Histamine H₂-Receptor Antagonism of T-593, an Anti-ulcer Agent: Studies on Aminopyrine Accumulation in Isolated Canine Gastric Mucosal Cells

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ABSTRACT—Histamine H₂-receptor antagonistic properties of the anti-ulcer agent T-593, (±)-(E)-1-[2-hydroxy-2-(4-hydroxyphenyl)ethyl]-3-[2][[5-(methylamino)methyl-2-furylmethyl][thio]ethyl]-2-(methylsulfonfyl)guanidine, were investigated on [¹⁴C]aminopyrine accumulation in isolated canine gastric mucosal cells and compared with those of ranitidine and famotidine. The potency of T-593-inhibition of [¹⁴C]aminopyrine accumulation stimulated by 10⁻⁴ M histamine, with an IC₅₀ value of 1.85 x 10⁻⁶ M, was approximately 5 times greater than that of ranitidine, but half that of famotidine. T-593 did not affect [¹⁴C]aminopyrine accumulation stimulated by carbachol or dibutyryl-cAMP. T-593 depressed the maximal response of the histamine concentration-response curve with a dose-related displacement to the right, indicating that the nature of the H₂-receptor antagonism of T-593 was insurmountable and included non-competitive inhibition. The inhibitory efficacy of T-593 was time-dependent and was retained after the cells were washed. The inhibitory potency of (−)-S-T-593, one of the enantiomers, on the [¹⁴C]aminopyrine accumulation stimulated by histamine was approximately twice that of racemic T-593 and it also behaved as an insurmountable H₂-receptor antagonist. However, the potency of (+)-R-T-593 was markedly weak. These results suggest that T-593 has H₂-receptor antagonism that is insurmountable and this agent slowly associates and dissociates with the receptor in isolated canine gastric mucosal cells and that the specific substance causing H₂-receptor antagonism is (−)-S-T-593.

Keywords: T-593, Histamine H₂-antagonist, Insurmountable antagonism, Canine gastric mucosal cell, Aminopyrine accumulation

H₂-receptor antagonists opened a new era in the control of gastric acid secretion, introducing a major pharmacological development in the therapeutic treatment of peptic ulcer disease (1–3). In addition, proton-pump inhibitors represent a further advancement due to their stronger inhibition of acid secretion (4, 5). The rate of recurrence of peptic ulcer after healing with the existing therapies employing H₂-receptor antagonists or proton-pump inhibitors is relatively high. Thus, in order to lessen this relapse rate, these anti-secretagogues are generally prescribed together with mucosal protective agents. Furthermore, although attention is called to the fact that peptic ulcer relapse is reduced by the eradication of Helicobacter pylori (6), no treatment regimen exists that is completely safe and effective. Therefore, T-593 has been developed as a second-generation H₂-receptor antagonist (7) possessing mucosal protective properties, which should provide additional benefits in peptic ulcer therapy. T-593 contains a 2-[2-hydroxy-2-(4-hydroxyphenyl)ethyl]methyl moiety in its chemical structure (Fig. 1).

It was demonstrated that T-593 had antisecretory activity, improved the gastric mucosal blood flow and inhibited the experimentally induced gastric and duodenal lesions in rat (8, 9). We previously demonstrated in isolated guinea pig right atria that T-593 showed a time-dependent and insurmountable (10) H₂-receptor antagonism on the positive chronotropic response (11). It was conjectured in that study that the long-lasting antisecretory action of T-593 in vivo was caused by the slowly dissociable and long-acting H₂-receptor antagonism of T-593. However, although T-593 was found to be a time-dependent and insurmountable antagonist against the right atrial H₂-receptor, whether it has the same effect against the gastric H₂-receptor remained unclear. In the present study, we investigated the properties of the H₂-receptor antagonism of T-593 on the accumulation
of $[^{14}C]$aminopyrine ($[^{14}C]AP$), a weak organic base (pKa = 5.0), as an indirect measure of acid secretion in isolated canine gastric mucosal cells, and compared the effects of T-593 with those of ranitidine and famotidine. Isolated gastric mucosal cells, which excellently respond to secretagogues, are a well-established experimental system for studies of the direct effect of test agents on parietal cell function. Since T-593 is a racemic compound composed of (−)-S and (+)-R-T-593, the characteristics of these enantiomers were studied separately on $[^{14}C]AP$ accumulation in the same preparation.

**MATERIALS AND METHODS**

**Chemicals**

T-593, (−)-S-T-593 (+)-R-T-593 and omeprazole were synthesized and famotidine was extracted from the commercially obtainable product, Gaster® (Yamanouchi Pharmaceutical Co., Ltd., Tokyo), in our laboratory. Ranitidine hydrochloride, histamine dihydrochloride, pirenzepine hydrochloride, carbachol hydrochloride, pentagastrin, sodium dibutyryladenosine-3′,5′-cyclic monophosphate (dbcAMP) and bovine serum albumin were purchased from Sigma Chemical Co. (St. Louis, MO, USA); Hank's solution and Dulbecco's modified Eagle medium (DMEM), from Nissui Pharmaceutical Co., Ltd. (Tokyo); [dimethylamine-$^{14}$C]aminopyrine, from Amersham International Plc. (Bucks, UK); Hionic fluor, from Packard Instrument Co. (Meriden, CT, USA). All other chemicals were of reagent grade and purchased from Wako Pure Chemicals, Ltd. (Osaka).

**Isolation of canine gastric mucosal cells**

Isolated canine gastric mucosal cells were prepared as described previously (12). Briefly, a mongrel dog (10–15 kg; Fuji Animal Farm, Shizuoka) was fasted overnight and sacrificed by exsanguination, and the stomach was excised quickly. The fundic mucosa of the stomach was stripped and minced, and the fragments were incubated in digestion buffer (DMEM containing 0.1% collagenase, 10 mM HEPES and 0.2% bovine serum albumin) aerated with 95% − 5% O$_2$−CO$_2$ for 20 min. The cells were dispersed by gently pipetting, filtered through a nylon mesh to remove large cell clumps and mucus, and centrifuged at 80 × g for 5 min. The cell clumps and mucus remaining on the mesh were also digested under the same conditions and this procedure was repeated twice. The cell pellets obtained subsequently by the three digestion procedures were collected, washed with DMEM containing 10 mM HEPES and 0.2% bovine serum albumin by centrifugation, and finally resuspended in the same medium. The viabilities of mucosal cells in some experiments were greater than 95% as determined by trypan-blue dye exclusion.

**Measurement of $[^{14}C]AP$ accumulation**

$[^{14}C]AP$ accumulation was measured by a modified procedure as described previously (13, 14). Gastric mucosal cell suspensions containing 5 × 10$^6$ intact cells were precultured with 0.05 μCi of $[^{14}C]AP$ in the presence of each test drug in vials for the designated period at 37°C. Then various secretagogues were added to the cell suspension. Cell suspensions aerated with 100% O$_2$ were cultured for 30 min in a shaking water bath at 37°C. The cell suspensions were transferred into tubes in ice to stop the reaction and then centrifuged at 250 × g for 2 min. After the cell sediments were washed with cold fresh medium to remove extracellularly trapped $[^{14}C]AP$ by centrifugation at 600 × g for 2 min, the pellets were solubilized in 1 N NaOH by heating at 60°C for 45 min. After addition of a Hionic fluoro cocktail, the radioactivity that accumulated in the cells was determined with an Aloka liquid scintillation counter. The control group consisted of cells from the same isolated preparation that
received [\(^{14}\)C]AP after the preculture period but without the antagonist, and the basal accumulation group consisted of cells without antagonist and secretagogue. In all experimental series, cells were also incubated with 10\(^{-4}\) M 2,4-dinitrophenol, a mitochondria uncoupler; the incorporation in the presence of this agent was considered to represent nonspecific incorporation and the result was subtracted from all test values. All cultures were run in triplicate, and the mean value was calculated. Where appropriate, the data are expressed as the percentage of secretagogue stimulation in the absence of the antagonist. The minimal response in each experiment was subtracted.

**Washing of cells in dissociation experiments**

The cells were washed to remove the antagonist from the H\(_2\)-receptor. Gastric mucosal cell suspensions containing 5 \times 10\(^5\) intact cells were precultured with each test drug in tubes for 15 min at 37°C. Then they were centrifuged at 80 \times g for 2 min and the supernatants were removed. The cells were resuspended in fresh ice-cold medium, and this washing procedure was repeated five times in all, requiring approximately 60 min. After the final wash, the cell suspensions were used for the determination of [\(^{14}\)C]AP accumulation. Since the drug concentration of the extracellular medium was diluted more than 10\(^5\) times by the washing procedure, the cells were not exposed to potentiating concentrations of the drug.

**Data analyses**

IC\(_{50}\) and EC\(_{50}\) values were calculated by a computerized linear regression of Logit analysis by the method of least squares. The data for H\(_2\)-antagonism were analyzed by the method of Arunlakshana and Schild (15), calculated from the ratio of EC\(_{50}\) values estimated from fitting a logistic function to each histamine concentration-response curve. The Schild plots are expressed as log (DR-1) against the logarithmic concentration of the drug, where "DR" is the dose ratio of the concentrations of histamine producing equal responses. The pA\(_2\) values were found from the x-intercepts of the Schild plots. The pD\(_{2}\) value was obtained as the half-inhibition of maximal stimulation induced by 10\(^{-3}\) M histamine calculated by linear regression (16).

**RESULTS**

**Stimulation of acid secretion by various secretagogues**

[\(^{14}\)C]AP accumulation as the index of acid secretion stimulated by histamine, carbachol, pentagastrin or dbcAMP was determined in canine gastric mucosal cells (Fig. 2). Histamine stimulated the [\(^{14}\)C]AP accumulation concentration dependently in the range of 10\(^{-6}\) to 10\(^{-3}\) M, whereas carbachol remarkably stimulated this accumulation maximally at 10\(^{-4}\) M. Pentagastrin produced little stimulation, but dbcAMP greatly stimulated the [\(^{14}\)C]AP accumulation, with a peak value at 10\(^{-4}\) M. The maximal accumulation stimulated by each secretagogue, in the decreasing order of carbachol, dbcAMP, histamine and pentagastrin, was consistent with that observed by others (12, 13).

**Inhibitory effect of T-593 on [\(^{14}\)C]AP accumulation stimulated by various secretagogues**

The inhibitory effect of T-593 on [\(^{14}\)C]AP accumulation stimulated by 10\(^{-4}\) M histamine in canine gastric mu-

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![Fig. 2. Comparison of stimulation by the secretagogues, histamine, carbachol, pentagastrin and dbcAMP on [\(^{14}\)C]AP accumulation in canine gastric mucosal cells. The stimulation procedure was carried out by culturing with the secretagogues and [\(^{14}\)C]AP for 30 min. The data represent the mean from triplicate determinations.](image.png)
Fig. 3. Comparison of the effects of increasing concentration of drugs T-593 (○), ranitidine (△), and famotidine (□) on [14C]AP accumulation stimulated by histamine in canine gastric mucosal cells. The cells were preincubated with [14C]AP for 15 min in the presence of the drug or vehicle control, and then 10⁻⁴ M histamine was subsequently added for 30 min. The basal accumulation (●) was found without histamine and any antagonists. The percentage of histamine-stimulated [14C]AP accumulation, subtracting the minimal response, is plotted as a function of the drug concentration, from triplicate determinations.

Fig. 4. Effects of T-593 (○), ranitidine (△), famotidine (□) and pirenzepine (▲) on [14C]AP accumulation stimulated by carbachol in canine gastric mucosal cells. The cells were preincubated with [14C]AP for 15 min in the presence of the drug or vehicle control, and then 10⁻⁵ M carbachol was subsequently added for 30 min. The basal accumulation (●) was found without carbachol and any antagonists. The percentage of carbachol-stimulated [14C]AP accumulation, subtracting the minimal response, is plotted as a function of the drug concentration, from triplicate determinations.

Fig. 5. Effects of T-593 (○), ranitidine (△), famotidine (□) and omeprazole (▲) on [14C]AP accumulation stimulated by dbcAMP in canine gastric mucosal cells. The cells were preincubated with [14C]AP for 15 min in the presence of the drug or vehicle control, and then 10⁻⁴ M dbcAMP was subsequently added for 30 min. The basal accumulation (●) was found without dbcAMP and any antagonists. The percentage of dbcAMP-stimulated [14C]AP accumulation, subtracting the minimal response, is plotted as a function of the drug concentration, from triplicate determinations.

Cosal cells was compared with that of famotidine and ranitidine (Fig. 3). T-593, ranitidine and famotidine inhibited [14C]AP accumulation stimulated by histamine with IC₅₀ values of 1.85 × 10⁻⁶, 9.63 × 10⁻⁶ and 9.31 × 10⁻⁷ M, respectively.

T-593, ranitidine and famotidine (10⁻⁷ to 10⁻⁴ M) did not affect [14C]AP accumulation stimulated by 10⁻⁵ M carbachol (Fig. 4) and slightly inhibited the accumulation stimulated by 10⁻⁴ M dbcAMP with a maximal inhibition of 20–32% (Fig. 5). In contrast, pirenzepine, a muscarinic receptor antagonist, inhibited [14C]AP accumulation stimulated by carbachol with an IC₅₀ value of 5.26 × 10⁻⁸ M, and omeprazole, a proton-pump inhibitor, inhibited the accumulation stimulated by dbcAMP with an IC₅₀ value of 1.33 × 10⁻⁷ M. The IC₅₀ values of the antagonists are shown in Table 1. T-593 showed H₂-receptor antagonist, like ranitidine and famotidine.

Table 1. Summary of the IC₅₀ values of the drugs on [14C]AP accumulation stimulated by the secretagogues in canine gastric mucosal cells

<table>
<thead>
<tr>
<th>Drug</th>
<th>Histamine 10⁻⁴ M</th>
<th>Carbachol 10⁻⁵ M</th>
<th>dbcAMP 10⁻⁴ M</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-593</td>
<td>1.85 × 10⁻⁶ M</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>9.63 × 10⁻⁶ M</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Famotidine</td>
<td>9.31 × 10⁻⁷ M</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Pirenzepine</td>
<td>NT</td>
<td>5.26 × 10⁻⁸ M</td>
<td>NT</td>
</tr>
<tr>
<td>Omeprazole</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>(−)-S-T-593</td>
<td>8.74 × 10⁻⁷ M</td>
<td>NT</td>
<td>1.33 × 10⁻⁷ M</td>
</tr>
<tr>
<td>(+)-R-T-593</td>
<td>7.22 × 10⁻⁴ M</td>
<td>NT</td>
<td>NT</td>
</tr>
</tbody>
</table>

NT, not tested.
Fig. 6. Histamine cumulative concentration-response curves with T-593 (A), ranitidine (B) and famotidine (C) in canine gastric mucosal cells. The cells were precultured with [14C]AP for 15 min in the presence of the drug or vehicle control, and then 10^{-7}-10^{-3} M histamine was subsequently added for 30 min. The percentage of histamine-stimulated [14C]AP accumulation, subtracting the minimal response, is plotted as a function of the drug concentration, from triplicate determinations. Inserts show the Schild-plot analysis. (A) ●: 0 M, ○: 10^{-7} M, △: 3×10^{-7} M, □: 10^{-6} M; (B) ●: 0 M, ○: 10^{-6} M, △: 3×10^{-6} M, □: 10^{-5} M; (C) ●: 0 M, ○: 10^{-7} M, △: 3×10^{-7} M, □: 10^{-6} M of each H_{2}-antagonist.
Table 2. Summary of the Schild-plot analysis for the drugs on [14C]AP accumulation stimulated by histamine in canine gastric mucosal cells

<table>
<thead>
<tr>
<th>Drug</th>
<th>pD'2</th>
<th>pA2</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-593</td>
<td>5.55</td>
<td>—</td>
<td>2.01</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>—</td>
<td>7.29</td>
<td>0.93</td>
</tr>
<tr>
<td>Famotidine</td>
<td>—</td>
<td>8.22</td>
<td>1.06</td>
</tr>
<tr>
<td>(−)-S-T-593</td>
<td>6.02</td>
<td>—</td>
<td>1.95</td>
</tr>
<tr>
<td>(−)-R-T-593</td>
<td>—</td>
<td>6.00</td>
<td>0.81</td>
</tr>
</tbody>
</table>

The pD'2 value was calculated from the response produced by 10⁻³ M histamine.

Mode of T-593 inhibition on [14C]AP accumulation stimulated by histamine

The histamine concentration-response curve for [14C]AP accumulation showed an increase from 10⁻⁷ to 10⁻³ M histamine. The inhibitory effects of T-593, ranitidine and famotidine on the histamine concentration-response curve were determined in canine gastric mucosal cells. T-593 depressed the maximal response of the histamine concentration-response curve with a dose-related displacement to the right, but ranitidine and famotidine produced this rightward shift in the curve dose-dependently without decreasing the maximal response (Fig. 6). A Schild-plot analysis of these curves yielded slopes of 2.01, 0.93 and 1.06 for T-593, ranitidine and famotidine, respectively (Table 2). The slope for T-593 indicated that the antagonism was insurmountable and non-competitive, and since the values of ranitidine and famotidine did

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**Fig. 8.** Washout of the effect of T-593 (○), ranitidine (△) and famotidine (□) on [14C]AP accumulation in canine gastric mucosal cells. The cells were preincubated with each drug for 15 min and washed with fresh medium until the extracellular medium was diluted over 10⁶ times. Then [14C]AP and 10⁻³ M histamine were added to the cells and cultured for 30 min. The percentage of histamine-stimulated [14C]AP accumulation, subtracting the minimal response, is plotted as a function of the drug concentration, from triplicate determinations.

**Fig. 7.** Effect of T-593 preincubation time on [14C]AP accumulation stimulated by histamine in canine gastric mucosal cells. The cells were preincubated with [14C]AP for 0 (●), 15 (○), 30 (△) and 60 (□) min in the presence of the agent or vehicle control, and then 10⁻³ M histamine was subsequently added for 30 min. The basal accumulation (●) was found without histamine and T-593. The percentage of histamine-stimulated [14C]AP accumulation, subtracting the minimal response, is plotted as a function of the agent concentration, from triplicate determinations.

**Fig. 9.** Comparison of the effects of increasing concentration of racemic T-593 (○), (−)-S-T-593 (●) and (±)-R-T-593 (△) on [14C]AP accumulation stimulated by histamine in canine gastric mucosal cells. The cells were preincubated with [14C]AP for 15 min in the presence of the agent or vehicle control, and then 10⁻³ M histamine was subsequently added for 30 min. The basal accumulation (●) was without histamine and any agents. The percentage of histamine-stimulated [14C]AP accumulation, subtracting the minimal response, is plotted as a function of the agent concentration, from triplicate determinations.
not differ significantly from unity, this confirmed that the antagonism observed was competitive. Hence, the \( pA_2 \) value, the negative logarithm of the dissociation constant, could not be calculated for T-593, but the \( pD'_2 \) value for T-593 was 5.55. The \( pA_2 \) values were 7.29 for ranitidine and 8.22 for famotidine. The Schild-plot slope and the \( pA_2 \) and \( pD'_2 \) values for the drugs are given in Table 2.

The time dependency of the precurtue with T-593 for the inhibitory effect on \([^{14}C]\)AP accumulation stimulated by \(10^{-4} \) M histamine was determined (Fig. 7). The inhibitory potency of T-593 precurtue for 15 min was more than that 3.8 times that of the non-precurtue preparation. When the precurtue time was extended to 30 min, the T-593 inhibition was tripled, but extending the precurtue time to 60 min did not increase the inhibition further. This showed that T-593 produced a time-dependent inhibition in canine gastric mucosal cells.

**Washout of potency of T-593 inhibition of \([^{14}C]\)AP accumulation**

After washing the cells in the culture medium to remove the drug, the remaining inhibitory effects of T-593, ranitidine and famotidine on the \([^{14}C]\)AP accumulation stimulated by \(10^{-4} \) M histamine in canine gastric mucosal cells precurtue for 15 min were determined (Fig. 8). T-593 inhibited the \([^{14}C]\)AP accumulation stimulated by histamine with an \( IC_{50} \) value of \(2.24 \times 10^{-6} \) M; this value scarcely differed from the \( IC_{50} \) value obtained without the washing. In contrast to T-593, the inhibitory effect of ranitidine disappeared completely by washing the cells. Famotidine inhibited the \([^{14}C]\)AP accumulation stimulated by histamine with an \( IC_{50} \) value of \(3.75 \times 10^{-5} \) M, a value that was markedly weaker after the cells were washed. These data suggest that the \( H_2 \)-receptor antagonism of T-593 was tight and difficult to reverse by washing the cells under the present conditions, unlike the inhibition caused by ranitidine and famotidine.

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**Fig. 10.** Histamine cumulative concentration-response curves with (−)-S-T-593 (A) and (+)-R-T-593 (B) in canine gastric mucosal cells. The cells were precurtue with \([^{14}C]\)AP for 15 min in the presence of the agent or vehicle control, and then \(10^{-7} - 10^{-3} \) M histamine was subsequently added for 30 min. The percentage of histamine-stimulated \([^{14}C]\)AP accumulation, subtracting the minimal response, is plotted as a function of the drug concentration, from truplicate determinations. Inserts show the Schild-plot analysis. (A) •: 0 M, ○: \(10^{-7} \) M, △: \(3 \times 10^{-7} \) M, □: \(10^{-6} \) M; (B) ●: 0 M, ○: \(3 \times 10^{-5} \) M, △: \(10^{-4} \) M, □: \(3 \times 10^{-4} \) M of each enantiomer.
Inhibitory effect of (-)-S- and (+)-R-T-593 on \[^{14}\text{C}\]AP accumulation stimulated by histamine

The inhibitory effect and mode of inhibition of (-)-S- and (+)-R-T-593 on the \[^{14}\text{C}\]AP accumulation stimulated by 10\(^{-4}\) M histamine were determined in canine gastric mucosal cells and compared with these actions by racemic T-593 (Fig. 9). The inhibitory potency of (-)-S-T-593 with an IC\(_{50}\) value of 8.74 \times 10^{-7} M was approximately double that of racemic T-593 and 800 times greater than that of (+)-R-T-593, which had an IC\(_{50}\) value of 7.22 \times 10^{-5} M (Table 1). In addition, (-)-S-T-593 caused a dose-related displacement to the right in the histamine concentration-response curve and depressed the maximal response with a Schild-plot slope of 1.95, data that agree well with what is observed for racemic T-593, consistent with an insurmountable antagonism (Fig. 10, Table 2). In contrast, (+)-R-T-593 with a pA\(_{2}\) value of 6.00 and a Schild-plot slope of 0.81 produced a rightward displacement of the histamine concentration-response curve without depressing the maximal response (Fig. 10, Table 2).

DISCUSSION

T-593 has been developed as an anti-ulcer agent with the expectation of healing ulcers by reducing the aggressive action of gastric acid and improving the defense of the gastroduodenal mucosa. We investigated the \[^{14}\text{C}\]AP accumulation in isolated canine gastric mucosal cells to analyze the mechanism of the inhibitory effect on acid secretion and the \(H_2\)-receptor antagonism of T-593 in comparison with the effects of ranitidine and famotidine.

The \[^{14}\text{C}\]AP accumulation is an indirect measure of the acid compartment within parietal cells and is an extremely useful method for studying the mechanism of acid secretion and its inhibition in vitro. This method has been adopted in many other studies. The regulation of acid secretion by fundic mucosal parietal cells involves three major pathways: histaminergic, cholinergic and gastrinergic (17). In the present study, we used histamine, carbachol (the analogue of acetylcholine not metabolized by esterase), pentagastrin (five N-terminal residues of gastrin peptide) and dbcAMP (the analogue of cAMP not metabolized by phosphodiesterase) as secretagogues added to canine gastric mucosal cells (Fig. 2). Unfractionated gastric mucosal cells are sufficient for measurements of \[^{14}\text{C}\]AP accumulation, since parietal cells are the only cell type with proton production (13); and it is reported that in both unfractionated and fractionated cells, histamine stimulates the \[^{14}\text{C}\]AP accumulation in an equipotent as well as a concentration-dependent manner (18, 19). In agreement with the report of other investigators, it was characteristic of canine gastric mucosal cells that acid secretion induced by a cholinergic stimulant was markedly greater than those induced by other stimulants (13).

The inhibitory effect of T-593 on \[^{14}\text{C}\]AP accumulation stimulated by \(10^{-4}\) M histamine with an IC\(_{50}\) value of 1.85 \times 10^{-6} M was approximately more than 5 times as efficacious as ranitidine, but was half as effective as famotidine (Fig. 3). The minimal secretion inhibited by each drug was less than the basal secretion without stimulant. This presumably reflected an addition of secretion stimulated by endogenous histamine on the true basal level. T-593, ranitidine or famotidine could not affect \[^{14}\text{C}\]AP accumulation stimulated by 10\(^{-5}\) M carbachol or 10\(^{-4}\) M dbcAMP (Figs. 4 and 5). The response stimulated by carbachol or dbcAMP was reduced in a concentration-dependent manner by pirenzepine or omeprazole, respectively. T-593, ranitidine and famotidine (10\(^{-6}\) to 10\(^{-4}\) M) inhibited to approximately 30\% of the \[^{14}\text{C}\]AP accumulation stimulated by dbcAMP; the most probable mechanism for this slight inhibition is that the small component of cAMP response is mediated via endogenous histamine action on the \(H_2\)-receptor. The enhancement of \[^{14}\text{C}\]AP accumulation stimulated by pentagastrin in canine gastric mucosal cells was not potent enough to employ for determining any inhibitory effects of drugs. It was confirmed that T-593 did not affect the \(H^+\),K\(^+\)-ATPase (proton-pump) activity of gastric vesicles purified from hog gastric mucosa (data not shown). These results showed that T-593 had no inhibitory effect on the cholinergic receptor and on the process of acid secretion after the second messenger, cAMP, of the \(H_2\)-receptor. It is likely that the \(H_2\)-receptor antagonism of T-593 is selective, like those by ranitidine and famotidine. However, since it cannot be denied that T-593 has an inhibitory effect on Gs or adenylyl cyclase, further experiments that examine its binding to \(H_2\)-receptors are necessary.

The mode of inhibition of T-593 on the histamine concentration-response curve for \[^{14}\text{C}\]AP accumulation was also determined in canine gastric mucosal cells. T-593 acted as an insurmountable antagonist of histamine, in contrast to ranitidine and famotidine, which are competitive antagonists (Fig. 6). The present observations with T-593 were expected, since it was also reported in our earlier report that T-593 is an insurmountable \(H_2\)-receptor antagonist on guinea pig atrium (11). The pD\(_{2}\) value of 5.55 for T-593 in the present study agrees well with the value of 5.50 for guinea pig atrium.

The inhibitory effect of T-593 on \[^{14}\text{C}\]AP accumulation stimulated by histamine increased as the period of pretreatment of the cells with T-593 was extended (Fig. 7). In contrast, it has been demonstrated that the inhibitory effect of ranitidine or famotidine on acid secretion in vitro is not dependent on pretreatment time (20).
Gastric H₂-Antagonism of T-593

In the dissociation experiments, the inhibitory efficacy of T-593 after removing the drug from the medium by washing the cells remained the same as that without the washing procedure. In contrast, the potencies of ranitidine and famotidine almost disappeared after washing (Fig. 8). Employing the present washing condition with ice cold media within 60 min, T-593 showed tight H₂-receptor antagonism in canine gastric mucosal cells. In the guinea pig atrial preparations, the efficacy of T-593 is reversed finally after washing the tissue (11). This discrepancy may be due to the washing conditions, but it is appropriate to consider that T-593 dissociates slowly from H₂-receptors.

These results suggest that the H₂-receptor antagonism of T-593 is insurmountable and that T-593 slowly associates and dissociates with H₂-receptors in isolated canine gastric mucosal cells, unlike the antagonism produced by ranitidine and famotidine. The non-competitive, insurmountable nature of the interaction was attributable to the slow attachment and release of T-593 relative to the H₂-receptors (21, 22). Incidentally, in the case of famotidine, after the cells were washed, only 54% of the stimulation by histamine was recovered. This supports the report that the H₂-receptor antagonism of famotidine on gastric mucosal cells is partially irreversible (23). It appears that the H₂-receptor can be divided into two subclasses according to the binding of antagonists.

The nature of H₂-receptor antagonism with existing drugs is divided broadly into two pharmacological classes. Thus cimetidine (2) and ranitidine (24) are competitive, fully surmountable antagonists in in vitro preparations, whereas IT-066 (25, 26) and FRG-8813 (27, 28) cause an insurmountable antagonism of histamine that is difficult to reverse by washing the cells or tissue. T-593 appeared to belong in the latter classification. Famotidine has also been described as an insurmountable antagonist of histamine on the guinea pig atrium (11, 29), but it behaved as a competitive H₂-receptor antagonist on the gastric mucosa (23, 30). We have also confirmed that T-593 behaves similarly in the gastric mucosal cells of rat, guinea pig or rabbit. In all cases, it was difficult to reverse its efficacy by washing the cells.

The characteristics of the two enantiomers, (−)-S- and (+)-R-T-593, were very interesting. The inhibitory potency of (−)-S-T-593 was approximately twice that of racemic T-593 and approximately 800 times greater than that of (+)-R-T-593 (Fig. 9, Table 1). Also, (−)-S-T-593 was an insurmountable H₂-receptor antagonist with a Schild-plot slope of 1.95, which agrees quite well with the behavior of racemic T-593 (Fig. 10). In contrast, the efficacy of (+)-R-T-593 was markedly weak and exhibited a small enhancement at a 10⁻² M concentration, which was qualitatively different from the effects of (−)-S-T-593 and racemic T-593 (Fig. 9). It was not clear whether (+)-R-T-593 had a competitive H₂-receptor antagonism or not, since the rightward displacement of the histamine concentration-response curve was not a parallel shift with unity of the Schild-plot slope (Fig. 10, Table 2). The different effects of the enantiomers provides convincing evidence that (−)-S-T-593 is the specific substance of H₂-receptor antagonism.

It is conceivable that the aminomethylfurane moiety, which is a component of both T-593 and ranitidine, binds to the H₂-receptor, since it can occupy the space between the polar group and the nitrogen of this moiety (31, 32). Since the 2-[2-hydroxy-2-(4-hydroxyphenyl)]ethyl moiety of T-593, which probably does not contribute to receptor binding, decides the affinity of the two enantiomers to the receptor, this moiety may be a steric hindrance when the molecule approaches the binding site. Further studies are necessary to analyze the H₂-receptor binding properties in terms of molecular biology. (−)-S- and (+)-R-T-593 may prove to be valuable probes for regulating H₂-receptor antagonism.

In conclusion, the present data have demonstrated the H₂-receptor antagonism properties of T-593; this antagonism is insurmountable and indicates a slow association and dissociation of the agent and receptor in isolated canine gastric mucosal cells. The specific substance of H₂-receptor antagonism is (−)-S-T-593. T-593 is expected to excellently potentiate the therapeutic treatment of peptic ulcer disease.

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