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EFFECTS OF OMEPRAZOLE ON GASTRIC MUCOSAL GROWTH AND DIFFERENTIATION IN DEVELOPING RAT

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SUMMARY: The effects of omeprazole on developing rat stomach mucosa were investigated. Infant rats were given subcutaneous injections of either omeprazole (25 mg/kg body weight/day) or vehicle once a day from the day after birth. As a result, omeprazole caused an elevation of mucosal pH and suppressed an increase in mucosal pepsinogen and its mRNA levels during stomach development. Histologically, these changes were associated with a reduction in mature pepsinogen-producing cells throughout stomach mucosa. Omeprazole also caused a delay in the expression of cathepsin E in surface mucous cells and an increase in labeled cells with bromodeoxyuridine. Thus, the present results indicate that omeprazole induces an increase in mucosal cell proliferation and delays the differentiation of developing rat stomach mucosa. Since the observed changes were remarkable especially from days 15 to 21 after birth when significant development of acid secretion occurs, the effects of omeprazole appear to be related with the potent acid inhibitory effect of the reagent.

INTRODUCTION: Pepsinogen, a precursor of pepsin, is synthesized in and secreted from stomach mucosa. It has been shown that the mucosal pepsinogen and its mRNA levels increase during development and these biochemical changes reflect the degree of morphological maturation and differentiation of stomach mucosa (1-4). Thus, pepsinogen synthesis is developmentally regulated and the enzyme is considered as a marker of terminal differentiation of stomach mucosa.

Omeprazole, a proton pump inhibitor, almost completely inhibits gastric acid secretion by altering the activity of H^+/K^+-ATPase, the common final step of acid

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secretion in the gastric parietal cells (5). Omeprazole also affects the function of pepsinogen-producing cells by reducing pepsinogen secretion and the gene expression (6). However, it is unclear whether omeprazole has any other effects on stomach mucosa. Gastric mucosal cells proliferate and differentiate under an acidic environment in the gastric lumen. Thus, it is probable that omeprazole-induced reduced acidity in gastric lumen has some influences on the proliferation and differentiation of the mucosal cells. To resolve this problem, we have investigated the effects of omeprazole on developing rat stomach mucosa with special reference to pepsinogen expression.

MATERIALS AND METHODS

Animals and treatment with omeprazole. Wiatar-strain female rats (Charles River Japan Co.) were mated and the birthday of pups was set on day 0. Pups were given subcutaneous injections of homogenized omeprazole in 0.5 % sodium carboxymethyl cellulose solution (pH 7.6) at a dose of 25 mg/kg body weight once a day from days 1 to 28. Pups in the control group were injected with vehicle alone. On days 5, 10, 13, 15, 17, 19, 21, 24 and 28, rats were anesthetized with ethyl ether and blood was collected by cardiac puncture. The stomach was quickly removed and cut open along the greater curvature. The mucosal pH levels were measured by pH test paper (Toyo Roshi Co. Tokyo, Japan) and the stomach was stored in liquid nitrogen until use for biochemical analysis. Serum gastrin levels were determined as described elsewhere (7).

Total RNA isolation. RNA was extracted from the stomachs by the guanidium isothiocyanate method and purified by CsCl density gradient ultracentrifugation, as described by Chirgwin *et al.* (8). A sample of 20 μg of RNA was denatured and subjected to electrophoresis on an agarose-formalin gel by the method of Goldberg (9). Then the RNA was transferred to a nylon membrane and subjected to hybridization analysis, as described elsewhere (3). The PvuII-XbaI fragment of pRPC1, which contains nearly full-length cDNA coding for rat pepsinogen (10) was labeled with [α-^{32}P]dCTP by nick-translation and used as the probe for hybridization. Pepsinogen mRNA levels were determined by densitometric scanning of autoradiograms.

Assay of pepsinogen. Rat stomach mucosa was minced, homogenized and centrifuged as described previously (2). The spernatant was used for assays of the potential activity of pepsinogen, with hemoglobin as substrate (1). Protein concentrations were measured by the method of Bradford (11).

Histological studies. For histological examinations, the removed stomachs were fixed in pure acetone and embedded in paraffin. The avidin-biotin-peroxidase complex (ABC) method with anti-pepsinogen antibody (4) and anti-cathepsin E antibody (12) was used to detect pepsinogen-producing cells and surface mucous cells, respectively. Paradoxical concanavalin A (Con-A) staining was also used to detect mucosal cells containing class III mucin.

Studies on cell proliferation. For studies on mucosal cell proliferation, three rats on each day were intraperitoneally injected with 5-bromo-2'deoxyuridine (BrdU, Sigma, St. Louis, USA, 100 mg/kg body weight) 1 hour before sacrifice. Nuclei in the cells that incorporated BrdU were immunohistochemically detected.

RESULTS: In newborn rats, mucosal pH of the gastric lumen was about 7 and mucosal pepsinogen level in the stomach was low, about 10 % of that in adult rats. The luminal pH level gradually decreased thereafter to about 4 around day 15 and remarkably reduced to about 2 around day 21, reflecting an increase in parietal cell number in control rats. The mucosal pepsinogen level steadily increased until around day 15 and then dramatically increased, reaching a plateau around day 21, confirming the previous observation (1-4) (Fig.1). Our previous investigation analyzing the effect of omeprazole on acid secretion revealed that subcutaneously injected omeprazole dose-dependently inhibited acid secretion in adult rat stomach and that the inhibition was complete at a daily dose of 25 mg/kg body weight of omeprazole (6). To attain complete anacidity in the gastric lumen, infant rats were given 25 mg/kg body weight of omeprazole subcutaneously every day. Under the experimental condition, omeprazole did not influence the growth of infant rats and the mucosal pH was maintained constantly about 7. Daily administration of omeprazole suppressed an increase in the mucosal pepsinogen levels of developing rats. The suppression was especially remarkable from days 15 to 21; the mucosal pepsinogen level in omeprazole-treated rats was about 50 % of that in the controls on day 17 (Fig. 1). Reflecting the reduced

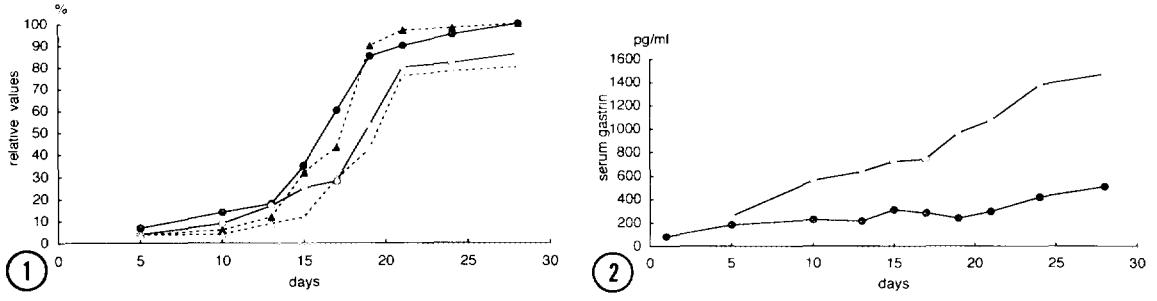

Fig. 1.

Mucosal pepsinogen and its mRNA levels during development in control and omeprazole-treated rats. ● ; mucosal pepsinogen in the controls, ○ ; mucosal pepsinogen in omeprazole-treated rats, ▲ ; pepsinogen mRNA in the controls, △ ; pepsinogen mRNA in omeprazole-treated rats. Mucosal pepsinogen and its mRNA levels were determined as described in the MATERIALS AND METHODS section. The values are shown relative to value of 28-day-old control rats.

Fig. 2.

Effect of omeprazole on serum gastrin levels during development. ●; control rats, ○; omeprazole-treated rats. The values are averages of results from 5-10 pups. Omeprazole induces a marked hypergastrinemia.

acidity in the gastric lumen, a marked increase in the serum gastrin levels was observed in omeprazole-treated rats; they gradually increased after birth and were 2-3 fold higher compared with those in control rats (Fig. 2).

To investigate whether the omeprazole-induced suppression in the mucosal pepsinogen levels was associated with the suppression in the mRNA levels, we analyzed RNAs from stomach mucosa of control and omeprazole-treated infant rats by Northern blot hybridization with rat pepsinogen cDNA as the probe. As shown in Fig. 1, pepsinogen mRNA levels were constantly lower in omeprazole-treated infants than those in the controls. The developing profile of the mRNA levels were closely correlated with that of the mucosal pepsinogen levels in both groups (Fig. 1).

Next, we analyzed the effect of omeprazole on developing rat stomach histologically. Omeprazole treatment increased the number of BrdU-labeled cells per a pit in both fundic and pyloric glands, indicating an increase in the mucosal cell proliferation (Table 1). This led to a gradual increase in the mucosal thickness of the treated animals throughout the experimental period, resulting in 10-20 % increase as compared with the controls at day 28. Immunohistochemistry with the specific antibodies for rat pepsinogen revealed various pepsinogen-producing cells throughout stomach

Table 1 Effects of omeprazole on cell proliferation in rat stomach

days	NC (Fundic)	OMP (Fundic)	NC (Pyloric)	OMP (Pyloric)
5	2.0±0.20	2.2±0.05	2.2±0.09	2.3±0.12
10	2.3±0.27	2.3±0.09	2.6±0.06	3.3±0.35
13	2.2±0.09	2.6±0.32	2.4±0.47	4.5±0.26 *
15	2.7±0.57	2.6±0.29	3.5±0.27	4.0±0.62
17	2.4±0.31	3.3±0.48	3.9±0.65	4.4±0.09
19	2.3±0.12	3.1±0.24 *	3.7±0.24	3.7±0.33
21	2.5±0.24	2.8±0.48	3.2±0.12	4.1±0.09 *
24	2.7±0.38	3.4±0.62	3.1±0.35	4.0±0.62
28	1.1±0.31	3.9±0.46 *	1.2±0.29	6.5±0.70 *

Results are mean ± SEM of the number of labeled cells with BrdU per a pit of three rats. Labeled cells were counted and averaged in 10 different glands from each rat in both fundic and pyloric region. Asterisks denote significant difference from control values ($p<0.05$). NC, Normal control. OMP, Omeprazole.

mucosa. In fundic glands, treatment with omeprazole markedly reduced the number of mature chief cells strongly immunoreactive for pepsinogen (Fig. 3). Instead, the number of cells weakly immunoreactive for pepsinogen was increased. Paradoxical Con-A staining revealed that these cells were positive for class III mucin which mature chief cells do not contain in their cytoplasm (Fig. 4). Thus, the cells could be identified as immature chief cells which synthesize both class III mucin and small amount of pepsinogen. Reflecting the increase in serum gastrin levels, the proportion of parietal cells in this region was also increased, especially after day 21. In control rats, pyloric gland cells contained both class III mucin and pepsinogen, and the amount of them

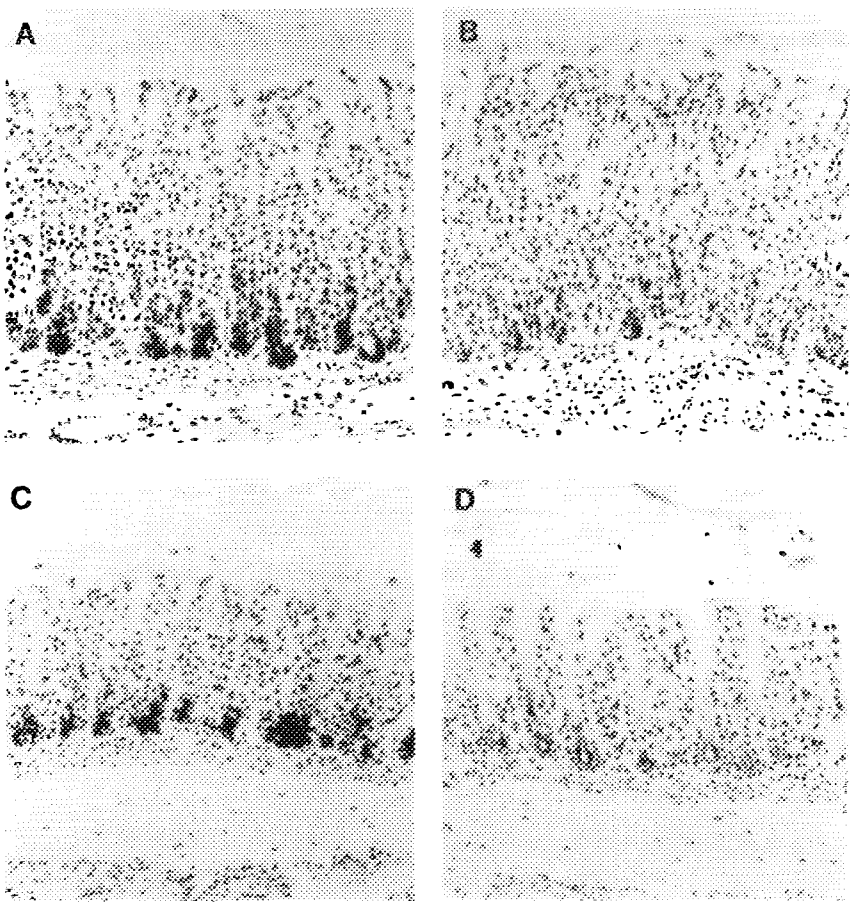

Fig. 3.

Immunohistochemistry of 19-day-old rat stomach mucosa with anti-pepsinogen antibodies . A and B ; fundic gland, C and D ; pyloric gland. A and C are from control animals. B and D are from omeprazole-treated animals. Omeprazole decreases the proportion of the cells strongly immunoreactive for pepsinogen.

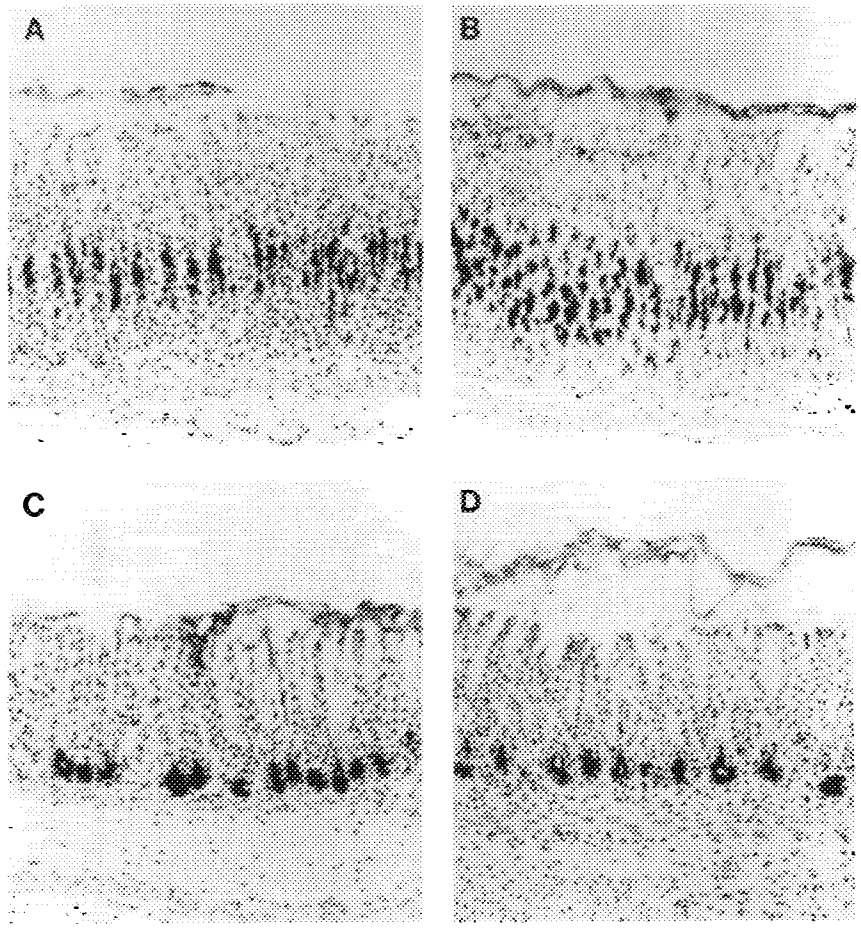

Fig. 4.

Paradoxical Con-A staining of the cells that contain class III mucin in the 19-day-old rat stomach mucosa. A and B ; fundic gland, C and D ; pyloric gland. A and C are from control animals. B and D are from omeprazole-treated animals. Omeprazole increases the proportion of the cells containing class III mucin.

appeared to increase with the development judging from the intensities of staining. Though omeprazole treatment did not alter the proportion of these cells, the intensities of staining for both were reduced, indicating a retardation in maturation (Fig. 3, 4). Immunohistochemistry with anti-rat cathepsin E antibodies revealed that omeprazole also reduced the expression of cathepsin E in surface mucous cells, indicating a delayed maturation of the cells (Fig. 5).

DISCUSSION: In this study we have demonstrated that administration of omeprazole to infant rats caused an elevation of mucosal pH and suppresses an

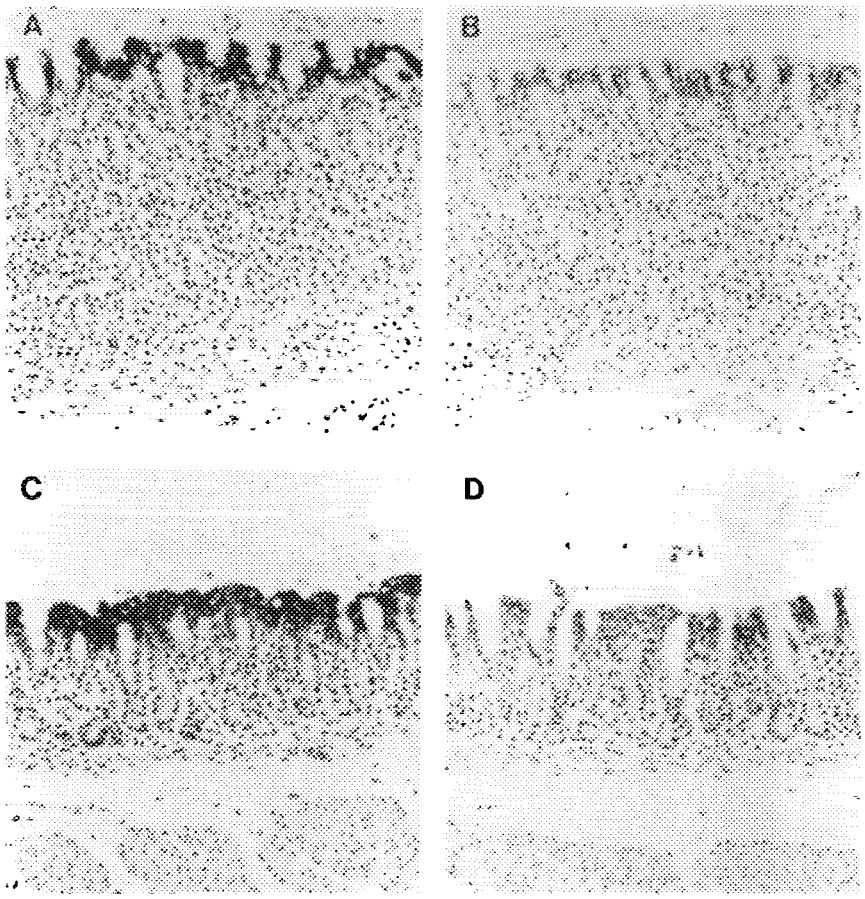

Fig. 5.

Immunohistochemistry of 19-day-old rat stomach mucosa with anti-cathepsin E antibodies. A and B ; fundic gland, C and D ; pyloric gland. A and C are from control animals. B and D are from omeprazole-treated animals. Omeprazole reduces the intensity of staining for cathepsin E in surface mucous cells.

increase in mucosal pepsinogen and its mRNA levels during stomach development. These omeprazole-induced biochemical changes were associated with the increase of morphologically immature pepsinogen-producing cells in both fundic and pyloric glands. The effect of omeprazole was not limited to pepsinogen-producing cells. It also affected surface mucous cells; omeprazole treatment reduced the expression of cathepsin E, which is considered as another marker of terminal differentiation of stomach mucosa (13), indicating a delay in maturation of the cells. In addition, the proportion of BrdU-labeled cells was increased by omeprazole treatment. Thus, the present results strongly indicate that omeprazole induces an increase in mucosal cell

proliferation and delays the normal processes of maturation of developing stomach mucosa. The mechanisms of action of omeprazole remain to be elucidated. Previous studies have demonstrated that H^+/K^+-ATPase activities can be detected in 18-day-old fetal rat stomach and acid secretion begins on day 20 of gestation (14). Acid secretion increases slowly after birth and significant development of acid secretion is observed from days 15 to 21 after birth when weaning takes place (15). In our experiment the effects of omeprazole are most evident during this period. In addition, essentially the same effects were obtained by administration of H_2-receptor antagonist (Ranitidine, 30 mg/kg body weight twice a day), another potent inhibitor of acid secretion (N. Kakei *et al.*, manuscript in preparation). Thus, it appears that the observed effects are not caused by a direct action of omeprazole. It is probable that omeprazole-induced achlorhydria plays an important role in delaying maturation and differentiation of stomach mucosa. Achlorhydria induces an elevation of the serum gastrin levels, leading to an observed increase of parietal cells. Thus, hypergastrinemia could be involved in the observed effects of omeprazole. However, it has been reported that stomach mucosa has no gastrin receptor detected before postnatal days 20 (16), and administration of exogenous gastrin did not induce notable changes on pepsinogen-producing cells and surface mucous cells (unpublished data). Thus, luminal acid or intraglandular acid itself might be one of the factors deeply involved in normal differentiation of stomach mucosa.

In conclusion, omeprazole treatment altered the developmental program of infant rat stomach mucosa probably by inhibiting acid secretion. Elucidation of the roles of acid in the development of stomach is a problem for future study.

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