Pharmacological Characterization of a Novel Long-acting Histamine H₁ Receptor Antagonist, KAA-276

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The pharmacological profile of a newly synthesized histamine H₁ receptor antagonist, KAA-276 (1-[1-(4-fluorophenylmethyl)-1H-benzimidazol-2-yl]-5-[2-[4-(2-carboxyethyl)phenyl]ethyl]-1,5-diazacyclooctane sulfate), was characterized. In a H₁ receptor binding assay in vitro, KAA-276 inhibited [³H]mepyramine binding to guinea pig cerebellar membrane preparations with an IC₅₀ of 0.66 nM. The inhibitory potency of KAA-276 was greater than that of terfenadine, but similar to that of astemizole and ketotifen. KAA-276 antagonized the histamine-induced constriction of ileum and trachea isolated from guinea pigs in a dose-dependent manner with a concomitant reduction in the maximum response. Furthermore, the inhibitory effect of KAA-276 on histamine induced constriction was potentiated depending on the duration of preincubation time and revealed an irreversible property. KAA-276 given orally, intraduodenally, and by inhalation significantly inhibited histamine-induced bronchoconstriction dose-dependently in guinea pigs. Inhalation of KAA-276 exhibited inhibitory activity with a rapid onset and long duration, while intraduodenal administration resulted in action with a slow onset. Therefore, KAA-276, an irreversible and selective histamine H₁ receptor antagonist, was shown to be a useful drug for therapeutic strategies against bronchial asthma when administered by the aerosol route.

Key words: histamine; antihistamine; aerosol; bronchoconstriction

Histamine is released by a variety of noxious stimuli, including chemicals and anaphylactic injury. The locally released histamine binds to specific receptors on cells and appears to initiate alteration in bronchial tone, vascular flow, and permeability. For this reason, histamine is thought to be one of the important chemical mediators in several inflammatory and allergic diseases, especially bronchial asthma. However, the use of conventional H₁ antihistamines in allergic disorders is still limited by various side effects. The major side effect is sedation or impaired performance, which is considered to be associated with H₁ receptor antagonism in the central nervous system. Other side effects are related to several other pharmacological properties including anticholinergic, antiadrenergic, and antiserotonergetic ones. Recently, new antihistamines, retaining long duration of action and lacking sedative effects, have been developed and are being used orally for the treatment of allergic disorders. However, these new antihistamines are not completely devoid of anticholinergic, antiadrenergic, and antiserotonergetic activities.

For the remedy of bronchial asthma, inhalation of steroids and β₂-stimulants have long been used; however, inhaled antihistaminic agents have never been used clinically. The superiority of the aerosol route of drug application has been recognized. Aerosol delivery of drugs avoids the systemic effects, allows dosing of the drug in higher concentrations to the local target area, and exhibits an immediate effect compared with oral administration.

We synthesized KAA-276 (1-[1-(4-fluorophenylmethyl)-1H-benzimidazol-2-yl]-5-[2-[4-(2-carboxyethyl)phenyl]ethyl]-1,5-diazacyclooctane sulfate), shown in Fig. 1, as a novel, selective, and long-acting antihistaminic agent. In the present study, we confirmed its affinity for histamine receptors and pharmacological antihistaminergic characteristics in vitro. In addition, we assessed the inhibitory property of inhaled and oral KAA-276 on the histamine-induced bronchoconstriction in guinea pigs, compared with that of antihistamines ketotifen and terfenadine given orally.

MATERIALS AND METHODS

Materials KAA-276, astemizole, and terfenadine were synthesized in Kissei Pharmaceutical Co., Ltd. The following compounds were purchased from commercial sources: ketotifen, cetirizine, mepyramine and chlorpheniramine, from Sigma Chemical, St. Louis, MO, U.S.A.; histamine hydrochloride from Wakoh Chemicals, Japan; and [³H]mepyramine (20—30 Ci/mmol) from New England Nuclear, Boston, MA, U.S.A. Other chemicals used were of analytical grade.

Animals Male Hartley guinea pigs (Japan SLC Inc., Hamamatsu, Japan), weighing about 350—450 g, were used for histamine H₁ receptor binding assays, and those weighing about 510—550 g for histamine H₂ receptor binding assay, histamine induced contraction of isolated organs, in vitro experiments, and histamine induced bronchoconstriction in vivo experiments. Male Sprague-Dawley rats (Charles River Japan Inc., Kanagawa, Japan) weighing 280—510 g were used for radioligand assays. The animals were housed in groups of five per cage with free access to commercial food pellets and tap water in a room with a 12-h light/12-h dark cycle.

Radioligand Binding Assay for Histamine H₁ Receptor

The cerebellum was rapidly removed from exsanguinated

Fig. 1. Chemical Structure of KAA-276

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guinea pigs (n=10), rinsed in ice-cold 50 mM phosphate buffer (pH 7.8), and homogenized in 10 ml of ice-cold 50 mM phosphate buffer. The homogenate was centrifuged at 2000×g for 10 min at 4 °C. The pellet obtained was resuspended with 50 mM phosphate buffer. The binding assay was performed in a final volume of 1 ml consisting of the cerebellar membrane preparation (0.3 mg protein) with various concentrations of KAA-276. After incubation for 60 min at 32 °C, [3H]mepyramine (70 nM) was added to the assay medium and the incubation was then continued for a further 60 min. The reaction was terminated by centrifugation (2000×g for 10 min) and sediment was washed twice with 50 mM phosphate buffer (pH 7.8). The radioactivity was counted after addition of 0.5 ml of aquazote 2 (New England Nuclear) with a liquid scintillation counter (1900CA, Packard). Nonspecific binding was determined in the presence of 100 μM astemizole, and specific binding was estimated by the difference between total and nonspecific binding. Protein content of the tissue suspension was determined using of a bichinonic acid protein assay (Pierce, Rockford, IL, U.S.A.). The assay was performed in duplicate. Scatchard plots were analyzed by linear least squares regression analysis. IC50 values were determined by computer fitting with a Graph Pad PRISM™ software package (Graph Pad Software Inc., San Diego, CA, U.S.A.).

Radioligand Binding Assay for Other Receptors
Brain, heart, and submandibular gland tissues obtained from male Sprague-Dawley rats or from male Hartley guinea pigs were used for radioligand binding assays. The best suited species tissue membrane preparations including receptor type and radioligands for each assay are summarized in Table 1. KAA-276, respective tritiated ligand and tissue homogenate were incubated under each condition. For all assays, the incubation was terminated by filtration through a glass fiber filter (Whatman GF/B) and washing with ice-cold buffer. The radioactivities of the filters were measured in a liquid scintillation counter. The specific binding was defined as the difference between the total counts in the absence of unlabeled ligand and the counts obtained in the presence of a specific inhibiting compound. Protein content of tissue suspension was determined using the Bradford method. These binding assays were performed in duplicate.

**Histamine-Induced Contraction of Isolated Guinea Pig Ileum**
Male Hartley guinea pigs were sacrificed by a blow to the neck, and the ileum was removed immediately. Segments of ileum about 1.0 cm in length were suspended in an organ bath containing 10 ml of Tyrode’s solution aerated with a mixture of 95% O2 and 5% CO2 at 37 °C. The response to a given histamine solution was isometrically recorded under a resting tension of 1 g for all preparations. Cumulative concentration–response curves for histamine were then obtained by increasing the concentration of the histamine. At least 3 contractions of about equal magnitude were produced before testing KAA-276. The drug was added to the organ bath 5 min before the addition of histamine. Contractile responses were expressed as a percentage of the maximal response.

In separate experiments, we studied the effects of preincubation time of KAA-276 on contraction by histamine (1 μM). The response to a given histamine was isometrically recorded under a resting tension of 1 g. KAA-276 was added to the organ bath 5, 30, 60 and 120 min before the addition of histamine. In the recovery experiment, KAA-276 at dose of 10 μM was added to the organ bath and then histamine (1 μM) was added at intervals of 10 min. The preparation was washed out following each histamine addition.

**Histamine Induced Contraction of Isolated Guinea Pig Trachea**
Trachea chain preparations were prepared from isolated guinea pig trachea and suspended in an organ bath containing 10 ml of Locke-Ringer’s solution aerated with a mixture of 95% O2 and 5% CO2 at 37 °C. The response to a given histamine solution was isometrically recorded under a resting tension of 1 g. At least 3 contractions of about equal magnitude were produced by the cumulative addition of histamine before testing KAA-276, which was added to the organ bath 30 min before histamine treatment. Contractile responses were expressed as a percentage of the response to 1 μM histamine.

**Histamine Induced Bronchoconstriction in Guinea Pigs**
Male Hartley guinea pigs were anesthetized with intraperitoneal administration of urethane (500 mg/kg) and α-chloralose (20 mg/kg). An endotracheal cannula was inserted into the guinea pig and connected to a respirator (SN-860, Shiono Seisakusyo, Tokyo, Japan), and then the animal was ventilated at a tidal volume of 10 ml/kg and at a frequency of 60 breaths/min. A side-hole catheter through the endotracheal cannula was linked to a pressure transducer (P-231D,
Table 2. Effect of KAA-276 and other Histamine H₁ Antagonists on [³H]Mepyramine Binding to Guinea Pig Cerebellum Membranes

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC₅₀ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KAA-276</td>
<td>0.66</td>
</tr>
<tr>
<td>Astemizole</td>
<td>0.20</td>
</tr>
<tr>
<td>Terfenadine</td>
<td>41.70</td>
</tr>
<tr>
<td>Ketotifen</td>
<td>0.10</td>
</tr>
<tr>
<td>Cetrizine</td>
<td>19.50</td>
</tr>
<tr>
<td>Mepyramine</td>
<td>2.04</td>
</tr>
<tr>
<td>Chlorpheniramine</td>
<td>2.63</td>
</tr>
</tbody>
</table>

Data show the mean of 2 separate experiments.

Gould, U.S.A.) to measure endotracheal pressure. Histamine (1 mg/ml) was atomized with an ultrasonic nebulizer (NE-U10B, OMRON, Kyoto, Japan) for 30 s and delivered to the anesthetized guinea pigs by inhalation.

KAA-276 (0.3—3 mg/kg), terfenadine (0.3—3 mg/kg) and ketotifen (0.01, 0.1 mg/kg) were given intraduodenally (i.d.) to anesthetized guinea pigs at 0.5, 2 and 4 h before histamine exposure and orally to conscious at 4, 8 and 24 h before. Furthermore, KAA-276 (0.0001—0.1%) was inhaled by the anesthetized guinea pigs for 1 min at 0.5, 2 and 4 h before histamine treatment. In the other experiment, KAA-276 (0.0001—0.1%) was inhaled by conscious guinea pigs for 15 min in a glass desiccator (inside diameter 30 cm) and histamine was delivered 4, 8 and 24 h thereafter. Inhalation time by conscious animals was determined by inhibition rate with KAA-276.

**Statistical Analysis** Dunnet’s multiple comparison test was used after ANOVA. *p* values of less than 0.05 were considered significant. ED₉₀ was calculated by Probit method.

**RESULTS**

**Affinity of KAA-276 for Histamine H₁-Receptor and Other Receptors** KAA-276 and other histamine H₁ receptor antagonists were compared by their IC₅₀ values in blocking [³H]mepyramine binding to histamine H₁ receptor sites in guinea pig cerebellum (Table 2). The IC₅₀ value of KAA-276 was 0.66 nM. The affinity of KAA-276 for histamine H₁ receptor was similar to that of astemizole and ketotifen, whereas it was more potent than that of terfenadine and cetirizine. KAA-276 had hardly any affinity for receptors (IC₅₀ > 10000 nM), histamine H₂, adrenergic (α₁, α₂, β₁ and β₂), serotoninergic (5-HT₁ and 5-HT₂), muscarinic (M₁ and M₂), dopaminergic (D₁ and D₂), nicotinic, or GABAergic (A and B). In the *in vitro* studies, KAA-276 had a higher affinity for histamine H₁ receptors than for other receptors.

**Effect of KAA-276 on Histamine Induced Contraction in Guinea Pig Ileum** As shown in Fig. 2, KAA-276 (0.1—3 μM), terfenadine (0.1—30 μM), and ketotifen (0.001—0.03 μM) dose-dependently caused a rightward shift in the histamine concentration-contractile response curve with a reduction in the maximal response. Furthermore, the inhibitory potency of KAA-276 on histamine-induced contraction depended on the preincubation time (Fig. 3A). That is to say, the IC₅₀ value of KAA-276 was 3.3, 0.56, 0.07 and 0.05 μM at 5, 30, 60 and 120 min of incubation with the drug, respectively. The time course for the recovery of the response to histamine following treatment with KAA-276 (10 μM) is shown in Fig. 3B. In spite of continual washing of the tissue with Tyrode’s solu-

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**Fig. 2** Effects of KAA-276 (KAA: A), Terfenadine (T: B), and Ketotifen (Ke: C) on Histamine-Induced Contractile Response in the Isolated Guinea Pig Ileum

Cumulative concentration-response curves for histamine were obtained by increasing its concentration of the histamine. The drug was added to an organ bath 5 min before the addition of histamine. Contractile responses were expressed as a percentage of the maximal response. Each point indicates the mean of 4 experiments.
Histamine-induced bronchoconstriction) to evaluate the efficacy of antihistamines in vivo. KAA-276 inhibited histamine-induced contraction by intraduodenal administration with ED50 of 1.38 and 1.9 mg/kg at 2 and 4 h in anesthetized guinea pigs; however, this antihistaminic effect was not expressed at 0.5 h. In oral administration, KAA-276 inhibited this contraction with an ED50 of 1.74, 2.31, and 2.21 mg/kg at 4, 8, and 24 h, respectively, in conscious guinea pigs (Table 3). However, the antihistaminic effect of KAA-276 was not evident at 0.5 h after oral administration. In the inhalation study, KAA-276 inhibited histamine-induced constriction in the anesthetized animals with an ED50 of 0.99% (0.27 mg/kg), 0.03% (0.09 mg/kg), and 0.03% (0.09 mg/kg) at 0.5, 2, and 4 h, and that in conscious animals with a value of 0.06% (0.18 mg/kg) and 0.08% (0.24 mg/kg) at 4 and 8 h, respectively, following inhalation (Table 3). The antihistaminic effect of inhaled KAA-276 appeared as easily as 0.5 h (ED50=0.09%) and diminished at 24 h (ED50=0.1%) following the administration (Fig. 5B). Ketotifen and terfenadine also significantly inhibited histamine-induced bronchoconstriction (Fig. 5C, D and Table 3). The inhibitory effect of ketotifen on this bronchoconstriction was maximal at 0.5 h following the intraduodenal administration and diminished gradually. Although terfenadine, like KAA-276, caused no significant inhibition at 0.5 h, it showed inhibitory activity with an ED50 of 24.3 mg/kg (i.d.) 2 h after the administration and one of 11.0 mg/kg (p.o.) 4 h following the administration (Table 3).

**DISCUSSION**

Histamine H1 antagonists have been widely used in the treatment of allergic disorders, particularly allergic rhinitis. However, the use of conventional antihistamines is limited because they have various side effects, such as sedation, local anesthetic action, and dryness of mouth. Recently, the new antihistamines, terfenadine, astemizole and cetirizine, the so-called "second-generation" H1 antagonists, have been developed and are being used in the treatment of allergic diseases. These new antihistamines do not induce sedation. With the goal of avoiding the antihistamine-induced adverse effects described above, we synthesized KAA-276, a novel histamine H1 receptor antagonist, and evaluated its pharmacological efficacy in vitro and in vivo.

In this study, we showed that KAA-276 specifically inhibited the binding of [3H]mepyramine to the histamine H1 receptor in guinea pig cerebellar membrane. The affinity of KAA-276 for histamine H1 receptor (IC50=0.66 nm) was similar to that of astemizole and ketotifen and higher than that of other antihistamines, terfenadine, cetirizine, mepyramine and chlorpheniramine. KAA-276 had no affinity (IC50>10000 nm) for adrenergic (α1, α2, β1, and β2), serotonergic (5-HT1 and 5-HT2), muscarinic (M1 and M2), or histamine H2 receptors. Although second-generation H1 antihistamines have more selectivity for the histamine H1 receptor than the conventional antihistamines, these new antihistamines are not completely devoid of antiadrenergic and antiserotonergic activity. In contrast to those antihistamines, KAA-276 displayed a remarkable selectivity for the histamine H1 receptor.

In vitro experiments, KAA-276 exerted an inhibitory effect on the histamine-induced contraction of the ileum and trachea of guinea pigs. KAA-276 inhibited the histamine-induced contractile response in a dose-dependent and noncompetitive manner. Terfenadine and ketotifen exhibited an inhibitory profile essentially similar to that of KAA-276 for the histamine-induced ileal contraction. In the isolated guinea pigs.
Fig. 5. Effects of KAA-276 (KAA: A, B), Terfenadine (T: C, and Ketotifên (Ke: D) on Aerosol Histamine-Induced Bronchoconstriction in Vivo in Guinea Pigs.

Bronchoconstriction was induced by inhaled histamine (1 mg/ml for 30 s). KAA-276 was given intraduodenally (i.d.) or orally (p.o.) and by inhalation (b) to anesthetized (0.5–4 h post-treatment time) and conscious (4–24 h post-treatment time) guinea pigs. Like KAA-276, ketotifen and terfenadine were also administered intraduodenally or orally. Each point indicates the percent inhibition of the contraction induced by aerosol histamine (1 mg/ml for 30 s) and is the mean of 5–10 animals. *: p<0.05 and **: p<0.01 vs. control.

Table 3. Inhibitory Effects of KAA-276, Ketotifên and Terfenadine against Histamine-Induced Bronchoconstriction in Anesthetized (A) or Conscious (B) Guinea Pigs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Route</th>
<th>ED₉₀ (mg/kg)</th>
<th>Time after drug administration (h)</th>
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<td></td>
<td></td>
<td></td>
<td>0.5</td>
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<tr>
<td>KAA-276</td>
<td>i.d.</td>
<td>&gt;3</td>
<td>2.38</td>
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<tr>
<td>KAA-276</td>
<td>inhalation</td>
<td>0.09</td>
<td>0.03</td>
</tr>
<tr>
<td>Ketotifên</td>
<td>i.d.</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>Terfenadine</td>
<td>i.d.</td>
<td>&gt;30</td>
<td>24.3</td>
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</table>

(B) Conscious guinea pigs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Route</th>
<th>ED₉₀ (mg/kg)</th>
<th>Time after drug administration (h)</th>
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<td></td>
<td>4</td>
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<tr>
<td>KAA-276</td>
<td>p.o.</td>
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<td>2.31</td>
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<td>0.08</td>
</tr>
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<td>Ketotifên</td>
<td>p.o.</td>
<td>&gt;0.1</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>Terfenadine</td>
<td>p.o.</td>
<td>11.0</td>
<td>&gt;30</td>
</tr>
</tbody>
</table>

ED₉₀: the concentration of drug causing 90% inhibition of aerosol histamine-induced bronchoconstriction.
oral administration. Moreover, in our inhalation study, suppression of bronchoconstriction by KAA-276 continued for more than 8 h. This long period of action is also reflected by the results obtained from its in vitro evaluation. On the other hand, ketotifen administered orally or intraduodenally exhibited its antihistaminergic effect with a rapid onset but for a short period. Conversely, the antihistaminergic effect of terfenadine was of long duration but of slow onset. This result is consistent with the findings of Cheng and Woodward who demonstrated that the antihistaminergic effect of terfenadine was slow in onset and long-lasting.

The second-generation H₂ antagonists are largely excluded from the brain when given at therapeutic doses, because they did not cross the blood-brain barrier appreciably, and thus they antagonize preferentially peripheral histamine H₁ receptors rather than central nervous system histamine H₁ receptors. In another of our experiments, KAA-276 was detected only slightly in the rat brain when administered via the intratracheal route (data not shown). Moreover, KAA-276 showed no significant prolongation in a hexobarbital-induced sleeping time test (<1000 mg/kg, p.o. data not shown). Therefore, KAA-276 is assumed to have no sedating action. In the present study, we showed topical application of KAA-276 to be beneficial in inhibiting histamine-induced bronchoconstriction in guinea pigs. Its topical application is also valuable to avoid not only the side effects related to the central nervous system but also the systemic effects.

In conclusion, KAA-276 is an irreversible and highly selective histamine H₁ antagonist. Although it is effective when given either orally or by inhalation, its inhalation is more useful because of the more rapid onset of effect and the longer lasting inhibition. Based on the pharmacological profiles shown in our studies, inhaled KAA-276 would be very useful in the treatment of bronchial asthma.

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REFERENCE