Original

**Effect of Omeprazole Ingestion on Healing of Gastric
Ulcers and Serum Gastrin Concentration in Rats**

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Abstract: We induced gastric ulcers in rats by administering a subserosal injection of 20% acetic acid and assessed the effects of omeprazole (OPZ) and of cimetidine on biosynthesis in the regenerating gastric mucosa and serum gastrin level. Immunohistological study of incorporation of Bromodeoxyuridine (BrdU) into DNA revealed that 3- and 5-day treatments with OPZ caused a significant decrease in the labeling index (LI) in the regenerating mucosa (38–48% of control, $p < 0.05$). Seven- and 14-day treatments with omeprazole caused a significant increase in the LI (170–186% of control, $p < 0.05$). In contrast, no significant effect was observed in cimetidine-treated rats throughout the entire experimental period. Serum gastrin levels were significantly elevated and reached a maximum (465 ± 73 pg/ml, 247% of control, $p < 0.05$) on the seventh day, and remained elevated (415 ± 133 pg/ml, 356% of control, $p < 0.05$) on the fourteenth day in OPZ-treated rats. Such changes were not observed in cimetidine-treated rats. These results suggest that increased serum gastrin, in response to the intragastric neutralization induced by OPZ administration, could accelerate cellular proliferation in the regenerating mucosa at the ulcer margin.

Key words: omeprazole, gastrin, stomach, acetic ulcer

Introduction

Omeprazole (OPZ), a substituted benzimidazole, suppresses intragastric acid secretion more strongly than H_2 -receptor antagonists by selectively interfering with the proton pump (H^+ , K^+ -ATPase) in parietal cells. OPZ has produced excellent results in treating peptic ulcer disease^{1,2)}, and has been shown to have other effects on the gastric mucosa in addition to intragastric neutralization^{3,4)}. Recently, there has been increasing interest in the effect of OPZ against peptic ulcers accompanied by *Helicobacter pylori* infection⁵⁾.

It is widely accepted that sufficient suppression of intragastric acid secretion leads to hypergastrinemia⁶⁻⁸⁾. In a previous report, after 4 weeks of treatment with OPZ, 20 mg/kg daily, serum gastrin levels rose to 186% of the pretreatment level in patients with duodenal ulcer⁹⁾. Gastrin is considered to be an important growth-promoting agent in the gastric mucosa^{10,11)}. Evidence that the constant administration of pentagastrin increased the oxyntic mucosal mass was initially presented by Crean et al.¹²⁾ Gastrin also promotes protein

synthesis in the oxyntic mucosa¹³⁾. The effect of exogenous hypergastrinemia on the rate of cell proliferation was studied in the gastric mucosa of normal humans, and a marked increase in the labeling index (LI) of ³H-thymidine was induced by pentagastrin infusion in endoscopic biopsied specimens of the fundic mucosa¹⁴⁾.

Taken together, gastrin might stimulate epithelial proliferation, even in the regenerating mucosa of an ulcer margin. Therefore, we designed the present study to determine whether the effect of OPZ on ulcer healing, especially in promoting regeneration of mucosa, is influenced by the serum gastrin level. Using an immunohistochemical stain with BrdU, we evaluated DNA synthesis at the regenerating mucosa of acetic acid ulcers of rats, and assessed the changes in the serum gastrin level after administration of OPZ.

Materials and Methods

Reagents and Antibodies

OPZ was a generous gift of Yoshitomi Pharmaceuticals (Osaka, Japan). Cimetidine (CMT) was purchased from Fujisawa Pharmaceuticals (Osaka, Japan). Bromo-deoxyuridine (BrdU) and 3,3'-diaminobenzidine tetrahydrochloride were purchased from Sigma (St Louis, MO, USA). All other materials were purchased from Sigma unless otherwise indicated.

Mouse anti-BrdU monoclonal antibody was purchased from Becton Dickinson (San Jose, CA, USA). Peroxidase-conjugated rabbit anti-mouse IgG antibody was purchased from Dakopatts (Glostrup, Denmark). These antibodies were diluted to 1 : 50 in phosphate-buffered saline (PBS) containing 1 % bovine serum albumin for the experiments.

Induction of Gastric Ulcer

Male Wistar rats seven weeks of age, 180–210 g, were used. Experimental gastric ulcers were induced by the subserosal injection of acetic acid according to Takagi *et al.*¹⁵⁾ In brief, rats were anesthetized with diethyl ether and laparotomized. Then, 0.02 ml of a 20% solution of acetic acid was injected into the subserosal layer at the fundoantral junction of the gastric anterior wall. After this treatment, the abdominal incisions were sutured and the rats were allowed free access to food and water.

Sample Collection

The rats were divided into 5 groups as shown in Table 1. In group 1, rats received 20 mg/kg bw of OPZ suspended in 2 ml of 0.5% methyl cellulose with 0.2% NaHCO₃ (pH 9), once a day by oral intubation. In group 2, rats received the same volume of the vehicle only and served as controls. In group 3, rats received 200 mg/kg bw of CMT suspended in 2 ml of water, once a day by oral intubation. In group 4, rats received the vehicle only and also served as controls. Administration of substances and vehicles was begun 24 h after ulcer induction and lasted for 3, 5, 7, or 14 days. Rats in group 5 received no drugs, only water. After being treated for various periods, the rats were deprived of food overnight until the subsequent procedure. Twenty hours after the last dose, 100 mg/kg bw of BrdU was injected intraperitoneally into each rat to label proliferating cells in the gastric mucosa. One hour later, the rats were sacrificed by cardiac puncture with an 18 GA (1.3 mm diameter) needle and blood was collected for determination of the serum gastrin concentration. Gastrin concentration was determined by [¹²⁵I]-radio immunoassay with a Gastrin RIA Kit (Dainabot Inc., Osaka, Japan). Then, the stomachs were excised

Table 1 Summer of the experimental design

Group	Subgroup	Treatment	Treatment Period (day)
Group 1	1	omeprazole (20mg/kg.bw)	3
	2	omeprazole (20mg/kg.bw)	5
	3	omeprazole (20mg/kg.bw)	7
	4	omeprazole (20mg/kg.bw)	14
Group 2	1	Vehicle	3
	2	Vehicle	5
	3	Vehicle	7
	4	Vehicle	14
Group 3	1	cimetidine (200mg/kg.bw)	3
	2	cimetidine (200mg/kg.bw)	5
	3	cimetidine (200mg/kg.bw)	7
	4	cimetidine (200mg/kg.bw)	14
Group 4	1	Vehicle	3
	2	Vehicle	5
	3	Vehicle	7
	4	Vehicle	14

Group 1 rats received 20 mg/kg omeprazole in 2 ml of 0.5% methyl cellulose with 0.2% NaHO₃. Group 2 rats received 2 ml of vehicle (0.5% methyl cellulose with 0.2% NaHO₃). Group 3 rats received 200 mg/kg cimetidine in 2 ml of water. Group 4 rats received 2 ml of vehicle (water) only. Each subgroup was composed of three to six rats.

immediately and opened along the greater curvature. After rinsing in saline, the stomachs were stretched on boards mucosal side up, and the ulcer indices (UI), which were determined by the length and the width of the ulcers, were determined. Then the stomachs on the boards were fixed in 70% ethanol for 24 hours at 4°C. Specimens of mucosa at the ulcer margin were cut and processed for immunohistochemical detection of BrdU and routine histological study by staining with haematoxylin and eosin.

Immunohistochemical Study

Immunohistochemical staining with anti-BrdU antibody was performed by the indirect immunoperoxidase method as follows: For each sample, two 4- μ m sections of fixed, paraffin-embedded tissues were stained according to the method reported by Tada et al.¹⁶⁾ One additional section was stained with hematoxylin and eosin for routine histological study. For BrdU staining, sections were deparaffinated, rehydrated and incubated in a solution of 0.3% hydrogen peroxide in 100% methanol for 30 minutes to block endogenous peroxidase activity. Then the sections were washed in PBS and incubated in 2N HCl for 1 hour to partially denature the DNA in the sections to obtain single-stranded regions of DNA. After another wash in PBS, the sections were incubated with mouse-anti BrdU monoclonal antibody for 1 hour at room temperature. After another wash with PBS, they were incubated with peroxidase conjugated-rabbit-anti-mouse IgG antibody for 30 min at room temperature. After additional washes, bound peroxidase was developed with 3, 3'-diaminobenzidine-tetrahydrochloride for 15 min. Subsequently, sections were counterstained with Mayer's haematoxylin, dehydrated, and mounted.

Cell Counting

Immunohistologically stained cells were viewed under a microscope (Olympus BH-2, Olympus, Tokyo, Japan). The microscopic images ($\times 100$) of the ulcer margins were photographed with a camera (Olympus C-35AD-4, Olympus, Tokyo, Japan) mounted on the microscope. We scanned these photographs with a flatbed image-scanner (Epson GT-8000, Seiko Epson Inc., Tokyo, Japan) and transferred them to the Macintosh computer using image-processing software (Adobe Photoshop 2.5J, Adobe Systems Inc., USA). Then we analyzed the transferred pictures utilizing image-analysis software (Image 1.55, NIH, USA). This procedure allowed us to obtain high resolution of the structures of the sections and to perform quantitative image analysis. Using the density slicing program, the BrdU-labeled cells were highlighted in red and backgrounds were left unchanged. These procedures were performed under the same conditions and settings through the entire experiment. According to Helpap *et al.*¹⁷⁾, we determined the proliferating zone as the area between uppermost and lowermost BrdU-labeled cells. The proliferative zone gradually increased in width as it got closer to the ulcer edge, and formed a wedge-shaped regenerating area where BrdU-positive cells had been densely distributed. After determining the regenerating area, we traced along the boundary of the area, and square measure of each area was obtained by the image-analyzing software. Subsequently, highlighted BrdU-labeled cells in thresholded images were automatically counted. The density of BrdU-labeled cells was calculated by dividing the number of counted cells by the square measure of the area, and expressed as BrdU-LI.

Statistical Analysis

Data were expressed as mean $\pm$ SD. Student's *t* test was used to evaluate the significance of differences. A probability of *p* less than 0.05 was taken as the level of significance. All analyses were performed utilizing the Stat View statistical software (Ver. 4.0, Abacus Concepts Inc., Berkeley, CA).

Results

General Comments

All treatments were well tolerated and no adverse effects were noted in any rats during the experimental period.

Ulcer Index

The time course of the UI is shown in Figure 1. On the third, fifth, and seventh day of the treatment, the UI of rats treated with either OPZ or CMT was a little lower than that of controls. However, there was no significant difference between the UI of each treated rat and that of the corresponding controls throughout the experimental period.

BrdU-Labeling Index

Proliferating, S-phase cells were positively stained in the ulcer margin (Fig. 2A), at the site of regenerating mucosa. A representative processed image is shown in Figure 2B and 2C (third day of OPZ treatment). In control rats for OPZ treatment, the LI reached a maximum on day 3 ($11.17 \pm 4.9 \times 10^2/\text{mm}^2$), then decreased gradually (Fig. 3A). In the OPZ-treated rats, while 3- and 5-day treatments significantly decreased LI (52% of control, day 3; 62% of control, day 5), 7- and 14-day treatments significantly increased LI (186%

Fig. 1. Time course of ulcer index (UI) in OPZ-treated rats (A) and CMT-treated rats (B) compared to the corresponding controls. The symbols used in the figure are: ●, OPZ-treated rats; ○, Controls of OPZ-treated rats; ■, CMT-treated rats; □, Controls of CMT-treated rats. Points and bars are means $\pm$ SD of three to five rats.

Fig. 2. Gastric mucosa of the ulcer margin on the third day of the treatment with OPZ, stained by indirect immunoperoxidase method with anti-BrdU antibody, showing the distribution of BrdU-labeled cells in the regenerative mucosa. A: Representative microscopic image of BrdU-staining. Original magnification, $\times 100$. Scale bar represents 100 μ m. B: Processed image of the microscopic picture using image-analyzing software (NIH image 1.55). The area traced by the line is proliferative zone at the ulcer margin. C: Image of analyzed image by NIH image 1.55, demonstrating the number of BrdU-labeled cells in the proliferative area.

Fig. 3. Time course of labeling index (LI) of OPZ-treated rats (A) and CMT-treated rats (B) compared to that of the corresponding controls.

The symbols used in the figure are: ●, OPZ-treated rats; ○, Controls of OPZ-treated rats; ■, CMT-treated rats; □, Controls of CMT-treated rats.

Points and bars are means $\pm$ SD of four to five rats. *: $p < 0.05$ vs control.

of control, day 7; 170% of control, day 14) (Fig. 3A).

In CMT-treated rats, there was no significant increase in LI throughout the experimental period, nor was there any significant difference between the LI of CMT-treated and control rats (Fig. 3B).

Serum Gastrin Concentration

In OPZ-treated rats, serum gastrin concentrations were elevated on day 5 and reached a maximum value (465 ± 73 pg/ml, 247% of control, $p < 0.05$) on day 7 and remained significantly higher on the fourteenth day (415 ± 133 pg/ml, 356% of control, $p < 0.05$) (Fig. 4A). In contrast, there was no significant difference in serum gastrin concentration between rats treated with CMT and controls (Fig. 4B).

Histological Study

There were no significant histological differences among the OPZ-treated group, the CMT-treated group and their respective vehicle control groups. General histological findings were as follows. One day after ulcer production, the mucosa was completely destroyed and only necrotic or charred tissue remained in the injured area. Three days after ulcer production, a slight inward rolling of the marginal mucosa was observed. In some sections, a thin layer of epithelial surface-cells had grown over the denuded connective tissue. Five days after ulcer production, the injured area appeared about half as wide as it had been earlier. No significant changes in the regenerative zone were observed as compared to day 3. Seven days after ulcer production, the defect in the mucosa had become smaller and most of the epithelial formations along this area seemed to have fully regenerated. At this stage and later, many buds, composed of mucous neck cells, appeared at the base and sides

Fig. 4. Time course of serum gastrin concentration of OPZ-treated rats (A) and CMT-treated rats (B) compared to the corresponding controls.

The symbols used in the figure are: ●, OPZ-treated rats; ○, Controls of OPZ-treated rats; ■, CMT-treated rats; □, Controls of CMT-treated rats.

Points and bars are means $\pm$ SD of three to four rats. *: $p < 0.05$ vs control.

of the regenerated mucosa, giving the appearance of branched glands. Fourteen days after ulcer production, the wounds were covered with regenerating mucosa in most cases. A thin layer of cells had bridged the gap in several sections.

Discussion

The present study showed that treatment with OPZ for at least 7 days increased the serum gastrin concentration and enhanced the rate of cellular proliferation in the regenerating mucosa. Various factors regulate gastric ulcer healing, such as cellular proliferation, microcirculation of gastric mucosa, mucus production, intragastric acidity and vascularization of regenerating mucosa. Above all, evaluation of cellular proliferation in the regenerating mucosa is one of the useful methods for assessing ulcer healing. Former investigators have used methods such as measuring incorporation of ^3H -thymidine by autoradiography^{4, 18, 19)} or BrdU with immunohistochemical staining^{20, 21)} to determine S-phase cell kinetics in the gastric mucosa. The combination technique of labeling with BrdU and indirect immunoperoxidase staining has been widely used in studying cellular proliferation and kinetics in the gastric mucosa. In the most commonly used method, LI was determined by the following steps. By use of a grid, BrdU-positive cells and background mucosal cells were counted in microscopic fields.

The percentage of labeled cells to the total number of background epithelial cells was then calculated as LI. However, this method has some problems. In the intact gastric mucosa, the proliferating zone is commonly defined as the region between the uppermost parietal cells and the uppermost zymogen cells²²⁾. At the ulcer margin, the distribution of BrdU-labeled cells was quite different from that in the intact gastric mucosa. The labeled cells were distributed much wider, more focally, and more unequally as compared to the intact mucosa. If the ulcer margin was examined by the common method described above,

the result would be greatly influenced by the microscopic field chosen for counting cells. Therefore, in the present study, we determined the proliferating zone as the area between uppermost and lowermost labeled cells at the ulcer margin, where BrdU-positive cells were focally and densely distributed. Then, we utilized image analyzing software to trace and measure the regenerating area, and counted the labeled cells inside the area. This method has several advantages. First, since the cells are counted by computer, it is much quicker. Second, we can avoid bias when we determine BrdU-positive cells, since the computer program automatically defines the labeled cells.

The LI at the proliferating zone in the regenerating mucosa increased significantly on the seventh day of treatment with OPZ and remained elevated on day 14. These results suggest that OPZ-administration may promote cellular proliferation in the regenerating mucosa, and sustain such promotion even at the late stage of ulcer formation. Unexpectedly, the LI of OPZ-treated rats on the third and fifth day of the treatment was rather low compared to controls. We can not properly explain this finding, but suggest that it may be linked to some unknown effect of OPZ at the early stage of ulcer healing. Furthermore, since the gastric mucosa at the acute stage of acetic ulcer is extremely injured and inflamed, intragastric neutralization induced by OPZ may have some unknown influence upon gastric mucosa. Further investigation is required to explain this finding.

Despite the marked change in LI, there was no significant difference in the histological structure of ulcers between OPZ-treated rats and controls. An increase in cellular proliferation observed in the present study may be insufficient to induce histological change. We presume that an increase in cellular proliferation maintained for sufficiently long period will produce a detectable effect on the histological structure.

In the present study, treatment with OPZ elevated serum gastrin levels which reached a maximum on day 7 and remained elevated until the end of the treatment. These results are consistent with a number of previous reports regarding the effect of OPZ on serum gastrin level. Walsh reported that the oral administration of OPZ to rats induced hypergastrinemia¹⁰. Li and Helander reported that plasma gastrin level was 5- to 6-fold elevated by OPZ administration⁴. In the present study, on the third day of treatment with OPZ, the serum gastrin level was not significantly different from that of controls. This result suggests that the suppressive effect of OPZ on intragastric acidity might not be optimal by the third day. Lind et al studied the relationship between reduction of gastric acid secretion and serum gastrin concentration during the administration of OPZ, and reported that at least 5 days of treatment was required to establish the stable suppression of acid secretion⁶. However, in both OPZ-treated and CMT-treated rats, the serum gastrin concentration decreased from the first to the third day, indicating that the conditions of gastrin secretion in the early phase of the experimental period (until the fifth day) differed from those in the late phase (days 7-14). Various factors such as the stress of laparotomy, the anesthesia and host defense against acute mucosal injury may influence gastrin secretion in the early phase and make it more complicated than in the late phase.

Administration of CMT did not affect LI through the entire experimental period. This result is supported by previous reports^{23, 24}. There was no significant difference in serum gastrin level between CMT-treated rats and controls. Seima et al. reported that serum gastrin concentration increased when CMT was administered intraperitoneally to rats²⁵. A study of patients with peptic ulcers showed that treatment with CMT increased serum

gastrin levels²⁶⁾. The methodological difference, such as the mode of drug administration, may explain our findings that CMT-treatment did not affect serum gastrin level. It is possible that the dosage of CMT we used is insufficient to inhibit acid secretion in rats.

A number of studies have examined the trophic effect of gastrin on the oxyntic mucosa. Most studies confirmed that either an increase in endogenous gastrin or exogenously administered gastrin stimulated cellular growth in the gastric mucosa. Administration of pentagastrin to rats led to a significant increase in incorporation of ³H-thymidine in the oxyntic gland area²⁷⁾. Other studies confirmed that endogenous hypergastrinemia induced by surgical manipulation produced trophic effects in the oxyntic mucosa that paralleled changes in circulating gastrin^{11, 26)}.

In conclusion, we have demonstrated that endogenous hypergastrinemia induced by the administration of OPZ increased the cellular proliferation in the ulcer margin that might accelerate the ulcer healing process. This observation might indicate an additional effect of OPZ in ulcer healing other than intragastric neutralization.

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