cetirizine	30	22	32.0 ± 2.4 (-4.8)	64.2 ± 2.3 (-1.3)	70.7 ± 1.7 (2.4)	71.4 ± 2.0 (1.9)
Control	—	30	28.7 ± 1.3	62.6 ± 2.0	74.3 ± 1.9	73.4 ± 1.7
Indomethacin	3	10	$20.6 \pm 2.0^{**}$ (28.2)	$40.9 \pm 2.1^{**}$ (34.7)	$50.0 \pm 1.8^{**}$ (32.7)	$53.3 \pm 1.7^{**}$ (27.5)

Each drug was given orally 1 hr before carrageenin-injection. Each data value indicates the mean $\pm$ S.E. * or **: Significant difference from the control at $P < 0.05$ or $P < 0.01$, respectively.

is well known that rat mast cell contains histamine, 5-HT and other chemical mediators and that these mediators are released by immunological and non-immunological stimuli (24). It has been reported that compound 48/80 induces the chemical mediators released by interaction with a specific receptor on the mast cells (25), and A23187 stimulates the release process by facilitating the influx of Ca^{2+} into the mast cells (26). These results indicate that the antiallergic effect of ZCR-2060 on immediate hypersensitivity is due not only to blockage of the histamine H_1 -receptor, but also to inhibition of chemical mediator release, and that this antiallergic effect is long lasting.

Recently, much attention has been paid to late phase reactions in the skin, nose and airway which are characterized by an infiltration of inflammatory leukocytes including eosinophils, basophils, neutrophils and mononuclear cells (7). Late phase reactions have been observed several hours after immediate hypersensitivity elicited by antigen challenge. Infiltration of activated inflammatory cells leads to the generation and release of multiple mediators that produce various allergic disorders in various target organs (7, 27). These biphasic responses are believed to reflect the clinical symptoms of chronic allergic disorders. Ray et al. (28) demonstrated that monoclonal anti-DNP IgE antibody mediated the biphasic responses of immediate and late phase reactions in mouse ears. Katayama et al. (29) reported that biphasic responses were hapten-specific and mast cell-dependent because WBB6F₁-W/W^v mice, which are mast cell deficient mice, showed no biphasic responses. Sakurai et al. (15) reported that prednisolone and dexamethasone inhibited both biphasic responses and that the classical histamine H_1 -receptor antagonists, such as diphenhydramine and homochlorcyclizine, inhibited the immediate phase response, but not the late phase response. In the present study, ZCR-2060 and prednisolone significantly inhibited both biphasic skin reactions in mice. It has been reported that cetirizine inhibited the allergen-induced migration of inflammatory cells in late phase reactions of skin and lung (7, 30, 31). It has been reported that histamine release could be identified in the biphasic responses of both nose and skin (32, 33). We observed that ZCR-2060 inhibited the antigen-induced migration of inflammatory cells in bronchoalveolar lavage fluids of actively sensitized guinea pigs (34). Moreover, ZCR-2060 inhibited carrageenin-induced rat paw edema, which is a non-allergic inflammation, and was found to be more potent than other antihistamines.

Therefore, these findings clearly show that ZCR-2060 inhibits immediate and late phase allergic responses, caused by histamine H_1 -receptor blockade, inhibition of chemical mediators release and antiinflammatory action. It also suggests that ZCR-2060 might be useful in the treatment of allergic skin reactions and rhinitis in humans.

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