Histamine H₂-Receptor Antagonism of T-593: Studies on Positive Chronotropic Responses in Guinea Pig Atria

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Histamine H₂-receptor antagonists, the original compound being brimamide (1), have brought remarkable progress in the therapy for peptic ulcer disease. Cimetidine, ranitidine, famotidine, nizatidine and roxatidine are presently available for clinical use. However, ulcer relapse has become a clinical problem after cessation of these first-generation H₂-antagonists (2–6) since fragility of mucosal defensive factors occur (7–9). Therefore, drugs with an enhancing effect on defensive factors are used together (10) to raise the quality of ulcer healing with less relapse rate. The newly synthesized anti-ulcer drug T-593, (±)-N-[2-hydroxy-2-(4-hydroxyphenyl)ethyl]-N'[2-[[5-(methylamino)methyl-2-furyl]methyl][thio]ethyl]-N''-(methylsulfonyl) guanidine (Fig. 1) (11), was developed to be a second-generation H₂-antagonist with the expectation to possess defensive effects for gastrointestinal mucosa.

The atrial chronotropic response and gastric acid out-
put are widely tested for studies on H2-receptors. In the present paper, we have determined pharmacological characteristics of T-593, revealing that T-593 showed unsurmountable antagonism on the guinea pig atrial chronotropic response to histamine. T-593 is a racemic compound composed of two kinds of enantiomers: (−)-T-593 and (+)-T-593. Differences in the H2-antagonistic activities of these chiral compounds have also been studied.

MATERIALS AND METHODS

Animals

Male Hartley guinea pigs were purchased from Nihon SLC (Hamamatsu). The animals were housed in a room at 22–25°C with a 12-hr light-dark cycle (light on 6:00 a.m.) and were given free access to food and water.

Measurement of heart rate

Isolated right atria from guinea pigs weighing 200 to 475 g were mounted under 1 g of tension in a 20-ml organ bath containing Krebs-Henseleit solution at 31°C and continuously gassed with a mixture of 95% O2 and 5% CO2. Heart rate was picked up with an isometric force-displacement transducer (SB-1T; Nihon Kohden, Tokyo) and recorded on a pen recorder via a carrier amplifier (RP-5, Nihon Kohden) and pulse-rate tachometer (RT-5, Nihon Kohden).

Inhibitory effects of H2-antagonist on agonist-induced chronotropic responses

Cumulative administration of histamine (1 × 10⁻⁷ – 3 × 10⁻¹⁻ M) or isoproterenol (3 × 10⁻¹⁰ – 3 × 10⁻⁸ M) was performed at 60-min intervals. After two constant control curves were obtained, T-593, ranitidine or famotidine (1 × 10⁻⁶ – 3 × 10⁻⁶ M) was applied for 30 min prior to further cumulative administration of histamine. The chronotropic response was reported as a percentage of the response without antagonist from the same preparation. Each concentration of antagonist was tested in 4–6 separate preparations. In the experiment on receptor protection, ranitidine at 3 × 10⁻⁶ M was pretreated for 10 min prior to the 30-min treatment with T-593 at 1 × 10⁻⁶ M. In the experiment on reversibility from H2-antagonism, chronotropic responses after washing out each of the H2-antagonists were observed at 60-min intervals with washing of the atrial tissue every 20 min.

IC₅₀, pD₂ (12) after Logit analysis and the slope of the Schild plot (13) at 30% response were calculated through computerized linear regression analysis by the least-squares method. Statistical significance was analyzed by Dunnett’s multiple comparison test.

Effect of T-593 on histamine H₂-receptors in guinea pig ileum

Longitudinal segments of ileum isolated from guinea pigs weighing 340 to 375 g were suspended under 0.5 g of tension in a 20-ml organ bath filled with Tyrode solution at 30°C, gassed with a mixture of 95% O₂ and 5% CO₂. After constant contractile responses induced by 3 × 10⁻⁶ M histamine were established, further responses were obtained after adding T-593 for 5 min. The contractions were recorded on a pen-recorder connected to an isotonic transducer (KN-259; Natsume, Tokyo). Statistical significance was analyzed by the paired t-test.

Chemicals

T-593, enantiomers of T-593 [(−)-T-593, (+)-T-593] and ranitidine (free base) were synthesized in our research laboratory. Famotidine was extracted from the commercially available product (Gaster®; Yamanouchi Pharmaceutical Co., Ltd., Tokyo) in our research laboratory. Histamine dihydrochloride and (±)-isoproterenol hydrochloride were commercially obtained from Sigma Chemical Co. (St. Louis, MO, USA). T-593, its enantiomers and famotidine after solubilization with 0.3 N HCl were neutralized with 0.1 N NaOH and diluted with saline. Ranitidine was neutralized with 0.1 N HCl and diluted with saline.

RESULTS

Slow onset of unsurmountable H₂-antagonism by T-593 on the chronotropic response to histamine in guinea pig atria

Effect of pretreatment time of T-593 at 1 × 10⁻⁶ M was determined on the positive chronotropic response to histamine in guinea pig atria (Fig. 2). The maximal response of the concentration-response curve was suppressed 7.9% after a 10-min treatment with T-593 and 29.0% after a 30-min treatment. However, further suppression was not observed by the prolonged treatment for 60 min.

Mode of inhibitory action of T-593, ranitidine or famotidine on the chronotropic response to histamine

The modes of antagonism of the H₂-antagonists T-593, ranitidine and famotidine were determined on the chronotropic response to histamine in guinea pig right atria. T-593 (3 × 10⁻⁷ – 3 × 10⁻⁶ M) and famotidine (1 × 10⁻⁷ – 3 × 10⁻⁶ M) suppressed the maximal response in a concentration-dependent fashion, indicating that these drugs are unsurmountable antagonists (Fig. 3, A and C). In contrast, ranitidine at the same concentrations of T-593 shifted the concentration-response curves to the right without suppression of the maximal response (Fig. 3B). Incubation of guinea pig atria with T-593 for 30 min did
not alter the basal heart rate, and the maturity (body weight) of the used animal did not affect the sensitivity of the atria to histamine (data not shown).

pD'2 values for T-593 and famotidine, slope of the Schild plot, pA2 value and IC50 values are summarized in Table 1. The pD'2 values were 5.50 for T-593 and 5.61 for famotidine. The IC50 values at 1 x 10^-6 M histamine were 1.67 x 10^-7 M for famotidine, 1.05 x 10^-6 M for T-593 and 1.59 x 10^-6 M for ranitidine in this potency order.

**Table 1. H2-antagonistic effects of T-593, ranitidine and famotidine on histamine-induced chronotropic responses in guinea pig atria**

| Antagonists | pD'2 | Slope of Schild plot | pA2 | IC50 (M) |
|-------------|------|----------------------|-----|----------|
| T-593       | 5.50 | 2.03                 | —   | (3.92 x 10^-7 - 2.82 x 10^-5) |
|             | (4.95 - 6.04) | (1.48 - 2.58) |     |          |
| Ranitidine  | —    | 1.22                 | 6.67| (5.95 x 10^-7 - 4.22 x 10^-5) |
|             |      | (0.92 - 1.52)       |    |          |
| Famotidine  | 5.61 | 1.74                 | —   | (1.67 x 10^-7) |
|             | (4.93 - 6.29) | (1.30 - 2.18) |     | (5.45 x 10^-7 - 5.14 x 10^-7) |

Each value was obtained from 5 preparations. 95% confidence limits are shown in parentheses.
The pD\textsubscript{2} value and IC\textsubscript{50} value for (-)-T-593 and slope of the Schild plot for (+)-T-593 are summarized in Table 2. The pD\textsubscript{2} value was 5.39, and the IC\textsubscript{50} value at 1 x 10\textsuperscript{-5} M histamine was 6.97 x 10\textsuperscript{-7} M for (-)-T-593, which had a 1.5-fold higher potency than racemic T-593. The slope of the Schild regression line of (+)-T-593 was 0.518, indicating that its mode of inhibition was not competitive.

 Confirmation of the action site of T-593

The action site of T-593 was confirmed. Isoproterenol-induced positive chronotropic responses were not affected by T-593 even at the high concentration of 3 x 10\textsuperscript{-5} M (Fig. 5), indicating that T-593 did not antagonize atrial adrenergic beta-receptors. Pretreatment with ranitidine for 10 min prior to the application of T-593 protected H\textsubscript{2} receptors from the unsurmountable antagonism by T-593 (Fig. 6). Furthermore, even at 3 x 10\textsuperscript{-4} M, T-593 did not inhibit the contraction of guinea pig ileum induced by 3 x 10\textsuperscript{-6} M histamine (Table 3). These results indicated that the action site of T-593 was the H\textsubscript{2}-receptor.

Comparison of reversibility of H\textsubscript{2}-antagonism of T-593, ranitidine and famotidine

Reversibility of H\textsubscript{2}-antagonism of T-593, ranitidine or
Table 3. Effect of T-593 on histamine H2-receptors in guinea pig ileum

| T-593 (M) | N | Contraction (%) |
|-----------|---|----------------|
| 3 × 10^-6 | 5 | 99.8±1.7       |
| 3 × 10^-5 | 5 | 100.8±2.2      |
| 3 × 10^-4 | 5 | 98.4±3.2       |

Each value represents the mean ± S.E. of the percentage to the control response from the same preparation at 3 × 10^-6 M histamine. N.S.: No significant difference (paired t-test).

DISCUSSION

Inhibitory modes of H2-antagonists are classified as traditional competitive antagonism (e.g., cimetidine (14), ranitidine (15) and loxatidine (16)) or as unsurmountable antagonism (e.g. famotidine (17) and IT-066 (18)). Unsurmountable antagonism, which was proposed by Gaddum et al. (19), is characterized by its slow onset of action, long-lasting inhibition and suppressive effect on the maximal response. It is suggested that these inhibitory mecha-

nisms are based on the slow dissociation of the antagonist from its receptors (20). In the present study, we have determined the histamine H2-antagonistic characteristics of T-593 on the positive chronotropic responses to histamine in guinea pig atria, in comparison with those of ranitidine and famotidine.

T-593 and famotidine suppressed the maximal response of the histamine-induced positive chronotropic response, indicating that these compounds were unsurmountable H2-antagonists (Fig. 3, A and C). In contrast, ranitidine shifted the histamine concentration-response curve to the right without suppression of the maximal response (Fig. 3B). The pA2 value of ranitidine at 30% response agreed with the value from previous reports (21, 22), and the slope of the Schild regression line was 1.22. This result revealed that ranitidine was a typical competitive H2-antagonist. We have determined the action site of T-593. T-593 did not affect the isoproterenol-induced chronotropic response (Fig. 5) and histamine-induced ileal contraction (Table 3), showing that T-593 did not interact with either adrenergic beta-receptors or histamine H1-receptors. Pretreatment with ranitidine prior to the application of T-593 protected histamine H2-receptors from the unsurmountable antagonism by T-593 (Fig. 6). These results demonstrated that T-593 acted selectively and specifically on histamine H2-receptors. Furthermore, the inhibitory response by T-593 was retained for over 6 hr after repeated washing of the atrial tissue (Fig. 7). This long-lasting inhibitory action suggests that T-593 is an antagonist that slowly dissociates from histamine H2-receptors.

Because T-593 is a racemic compound composed of two enantiomers: (−)-T-593 and (+)-T-593, it is necessary to determine if the two enantiomers show differences in H2-antagonism. (−)-T-593 showed more potent histamine H2-antagonism than racemic T-593 (Fig. 4A). (+)-T-593 slightly shifted the histamine concentration-response curves to the right (Fig. 4B), but the slope of its Schild regression line was 0.518 (Table 2), indicating that the inhibitory mode of (+)-T-593 was not competitive. We consider that this inhibition by (+)-T-593 is non-specific antagonism to histamine H2-receptors, and the antagonistic activity by racemic T-593 is mainly attributed to that by (−)-T-593. Batzri et al (21) suggested that gastric H2-receptors possess two binding sites from the data that higher concentrations of H2-antagonists were required to inhibit [3H]histamine binding than to inhibit cyclic AMP stimulation, and they also described that occupation of the high-affinity binding sites was sufficient to inhibit the changes in cellular cyclic AMP. Our results showing the different inhibitory action of each enantiomer indicate that hydroxy moiety on asymmetric carbon of T-593 may interact with one of the binding sites where histamine activates H2-receptors. Bristow et al. (23) sug-

famotidine (3 pM, respectively) was compared at every 1 hr after a 30-min treatment with the H2-antagonists (Fig. 7). Long-lasting inhibition was observed for over 6 hr by T-593 and for 5 hr by famotidine. In contrast, the inhibitory effect by ranitidine was not maintained after washout of the atrial tissue. T-593 is a slowly dissociable antagonist of H2-receptors.
gested that ranitidine and cimetidine may bind to different sites in guinea pig heart membrane preparation. Protection by ranitidine from the unsurmountable antagonism by T-593 indicates that one of the binding sites for T-593 is similar to that of ranitidine. The fura moity in both T-593 and ranitidine may interact with these binding sites.

The mode of antagonism by famotidine is controversial. In the present experiments, famotidine showed unsurmountable antagonism. This result is supported by Pendleton et al. (17). However, some reports show that famotidine is a competitive antagonist to atrial H₂-receptors (24, 25). In gastric secretion, famotidine does not show unsurmountable antagonism (26, 27). Inconsistency in the mode of action in these two kinds of tissues may be caused by a difference in the dissociability from each histamine H₂-receptor, but the precise mechanism remains unknown.

In conclusion, T-593 showed unsurmountable antagonism on the histamine-induced positive chronotropic response in guinea pig right atria. Its inhibitory action was more continuous than those of ranitidine and famotidine. Further studies are necessary to determine the anti-gastric secretory activity and anti-ulcer activity of T-593.

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