Antiallergic Effect of ZCR-2060: Antihistaminic Action

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ABSTRACT—The antihistaminic effect of 2-[2-[4-(diphenylmethyl)-1-piperadinyl]ethoxy] benzoic acid maleate (ZCR-2060), a newly synthesized antiallergic agent, was investigated in both in vitro and in vivo studies. ZCR-2060 clearly antagonized histamine-induced contraction of isolated guinea pig ileum and trachea. In contrast, carbachol-, BaCl2- and 5-hydroxytryptamine-induced contractions of isolated guinea pig ileum were slightly inhibited by higher concentrations of ZCR-2060. 3H-Mepyramine specific binding to membranes from guinea pig lung and brain were markedly inhibited by ZCR-2060 in a concentration-dependent fashion. In the in vitro studies, the antihistaminic effect of ZCR-2060 was greater than those of cetirizine and terfenadine, but was less than that of ketotifen. In the in vivo studies, ZCR-2060 significantly inhibited the histamine-induced cutaneous reaction in rats, when administered orally 1 hr before the histamine injection. Moreover, ZCR-2060 has a long-lasting antihistaminic effect. In the in vivo studies, the antihistaminic effect of ZCR-2060 was found to be greater than that of cetirizine and terfenadine, and it was the same as that of ketotifen. Thiopental-induced sleep and spontaneous ambulatory activity in mice, however, were unaffected by ZCR-2060 at higher doses. These results indicate that ZCR-2060 has a potent, selective and long acting histamine H1-receptor antagonistic action without causing any unwanted CNS side effect.

Keywords: Antiallergic agent, Histamine H1-receptor antagonist, Non-sedative antihistamine, ZCR-2060

Histamine is an important chemical mediator in allergic reaction and inflammation. It is synthesized and stored in the secretory granules of mast cells in a variety of tissues or circulating blood basophils (1). Anaphylactic response is triggered by an interaction between the antigen and IgE antibody fixed to mast cells or circulating basophils (2, 3). Histamine is released from mast cells and circulating basophils induced by these interactions and acts through a specific H1-receptor to cause smooth muscle contraction, increase vasopermeability and mucus production (4, 5). This evidence led two theoretical possibilities for prophylaxis of anaphylactic reactions: an inhibition of histamine release from mast cells or basophils and a blockade of histamine receptors of the target organ. Disodium cromoglycate (DSCG), which inhibits the anaphylactic release of histamine without an antagonistic action against histamine, is often used for controlling certain kinds of allergic disorders (6). In addition to an inhibitor of histamine release, an H1-receptor antagonist is also useful for the treatment of some allergic diseases. The H1-receptor antagonist was first described by Bovet and Staub in 1937 (7). Since then, many histamine H1-receptor antagonists have been developed. Classical histamine H1-receptor antagonists such as mepyramine, chlorphenilamine and diphenhydramine have been widely used for the treatment of allergic rhinitis and skin diseases (8). However, these drugs readily cross the blood-brain barrier and have the capacity to cause symptoms of central nervous system (CNS) depression such as sedation. Although ketotifen and mequitazine are more potent and more specific than the classical H1-receptor antagonists, these compounds still produce sedation (9, 10). Recently, newer potent histamine H1-receptor antagonists such as terfenadine, cetirizine and astemizole have been developed which do not readily cross the blood-brain barrier and therefore do not produce CNS effects (11–13). These drugs were found to be effective in the management of allergic disorders.

In the present study, the antihistaminic effect of a newly synthesized compound, 2-[2-[4-(diphenylmethyl)-1-piperadinyl]ethoxy] benzoic acid maleate (ZCR-2060, see structural formula in Fig. 1), was investigated in both in
vitro and in vivo studies and compared with those of ketotifen, terfenadine and cetirizine.

**MATERIALS AND METHODS**

**Animals**

The animals used in this study were male Hartley guinea pigs (weighing 250 to 350 g; Japan SLC, Hama-matsu), male SD rats (weighing 120 - 160 g; Charles River Japan, Atsugi), male ICR mice (4-week-old, Charles River Japan) and male ddY mice (4-week-old, 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 Ind (Saitama). Mepyramine maleate salt (mepyramine; Sigma, St. Louis, MO, USA), diphenhydramine hydrochloride (diphenhydramine; Nacalai Tesque, Kyoto) and chlorpromazine hydrochloride (chlorpromazine, Sigma) were purchased commercially.

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

**Contraction of isolated guinea pig ileum**

Male Hartley guinea pigs were sacrificed by bloodletting, and the ileum was removed. The ileal segments, approximately 1.5 - 2 cm in length, were set up in a 31°C organ bath containing 10 ml of Tyrode's solution that was bubbled with air. The segments were equilibrated for 30 min before the start of the experiments. Changes in the tone of the preparation with 0.5 g initial resting tension were measured with a model TD-112S isotonic transducer (Nihon Kohden, Tokyo). The drugs were added to the organ bath 3 min prior to the addition of histamine dihydrochloride (histamine, Nacalai Tesque), carbamylcholine chloride (Carbachol, Sigma) or BaCl₂ 2H₂O (BaCl₂, Nacalai Tesque). Cumulative concentration-response curves were obtained for the agonists. pA₂ or pD′₂ values were calculated by the Schild plot method (14) or the procedure of Van Rossum (15). The ileal contraction induced by 5-hydroxytryptamine (5-HT, Sigma) was carried out at a concentration of 3 × 10⁻⁶ M. Each drug was added for 3 min before 5-HT was applied.

**Histamine-induced contraction of isolated guinea pig trachea**

Male Hartley guinea pigs were sacrificed by bloodletting, and the trachea was removed. Tracheal strip chains were prepared according to the method of Takagi et al. (16) and were set up in a 37°C organ bath containing 10 ml of Tyrode's solution that was bubbled with air. The preparations were initially loaded with 0.5 g tension and allowed to equilibrate before the start of the experiments. Changes in tone of the preparation were measured with a model TD-112S isotonic transducer (Nihon Kohden). The preparations were contracted with a submaximally effective concentration of histamine (3 × 10⁻⁶ M). Each drug was applied cumulatively when the contraction response reached the plateau state.

**Preparation of guinea pig lung and brain membrane**

Male Hartley guinea pigs were sacrificed, and the lungs and brains were removed. The membrane suspensions of the lung and brain were prepared according to the method of Laudron et al. (17) and Ahn and Barnett (18), respectively. Briefly, the lungs were minced into small segments and homogenized in 10 volumes of 0.25 M sucrose with an Ultra-Turrax T-25 homogenizer (Ika-Labortechnik, Tokyo). After centrifugation at 500 × g for 10 min at 4°C, the pellets were rehomogenized in 10 volumes of 0.25 M sucrose and centrifuged again. Both pooled supernatants were centrifuged at 50,000 × g for 15 min at 2°C. The pellets were suspended in 3 ml of buffer for each gram of original wet weight and stored at -80°C.

**3H-Mepyramine binding assay**

The 3H-mepyramine binding assay was performed according to the method of Tran et al. (19). Briefly, the membrane suspensions of brain (15 mg original wet weight/0.4 ml) and lung (0.5 mg protein/0.4 ml) were prewarmed for 5 min at 25°C, and further incubation was
done for 30 min with 50 µl of 20 nM 3H-mepyramine (specific activity of 917.6 GBq/mmol; NEN, Boston, MA, USA) and 50 µl of each drug. The reaction was terminated by addition of 4 ml of ice-cold buffer and the suspension was then filtered rapidly under vacuum through Whatman GF/B filters with 3 x 4 ml rinses of cold buffer. Radioactivity trapped on the filters was counted in 5 ml of ATOMLIGHT (NEN). Specific binding of 3H-mepyramine was estimated as the difference between radioactivity bound in the absence or presence of 2 µM promethazine hydrochloride (Sigma). The radioligand competition activities of the drugs were expressed as Ki, defined as 

\[ K_i = IC_{50} / \left( 1 + [^{3}H\text{-mepyramine}] / K_d \right) \]

where 3H-mepyramine was the concentration of the radioligand used (20). The dissociation constant (Kd) and the density of 3H-mepyramine binding (Bmax) were determined by the nonlinear least squares regression program MULTI (21).

**Histamine-induced cutaneous reaction in rats**

Male SD rats were injected intradermally with 5 µg/site of histamine into the shaved back, immediately after i.v.-injection of 1 ml of 1% Evans blue saline solution. Thirty minutes later, the animals were sacrificed, and reaction sites were excised for measurement of extravasated dye. The amount of dye leaked was determined according to the method of Katayama et al. (22). ZCR-2060 was administered orally 1 hr or at another indicated time before histamine injection. Other drugs were administered orally 1 hr before histamine injection.

**Thiopental-induced sleep in mice**

The experiment was performed according to the method of Tasaka et al. (23). Briefly, male ICR mice were injected intravenously with 40 mg/kg of thiopental sodium (Tanabe, Osaka). The sleeping time was measured between the loss and return of the righting reflex. Each drug was administered orally 1 hr before thiopental injection.

**Exploratory behavior in mice**

Exploratory behavior was performed according to the method of Hall (24). Briefly, male ddY mice were placed in a Hall's open-field apparatus which was divided into 19 blocks of approximately equal area. The amount of ambulatory activity was measured for 90 sec. Each drug was administered orally 1 hr before the test.

**Statistics**

The results are expressed as means±S.E. Either Student's or Welch's t-test after the F-test were used. P < 0.05 was considered to be significantly different.

### RESULTS

**Effect on histamine-induced contraction of isolated guinea pig ileum**

As shown in Fig. 2, ZCR-2060 produced competitive inhibition at low concentrations and non-competitive inhibition at high concentrations of histamine-induced contractions of isolated guinea pig ileum. As summarized in Table 1, The calculated pA2 and pD2 values of ZCR-2060 were 7.27 and 5.70, respectively. Ketotifen and terfenadine showed both inhibitory actions. In contrast, cetirizine showed competitive inhibition at concentrations lower than 10⁻⁵ M. On the basis of the pA2 value, the inhibitory activity of ZCR-2060 was less than that of ketotifen, but was more potent than those of terfenadine and cetirizine.

**Effect on carbachol-, BaCl₂- and 5-HT-induced contraction of isolated guinea pig ileum**

As shown in Fig. 3, ZCR-2060 at a concentration of

![Graph](image-url)
$10^{-5}$ M slightly inhibited the carbachol-induced contraction of isolated guinea pig ileum, but did not inhibit the BaCl$_2$-induced contraction. As shown in Fig. 4, both ZCR-2060 and cetirizine slightly inhibited the 5-HT-induced contraction of isolated guinea pig ileum. In contrast, ketotifen and terfenadine clearly inhibited the 5-HT-induced contraction of isolated guinea pig ileum in a concentration-dependent fashion.

**Fig. 3.** Effect of ZCR-2060 on carbachol- and BaCl$_2$-induced contraction of isolated guinea pig ileum. Each point indicates the mean of 3 to 5 experiments. ○ and △: Control, ● and ▲: ZCR-2060 ($10^{-5}$ M).

**Fig. 4.** Effect of ZCR-2060, ketotifen, terfenadine and cetirizine on 5-HT-induced contraction of isolated guinea pig ileum. Each point indicates the mean of 4 experiments. ○: ZCR-2060, ●: Ketotifen, △: Terfenadine, ▲: Cetirizine.

**Table 2.** Effects of ZCR-2060, ketotifen, terfenadine and cetirizine on histamine-induced contraction of isolated guinea pig trachea

| Drugs     | N | IC$_{50}$ (× $10^{-7}$ M) | 95% C.L. (× $10^{-7}$ M) |
|-----------|---|---------------------------|--------------------------|
| ZCR-2060  | 6 | 1.1                       | 0.8 - 1.5                |
| Ketotifen | 5 | 0.009                     | 0.006 - 0.01             |
| Terfenadine | 5 | 13.1                      | 8.2 - 21.0               |
| Cetirizine | 5 | 5.1                       | 3.3 - 7.7                |

Effect on histamine-induced contraction of isolated guinea pig trachea

As shown in Fig. 5, ZCR-2060 and other drugs inhibited the histamine-induced contraction of isolated guinea pig trachea in a concentration-dependent fashion. As summarized in Table 2, the IC$_{50}$ values of ZCR-2060, ketotifen, terfenadine and cetirizine were 0.11, 0.009, 1.31 and 0.51 μM, respectively. Note that the inhibitory activity of ZCR-2060 was less than that of ketotifen, but was greater than those of terfenadine or cetirizine.

Effect on $^3$H-mepyramine specific binding

The ability of ZCR-2060 to compete with $^3$H-mepyramine in specific binding was compared with those of ketotifen, terfenadine, cetirizine and unlabelled mepyramine.

As shown in Figs. 6 and 7, $^3$H-mepyramine specific binding to membranes from guinea pig lung and brain was markedly reduced in a concentration-dependent
fashion by ZCR-2060 and other H₁-antihistamines. $K_i$ values of the drugs for $^3$H-mepyramine binding in the lung and brain are summarized in Table 3. ZCR-2060, as well as ketotifen, terfenadine and cetirizine displayed a higher affinity for the brain than for the lung. Unlabelled mepyramine, however, has similar affinity for lung and brain. The rank of the drugs in terms of activity in the lung and brain is as follows: mepyramine = ketotifen $>>$ ZCR-2060 $>$ Terfenadine $=$ cetirizine.

| Drugs          | $K_i$ value (nM) |
|----------------|------------------|
|                | Lung             | Brain            |
| ZCR-2060       | 41.6 ± 8.3       | 19.3 ± 4.4       |
| Ketotifen      | 0.6 ± 0.1        | 0.3 ± 0.1        |
| Terfenadine    | 213.8 ± 55.1     | 47.7 ± 8.1       |
| Cetirizine     | 194.0 ± 42.5     | 75.5 ± 9.5       |
| Mepyramine     | 0.6 ± 0.1        | 0.5 ± 0.1        |

Each data value indicates the mean ± S.E. of 4 experiments. $K_i$ values of lung and brain membrane were 0.8 ± 0.18 and 0.5 ± 0.04 nM, respectively. $B_{max}$ values of lung and brain membrane were 90.1 ± 6.4 fmol/mg protein and 6.0 ± 0.12 pmol/g tissue, respectively.

**Effect on histamine-induced cutaneous reaction in rats**

As shown in Table 4, cutaneous reactions induced by histamine were inhibited significantly by ZCR-2060, ketotifen, terfenadine and cetirizine when the drugs were administered orally 1 hr before histamine injection. The $ID_{50}$ values of ZCR-2060, ketotifen, terfenadine and cetirizine were 0.3, 0.3, 1.8 and 0.5 mg/kg, respectively. The

| Drugs          | Dose (mg/kg) | Amount of dye (µg/site) | Inhibition (%) | $ID_{50}$ (mg/kg) |
|----------------|-------------|-------------------------|----------------|-------------------|
|                |             |                         |                | (95% C.L.)        |
| Control        | —           | —                       | —              | —                 |
| ZCR-2060       | 0.1         | 7.5 ± 0.8               | 22.7           | 0.3               |
|                | 0.3         | 4.1 ± 1.2*              | 57.7           | (0.02 – 4.2)      |
|                | 1.0         | 2.5 ± 1.2**             | 74.2           |                   |
| Ketotifen      | 0.03        | 8.8 ± 0.9               | 7.4            | 0.3               |
|                | 0.1         | 5.7 ± 1.4               | 40.0           | (0.07 – 1.1)      |
|                | 0.3         | 5.9 ± 3.1               | 37.9           |                   |
|                | 1.0         | 1.9 ± 0.5**             | 80.0           |                   |
| Terfenadine    | 0.3         | 4.4 ± 1.5               | 17.0           | 1.8               |
|                | 1.0         | 3.3 ± 0.7               | 37.7           | (1.2 – 2.7)       |
|                | 3.0         | 2.3 ± 0.7*              | 56.6           |                   |
|                | 10.0        | 0.8 ± 0.6**             | 84.9           |                   |
| Cetirizine     | 0.3         | 4.9 ± 1.1               | 17.0           | 1.8               |
|                | 1.0         | 1.0 ± 0.4*              | 86.3           | (0.03 – 8.0)      |
|                | 3.0         | 1.3 ± 0.5*              | 82.2           |                   |

$ID_{50}$ values of ZCR-2060, ketotifen, terfenadine and cetirizine were 0.3, 0.3, 1.8 and 0.5 mg/kg, respectively. Each drug was given orally 1 hr before histamine injection. Each data value indicates the mean ± S.E. of 4 animals. * or **: Significant difference from the control at $P < 0.05$ or $P < 0.01$, respectively.
The efficacy of ZCR-2060 was greater than those of terfenadine and cetirizine, and it was almost the same as that of ketotifen. As illustrated in Fig. 8, significant inhibitory action by ZCR-2060 at a dose of 1 mg/kg was observed until 8 hr before histamine injection.

**Effect on thiopental-induced sleep in mice**

As shown in Table 5, thiopental-induced sleep was unaffected by ZCR-2060, cetirizine and terfenadine at a dose of 100 mg/kg. In contrast, ketotifen at a dose of 12.5 mg/kg markedly prolonged thiopental-induced sleeping time.

**Effect on exploratory behavior in mice**

As shown in Table 6, ZCR-2060 and cetirizine caused no significant change in the amount of ambulation in mice. On the other hand, terfenadine (300 mg/kg), ketotifen (100 mg/kg) and diphenhydramine (100 mg/kg) elicited a significant reduction in the amount ofambulation. In addition, chlorpromazine strongly decreased the amount of ambulation at a dose of 10 mg/kg.

**DISCUSSION**

Classical H₁-receptor antagonists are able to interact with various receptors such as 5-hydroxytryptaminergic, cholinergic, a-adrenergic and dopaminergic receptors and cause adverse CNS effects such as sedation (25, 26). These adverse effects have severely limited the use of this medication. The newer H₁-receptor antagonists display high affinity for the H₁-receptor with fewer effects on the other receptors and produce no CNS effect (8). However, ketotifen and terfenadine at high doses have an affinity for cholinergic and 5-HT receptors. Cetirizine, in contrast, has no affinity for CNS-receptors.

In the present study, the antihistaminic effect of ZCR-2060 was investigated in both in vitro and in vivo studies. In the in vitro studies, ZCR-2060 clearly inhibited the histamine-induced contraction of isolated guinea pig ileum and trachea without affecting ileal contractions induced by calbachol, 5-HT and BaC1₂. These results indicate that ZCR-2060 does not show unwanted side effects that may related to the blockage of cholinergic or 5-HT receptors. In addition, ZCR-2060 and other tested drugs clearly inhibited [³H]-mepyramine specific binding to the membranes from guinea pig lung and brain in a concentration-dependent fashion. However, ZCR-2060, ketotifen, terfenadine and cetirizine showed more affinity for the brain than for the lung. In [³H]-mepyramine displacement, loratadine (27) and setastine (28), which are low sedative antihistamines, have a great affinity for peripheral histamine H₁-receptors and minimal affinity for CNS H₁-recep-

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**Table 5. Effects of ZCR-2060, ketotifen, terfenadine and cetirizine on thiopental-induced anesthesia in mice**

| Drugs    | Dose (mg/kg) | N  | Sleeping time (sec) |
|----------|--------------|----|---------------------|
| Control  | —            | 23 | 680.3 ± 93.1        |
| ZCR-2060 | 100          | 10 | 768.5 ± 159.6       |
| Ketotifen| 12.5         | 10 | 1232.2 ± 154.9**    |
| Terfenadine | 100       | 10 | 868.5 ± 175.9       |
| Cetirizine| 100          | 10 | 676.0 ± 142.7       |

Each drug was given orally 1 hr before thiopental injection. Each data value indicates the mean ± S.E. **: Significant difference from the control at P < 0.01.

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**Table 6. Effects of ZCR-2060, ketotifen, terfenadine, cetirizine, diphenhydramine and chlorpromazine on ambulatory activity in mice**

| Drugs           | Dose (mg/kg) | N  | Ambulation/90 sec |
|-----------------|--------------|----|------------------|
| Control         | —            | 8  | 35.6 ± 4.0       |
| ZCR-2060        | 300          | 8  | 45.8 ± 7.6       |
| Ketotifen       | 100          | 8  | 18.8 ± 5.1*      |
| Terfenadine     | 300          | 8  | 20.8 ± 4.9*      |
| Cetirizine      | 300          | 8  | 30.5 ± 3.2       |
| Diphenhydramine | 100          | 8  | 18.1 ± 4.2**     |
| Chlorpromazine  | 10           | 8  | 11.0 ± 5.0**     |

Each drug was given orally 1 hr before the test. Each data value indicates the mean ± S.E. * or **: Significant difference from the control at P < 0.05 or P < 0.01, respectively.
tors. Thus the low sedative effect of these compounds may be due to these findings and poor penetration into the CNS. It has been reported previously that cetirizine and terfenadine did not have selectivity for peripheral histamine H<sub>1</sub>-receptor (29). In the in vitro studies, the rank order of antihistaminic activity was as follows: ketotifen >> ZCR-2060 > cetirizine > terfenadine.

In the in vivo studies, the histamine-induced cutaneous reaction in rats was clearly inhibited in a dose-dependent fashion by ZCR-2060 when it was administered orally 1 hr before the histamine-injection. The rank order of inhibitory activity was as follows: ZCR-2060 = ketotifen > cetirizine > terfenadine. The antihistaminic activity shown in the in vitro and in vivo studies indicate that ZCR-2060 is efficiently absorbed by the gastrointestinal tract and widely distributed in the tissues. In addition, the inhibitory activity of ZCR-2060 lasted for more than 8 hr in rats. Omata et al. (30) and Yoshida et al. (31) reported that ZCR-2060 was effective in the treatment of various experimental immediate and late phase allergic responses in animals. It is clear that the H<sub>1</sub>-receptor antagonistic action of ZCR-2060 contributes to the antiallergic and antiasthmatic actions.

Moreover, CNS actions of ZCR-2060 were studied in the in vivo models. Gross observations in mice were chosen as a part of the criteria, because H<sub>1</sub>-receptor blocking with CNS actions prolonged sleep or decrease spontaneous motor activity (32). ZCR-2060 and cetirizine at about 100 times the dose for their antihistaminic action did not affect thiopental-induced sleep or spontaneous ambulatory activity in mice. At the same dose, ketotifen showed the adverse effect in both experiments. Terfenadine at 300 mg/kg reduced spontaneous ambulatory activity in mice. Our results with ketotifen and terfenadine agree with previous reports (33, 34). Furthermore, in our primitive study, the electroencephalogram (EEG) in conscious dogs was not affected by ZCR-2060 (unpublished data). Mann et al. (11) reported that the newer antihistamines, which are moderately lipophobic compounds, effectively block the peripheral H<sub>1</sub>-receptors without crossing the blood-brain barrier, and higher doses of the compounds can be used in the medication. These results suggest that ZCR-2060 has no detectable actions on the CNS side effect, because it might be due to its poor penetration through the blood brain barrier.

In conclusion, ZCR-2060 was shown to be a potent, selective and long acting histamine H<sub>1</sub>-receptor antagonist without anticholinergic and 5-hydroxytryptaminergic activity. Furthermore, the compound causes few, if any, unwanted CNS side effects.

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