Effects of Z-300, a New Histamine H2-Receptor Antagonist, on Mucin Biosynthesis in Rat Gastric Mucosa

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ABSTRACT—We examined the effects of Z-300 (N-[3-[3-(piperidinomethyl)phenoxy]propyl]-2-(2-hydroxyethyl-1-thio)acetamido-2-(4-hydroxy benzoyl)benzoate), a newly-synthesized selective histamine H2-receptor antagonist, on mucin in rat gastric mucosa. Deep corpus mucin content increased significantly to 127% of the control after the administration of 30 mg/kg of Z-300, whereas that in the antral mucosa did not increase. The addition of Z-300 significantly increased [3H]-labeled mucin in the corpus region. In the antrum, biosynthetic activity showed no significant change by 10-8–10-5 M of Z-300. These results suggest that Z-300 not only inhibits acid secretion but may also promote gastric mucus metabolism in the corpus region.

Keywords: Z-300, Histamine H2-receptor antagonist, Mucin biosynthesis

Z-300, N-[3-[3-(piperidinomethyl)phenoxy]propyl]-2-(2-hydroxyethyl-1-thio)acetamido-2-(4-hydroxy benzoyl)benzoate (Fig. 1), is a highly selective and competitive histamine H2-receptor antagonist (H2-antagonist) that exhibits potent antisecretory activity in rats and dogs (1). Okabe et al. (1) showed that this compound markedly protected the gastric mucosa not only against the pathogenesis related to gastric secretion, such as water-immersion stress, indomethacin or aspirin, but also against HCl-ethanol-induced gastric lesions in rats. This finding suggests that Z-300 may have a so-called cytoprotective action (2) as well as anti-acid secretory activity. Although the exact mechanism of mucosal cytoprotection is unknown, it presumably involves one or more of the naturally occurring defensive factors of gastric mucosa such as mucus metabolism. Mucin, a major component of gastric mucus, is a high molecular weight compound of unique structure and is considered to be an important mucosal defensive factor (3). The present study was conducted to provide some clarification of the effects of Z-300 on mucin in rat gastric mucosa, by determining the changes in the gastric intramucosal mucin content and the biosynthetic activity of mucin.

Seven-week-old male Wistar rats (SLC, Shizuoka), each weighing approximately 160 g, were used. All were fasted for 24 hr before the experiments and had free access to water during this time. To study the effect of Z-300 on the gastric intramucosal mucin content, the drug, suspended in a 0.5% carboxymethylcellulose (CMC) solution, was administered orally at 10 or 30 mg/kg body

![Fig. 1. Chemical structure of Z-300.](image-url)
weight. The control rats received 0.5% CMC alone. The animals were killed 1 hr after the last administration, and then their stomachs were removed. After scraping off the surface mucus gel, the remaining deep mucosa of the stomach was separated into the corpus and antral regions. According to our previously described method (4), the mucin was extracted using Triton X-100-containing buffer and isolated with Bio-Gel A-1.5 m column chromatography. Three peaks appeared on each elution curve (4). The first, which eluted near the void volume, was collected and designated as Fr-1. Most of the hexose-containing materials in Fr-1 have already been characterized as mucin (5). Thus, the mucin content was determined for each sample by dividing the hexose content in Fr-1, as measured by the phenol-sulfuric acid method, and expressed as micrograms hexose per stomach. The results were expressed as means ± S.D. The one way analysis of variance (ANOVA) with Dunnett's test was used for statistical analysis. Figure 2 shows the mucin content in the corpus region of rat gastric mucosa 1 hr after the treatment with or without Z-300 (10 or 30 mg/kg orally). Deep corpus mucin content increased significantly to 127% of the control after the administration of 30 mg/kg of Z-300, whereas that in the antral mucosa did not increase (data not shown). This finding suggests that Z-300 not only inhibits the gastric acid secretion, as H2-antagonists do, but may also promote gastric mucus metabolism in the corpus region.

For further clarification of the effects of this compound on mucin metabolism, the biosynthetic activity of mucin was determined by the organ culture technique. Ranitidine hydrochloride (ranitidine) was used as a reference compound, because it was shown to lack a protective effect against necrotizing agent-induced gastric mucosal damage (6). The stomachs of the rats fasted for 24 hr before the experiments were removed immediately. They were then cut along the greater curvature, and the luminal surface was gently washed with Ca2+/Mg2+-free phosphate-buffered saline (PBS(−)). The glandular part was selected, separated into the corpus and antrum and cut into small sections of 2 × 2 mm. The tissue culture method of Eastwood and Trier was used with modification (7). Eight tissue fragments, the mucosal surface facing up, were placed on a stainless steel grid in the central well of a plastic culture dish (60 × 15 mm; Falcon, Lincoln Park, NJ, USA) containing 0.75 ml of the culture medium. The medium consisted of 90% Eagle's minimum essential medium and 10% dialyzed fetal calf serum, with 370 KBq/ml of D-[1,6-3H(N)]glucosamine hydrochloride (1950 GBq/mmol; New England Nuclear, Boston, MA, USA). For the addition of H2-antagonists to the culture medium, the drugs were dissolved in dimethyl sulfoxide (DMSO) and added at concentrations of 10−8, 10−7, 10−6 and 10−5 M to the dishes, bringing the final concentration of DMSO to 0.01%. DMSO solution without drug addition was added to the control well. All the dishes were maintained at 37°C for 5 hr in 5% CO2 and 95% air. On completion of the culture period, the tissue fragments on a grid were harvested from the medium, gently rinsed with PBS(−) and boiled at 100°C for 3 min in 0.4 ml of 0.05 M Tris-HCl buffer, pH 7.2. According to our previously described method (7), the mucin was extracted and isolated with Bio-Gel A-1.5 m column chro-

Fig. 2. Effect of Z-300 on gastric mucin content in the corpus region. Values are expressed as percentages of the control and represent means ± S.D. from 4 different samples. The mucin content of control animals expressed as μg hexose/rat was 394 ± 56. *P < 0.05, as compared with the control value.

Fig. 3. Influences of Z-300 and ranitidine on [3H]glucosamine incorporation into mucins in the corpus region. Values are expressed as percentages of the control and represent means ± S.D. from 8 different samples. *P < 0.05 and **P < 0.01, as compared with the control value by one way analysis of variance (ANOVA) with Dunnett’s test. □ Ranitidine, □ Z-300.
matography. Finally, the radioactivity recovered in the void volume of the column, which had been demonstrated to be the radiolabeled mucin (7), was determined by a scintillation counter (Model LS-2800; Beckman, Fullerton, CA, USA), using Aquasol-2 (New England Nuclear) as the scintillant. To compare the synthetic activity of mucin, the total radioactivity of this fraction was divided by the tissue protein content of each homogenate to give the value relative to that of the control. Protein content in the tissue homogenate was determined with a Pierce BCA protein assay kit with bovine serum albumin as the standard.

Figure 3 shows the biosynthetic activity of mucin in the corpus as measured by [H]glucosamine incorporation. The biosynthetic activity of mucin in the control was $18.3 \pm 1.8 \text{ dpm/\mu g tissue protein}$. The addition of Z-300 significantly increased [H]glucosamine incorporation into mucin in the corpus region. On the other hand, ranitidine yielded no significant change in the mucin biosynthesis (Fig. 3). A similar result for ranitidine was obtained by Heim et al. (8) using isolated pig gastric mucosal cells. Z-300 has been shown to markedly protect the gastric mucosa against necrotizing agent in rats (1). Many reports have indicated that ranitidine lacks a protective effect against necrotizing agent-induced gastric mucosal damage in the rat (6). These results suggest that an H$_2$-antagonist that has a cytoprotective ability might promote mucin metabolism. Previously, we reported on exogenous prostaglandins that are capable of inducing direct cytoprotection by increasing significantly the biosynthetic activity of mucins in the corpus and antrum of the rat stomach (9). Moreover, our recent studies indicated that tetrogastrin protects the gastric mucosa from necrotizing agents (10) and significantly increases the biosynthetic activity of mucin (7). These results, together with our present results, suggest that the activation of the accumulation and biosynthesis of gastric mucin by a drug is closely related to the presence of the mucosal protective activity, so-called cytoprotection.

Heim et al. (8) found that histamine functions via histamine H$_2$-receptors as a stimulant of protein and glyco-protectin production in isolated pig gastric non-parietal cells. In this study, the effect of Z-300 in the corpus is not due to H$_2$-antagonism since another H$_2$-antagonist did not affect mucin production. Recently, the targets of H$_2$-antagonists were indicated to be not parietal cells, but the cells of the immune system in the gut (11). It is still uncertain whether Z-300 has a direct effect on mucus-producing cells or an indirect effect through the action of other cells, but gastrin, mainly released from G-cells located in the antrum, could not possibly be responsible for Z-300-induced stimulation of mucin biosynthesis that occurs in the isolated corpus mucosa. The mucin biosynthesis induced by the new H$_2$-antagonist Z-300 in the oxyntic region of rat stomach appears to be a point of considerable importance.

In the present study, no significant change could be detected in the mucin biosynthesis of the antrum by the addition of $10^{-8}$ – $10^{-5}$ M of either Z-300 or ranitidine (specific activity: $29.4 - 32.3 \text{ dpm/\mu g tissue protein}$). Recently, we found that gastrin, known to be an acid stimulatory hormone, accelerated mucin biosynthesis only in the oxyntic region, but yielded no significant change in the antral region of rat stomach. Moreover, the CCK-B/gastrin receptor was shown to be involved in this mechanism (7). In the previous study, mucins obtained from the corpus and antrum of rat gastric mucosa were shown to differ in the chemical composition of their carbohydrate moieties and subunit structures (12, 13). These results and an immunohistochemical observation (14) strongly indicate that there are different types of mucin producing cells in the corpus and antrum, and the regulatory mechanism on mucin biosynthesis might differ between these two types of cells. If Z-300 has a direct effect on the mucin producing cells in the oxyntic region, it might be a very useful tool for the further clarification of the regulatory mechanism of mucin synthesis.

In conclusion, the present findings demonstrated that Z-300 not only inhibits acid secretion but may also promote gastric mucous metabolism in the corpus region.

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