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Effect of Omeprazole on Secretion, Synthesis and the Gene Expression of Pepsinogen in the Guinea Pig Stomach Mucosa

SHINKO TSUKADA[†], MASAO ICHINOSE[†], NOBUYUKI KAKEI[†], MASAE TATEMATSU[‡], NORIAKI TEZUKA[‡], MASASHI MATSUSHIMA[†], KAZUMASA MIKI[†], KIYOSHI KUROKAWA[†], MASAYUKI NOZAWA[§,‖], TAKASHI KAGEYAMA[††], KENJI TAKAHASHI[¶], HIROSHI FUKAMACHI[‡‡], AND PAUL K. NAKANE[§]

[†]*First Department of Internal Medicine, Faculty of Medicine* · [¶]*Department of Biophysics and Biochemistry;* [‡‡]*Zoological Institute, Faculty of Science, University of Tokyo, Tokyo 113;* [‡]*Laboratory of Pathology, Aichi Cancer Center Research Institute, Nagoya 464;* [§]*Department of Anatomy, Nagasaki University School of Medicine, Sakamoto, Nagasaki 852;* [††]*Department of Biochemistry, Primate Research Institute, Kyoto University, Inuyama, Aichi 484, Japan*

The effects of omeprazole, a proton pump inhibitor, on gene expression, protein synthesis, intracellular storage and secretion of pepsinogen in guinea pig stomach were investigated.

After treatment with omeprazole for five days, acid and pepsinogen secretion into the gastric lumen was significantly reduced. Concomitant with this, there was an increase in intracellular pepsinogen as demonstrated by increased pepsin activity in the gastric mucosa, more intense immunohistochemical staining by antibodies specific for pepsinogen and accumulation of secretory granules in the cells producing pepsinogen. In these cells, the amount of pepsinogen mRNA was reduced as revealed by Northern blotting and *in situ* hybridization. Ultrastructurally the endoplasmic reticulum of these cells was poorly developed, the findings being consistent with a reduction in protein synthesis. It appears that omeprazole inhibits the secretion of pepsinogen, increasing the intracellular store and leading to the reduction in gene expression probably by a feedback mechanism and consequent reduction in pepsinogen synthesis. Since these changes were most evident in the acid-secreting fundic gland mucosa, as compared with other mucosae secreting only pepsinogen, namely pyloric and duodenal mucosa, it appears probable that these changes are linked with omeprazole-induced reduction in the acid secretion.

KEY WORDS—Omeprazole; pepsinogen; *in situ* hybridization.

INTRODUCTION

Omeprazole, a derivative of benzimidazole, powerfully inhibits gastric secretion not only in experimental animals but also in humans by blocking the action of H^+, K^+ ATPase, the enzyme involved in the final step of acid secretion in gastric parietal cells.[1] Because of the potent and long-lasting inhibitory activity on acid secretion, omeprazole provides a useful addition to the treatment option of acid peptic diseases such as peptic ulcers, reflux esophagitis and the Zollinger-Ellison syndrome. Since omeprazole is an inhibitor of the proton pump, a large number of studies on the effect of the agent on gastric acid secretion exist. However, there are relatively few studies on the effects of omeprazole on the function of other stomach cells such as the production and secretion of pepsinogen.[2–6] Among those existing, the results are conflicting. Omeprazole was reported to increase the secretion of pepsinogen from the perfused rat stomach.[5] On the other hand, the drug was reported to reduce the secretion in the rat and human.[2,3] The reason for the observed difference was unclear and should be clarified. However, it may be partly due to the difference in the species and the experimental system used. Since the drug was also reported to have no direct effect on the secretion of pepsinogen from dispersed chief cells from rabbit stomach,[4] the inhibitory effect on acid secretion by omeprazole may indirectly affect the cells producing pepsinogen. To clarify this problem, we investigated the effect of omeprazole

‖ Present address, Daiichi Pure Chemical Co., Tokyo 103, Japan.

Addressee for correspondence: Dr Shinko Tsukada, First Department of Internal Medicine, Faculty of Medicine, University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113, Japan.

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in three distinct areas where pepsinogen is produced: fundic gland mucosa where parietal cells are abundant; and pyloric and Brunner's glands where no parietal cells are present. In addition, since pepsinogen is a typical secretory protein, we can assume that its synthesis and secretion depends on a series of processes consisting of transcription and control of the mRNA. Thus, we analysed the effect of omeprazole on pepsinogen mRNA expression for the better understanding of the mechanisms of its action on pepsinogen synthesis and secretion.

MATERIALS AND METHODS

Animals

Adult male guinea pigs (Hartley strain, weighing around 350 g) were used. Animals were injected subcutaneously with various doses of omeprazole (0·1–25 mg kg^{-1} body weight (B.W.) day^{-1}) for five successive days. On the last day the animals were fasted overnight. They were anaesthetized with ethyl ether. After blood was withdrawn by cardiac puncture, they were killed. The stomach and duodenal tissues were removed and washed with ice-cold phosphate-buffered saline (PBS). The fundic and pyloric areas were separated and processed independently. The tissues were divided into three parts: some were fixed with 10 per cent formalin; some were fixed with 2·5 per cent glutaraldehyde in 0·1 M cacodylate buffer (pH 7·4) at 4°C for 2 h; and some were stored in liquid nitrogen until use for biochemical analysis.

Gastric Secretory Studies

For gastric secretory studies, the animals were fasted overnight. Under pentobarbital anaesthesia, the abdomen was incised and the pylorus was ligated. After 2 h, animals were killed with an overdose of ethyl ether, the gastric contents were collected and the volume was determined. The concentration and output of hydrogen ion into the gastric juice were determined by titration against 0·1 M NaOH to pH 7·0. Potential peptic activity of pepsinogen was determined by the method of Anson with a slight modification.[7,8]

Histological Studies

The tissues fixed with 10 per cent formalin were embedded in paraffin wax and were processed for staining with hematoxylin and eosin or for immunohistochemistry. For the latter, deparaffinized sections were first reacted with rabbit anti-pepsinogen antibodies[9] and the sites of the antibody binding were visualized by the avidin-biotin-peroxidase complex (ABC) method[10] using 3,3′diaminobenzidine as chromogen.[11] As a negative control, preimmune rabbit serum was used instead of the antiserum.

Electron Microscopic Studies

The glutaraldehyde-fixed tissues were washed overnight in 0·1 M cacodylate buffer (pH 7·4) and post-fixed with 1 per cent osmium tetroxide in the buffer for 1 h at 4°C. They were dehydrated and embedded in Epon 812. Thin sections were cut on an ultramicrotome, stained with uranyl acetate and lead citrate, and examined with a JEOL 100CX electron microscope.

Determination of the Pepsinogen Level in the Stomach Mucosa

A portion of the tissue stored in liquid nitrogen was homogenized, centrifuged at 105 000 *g* and the supernatant was used for the assay of the enzyme activity. Pepsinogen isozymogen patterns were determined by polyacrylamide gel electrophoresis as described previously.[7,8] Protein was measured by the method of Lowry *et al.*[12]

Isolation and Analysis of RNA

From the tissues stored in liquid nitrogen, total RNA was isolated by the guanidium/cesium chloride method.[13] A sample of 10 μg of RNA was denatured and subjected to electrophoresis on an agarose formalin gel by the method of Goldberg.[14] Then the RNA was transferred to a nitrocellulose filter, baked and hybridized as described elsewhere.[8] The cDNA insert for guinea pig pepsinogen in recombinant DNA clone GP477[15] was excised by digestion with the restriction endonuclease PstI, labelled by nick-translation with [α-^{32} P]dCTP,[16] and used as the hybridization probe. The insert of about 1·07 kb contains almost the entire length of the coding region.

In Situ *Hybridization*

On the sixth day of treatment with omeprazole, one group of animals was anaesthetized with ethyl

ether and perfused intracardially with about 100 ml of 4 per cent paraformaldehyde (PFA) in PBS. The stomach and duodenum were removed and quick frozen in a mixture of dry-ice and ethanol. Frozen sections, 6 μm in thickness, were mounted onto gelatin-coated glass slides and hybridized with thymine-thymine (T-T) dimerized probe DNA as described previously.[17] A mixture of oligonculeotide probes antisense to the reversed sequence from the region of No. 322–No. 366, No. 686–No. 730 and No. 935–No. 988[15] were used; each 3′end was linked to TTATTA, and the 5′end was linked to ATTATTATT. The sense probe was TTATTA-No. 686–No. 730-ATTATTATT.

Serum Gastrin Concentration

Blood was collected from all the animals except for those for the *in situ* hybridization study. Before killing, blood was withdrawn by cardiac puncture, the serum was separated and stored at −40°C until measurement. The serum gastrin level was determined by radioimmunoassay using anti-human gastrin antibodies.

RESULTS

Omeprazole inhibited the gastric secretion (both volume and acid outputs) in a dose-dependent

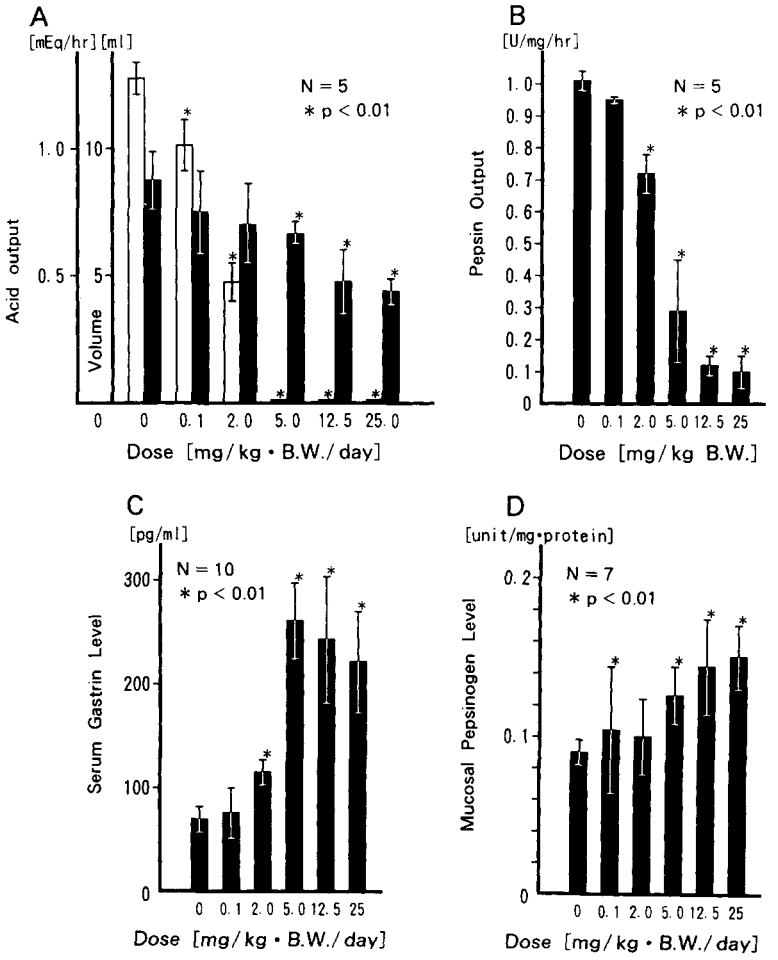

Figure 1. Effects of omeprazole on guinea pig gastric secretion, serum gastrin levels and mucosal pepsinogen levels. A, effects on acid secretion. □, total acid output (mEq h^{-1}); ■, total volume of gastric secretion (ml). B, effects on pepsinogen secretion. C, effects on serum gastrin levels. D, effects on mucosal pepsinogen levels. *Statistically different from controls ($p < 0.01$). All values represent mean ± SEM.

manner in the guinea pig stomach *in vivo*. Acid secretion was completely inhibited by the subcutaneous injection of omeprazole at a dose of 5·0 mg kg^{-1} B.W. day^{-1} for five days (Figure 1A). In the same dose range of omeprazole, a dose-dependent inhibition of pepsinogen secretion was also observed (Figure 1B). The inhibitory effect of the agent on pepsinogen secretion was incomplete and the degree of the inhibition was 85 per cent at a dose of 12·5 mg kg^{-1} B.W. day^{-1} as compared with the control guinea pigs. Even in the animals treated with a high dose of 50 mg kg^{-1} B.W. day^{-1} of omeprazole, complete inhibition of pepsinogen secretion was not observed. With the reduction in gastric acid secretion, a dose-dependent increase in the serum gastrin level was observed (Figure 1C). The mucosal pepsinogen level showed a stepwise increase with each increase in the dose of omeprazole (Figure 1D). Electrophoretic analysis of the crude homogenate of gastroduodenal mucosa revealed several proteolytic bands. Each band corresponds to an isozyme of pepsinogen. In omeprazole-treated animals, all the proteolytic bands of pepsinogen isozymes increased in intensity, indicating a uniform increase of all the isozymes (Figure 2). The increase in the mucosal pepsinogen level was most evident in the fundic mucosa (90 per cent increase) and was less in the pyloric mucosa (13 per cent increase). No increase was observed in the duodenal mucosa.

With the subcutaneous administration at a dose of 12·5 mg kg^{-1} B.W. day^{-1}, maximum inhibition of acid and pepsinogen output was observed around 6 h after the injection and this effect was maintained by the daily administration of the

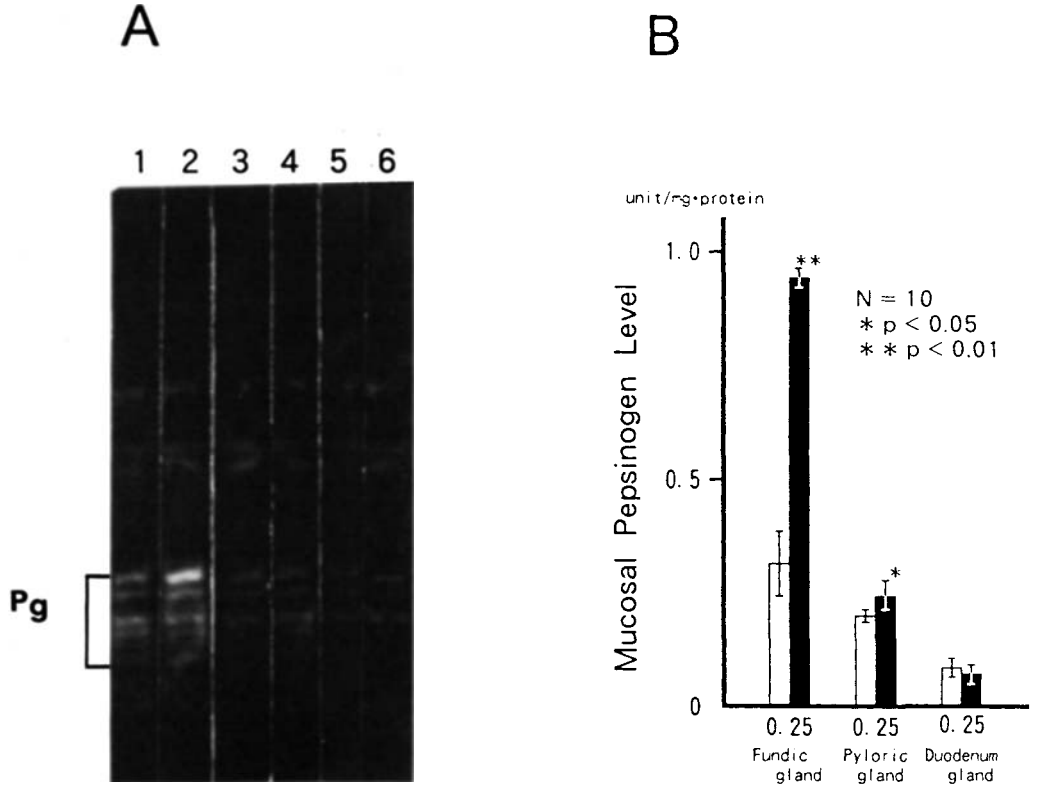

Figure 2. Effect of omeprazole on the mucosal pepsinogen level in different parts of the gastroduodenal mucosa. A, an analysis of the mucosal homogenate by polyacrylamide gel electrophoresis (PAGE). Protein samples of 10 μg from stomach mucosa and 50 μg from duodenal mucosa were analysed by PAGE. Lanes: 1 and 2, fundic gland stomach; 3 and 4, pyloric gland stomach; 5 and 6, duodenum. 1, 3 and 5 are from normal controls. 2, 4 and 6 are from omeprazole-treated (25 mg kg^{-1} B.W. day^{-1}) animals. Pg stands for pepsinogen isozymes. B, alteration of the pepsinogen level in the gastroduodenal mucosa by omeprazole. *Statistically different from controls ($p < 0·05$). **Statistically different from controls ($p < 0·01$). All values represent mean ± SEM.

same dose. With the inhibition of gastric secretion, there was a gradual increase in serum gastrin and mucosal pepsinogen level, both reaching a maximum around 24 h after injection.

In the omeprazole-treated animals, there was no change in number and proportion of pepsinogen-producing cells after treatment for five days. However, a large proportion of the pepsinogen-producing cells was more intensely stained with anti-pepsinogen antibodies than those of control mucosa, indicating an increase in the intracellular pepsinogen content (Figure 3). The difference in the staining intensity with the antibodies was most evident in the fundic mucosa and less so in the pyloric and duodenal mucosae, confirming the results of the mucosal pepsinogen levels in these regions. Consistent with these findings, electron microscopic analysis revealed an increase

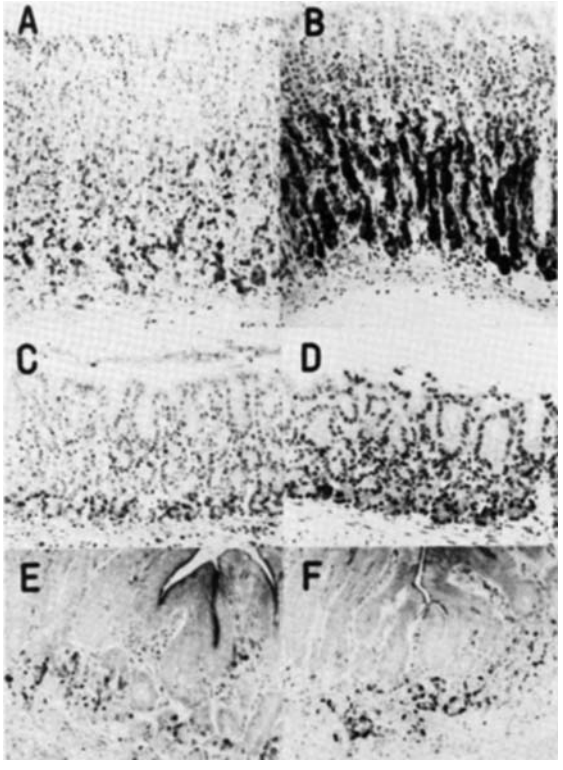

Figure 3. Immunohistochemistry of the cells that produce pepsinogen in the guinea pig gastroduodenal mucosa. A and B, fundic mucosa (×200); C and D, pyloric mucosa (×200); E and F, duodenal mucosa (×200). A, C and E are from control animals. B, D and F are from omeprazole-treated (25 mg kg^{-1} B.W. day^{-1}) animals. Cells staining with antibodies against guinea pig pepsinogen by the ABC method are seen.

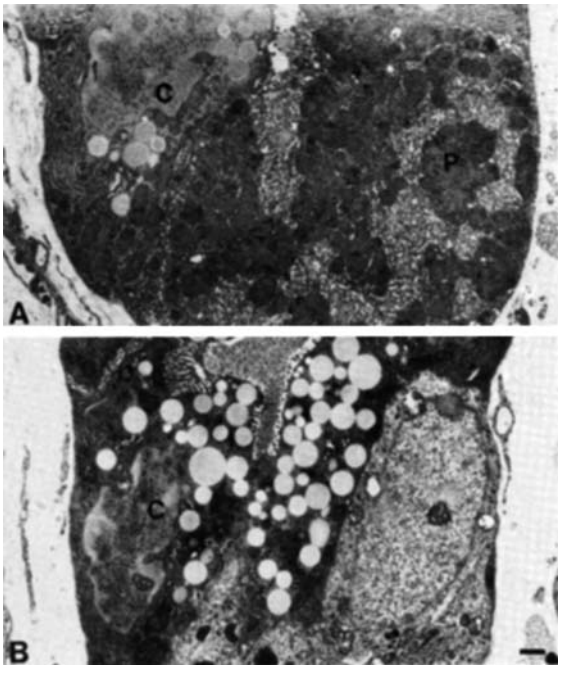

Figure 4. Ultrastructure of fundic gland cells at the bottom of glands in a control guinea pig (A) and in an omeprazole-treated guinea pig (B). Note that endoplasmic reticulum and intracellular canaliculi are well-developed in chief (C) and parietal cells (P), respectively, in control animals. Bar = 1 μm.

in the number and size of secretory granules in the pepsinogen-producing cells from the omeprazole-treated animals (Figure 4). These granules were observed near the secretory border of the cell, while those of the control animals were distributed rather evenly within the cell. In the omeprazole-treated pepsinogen-producing cells, the endoplasmic reticulum was poorly developed and free ribosomes were increased in number. In the fundic mucosa, extensive vacuolization of the parietal cells was also observed (Figure 5).

We have analysed pepsinogen mRNA expression under the same experimental conditions. Northern blot analysis of the RNAs revealed that there was a reduction in pepsinogen mRNA expression by the omeprazole treatment. As shown in Figure 6A, this reduction was also dose-dependent and was maximum at a dose of 25 mg kg^{-1} B.W. day^{-1}. Omeprazole rapidly inhibited the expression of pepsinogen mRNA. With the administration of omeprazole, pepsinogen mRNA level decreased steadily and reached a minimum level around 24 h after the injection (not shown). The degree of

inhibition of pepsinogen mRNA expression was different depending on the part of the mucosa analysed and was most evident in the fundic gland mucosa (about 90 per cent inhibition as revealed by an analysis of the same filter using an image analyser, BAS 2000, Fuji Film Co., Tokyo), and less so in the pyloric gland (about 40 per cent inhibition) (Figure 6B). In Brunner's gland, no reduction in the pepsinogen mRNA level was observed. An analysis using *in situ* hybridization revealed that the signals for pepsinogen mRNA were found in the chief and mucous neck cells in the fundic gland mucosa and also in the other pepsinogen-producing cells in the pyloric and duodenal mucosae. Omeprazole reduced the intensity of these signals and the reduction was most remarkable in the fundic gland mucosa (Figure 7), confirming the results of Northern blot hybridization.

DISCUSSION

The present results have demonstrated that omeprazole inhibits pepsinogen secretion together with acid secretion in guinea pig stomach in a

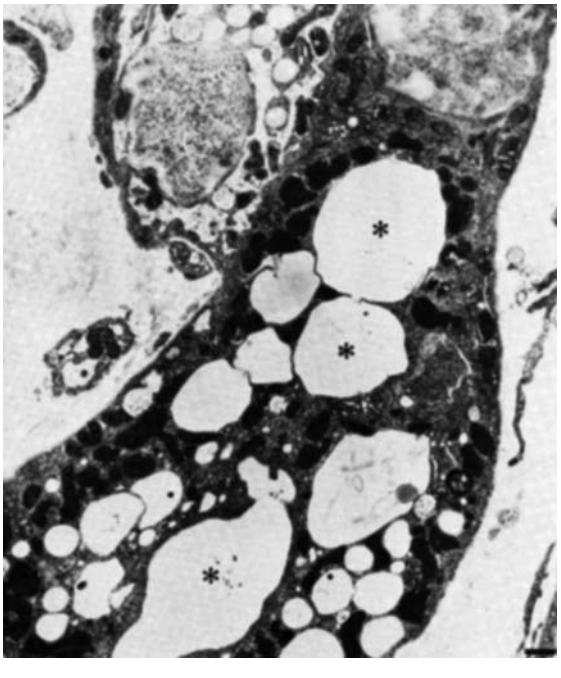

Figure 5. Ultrastructure of fundic gland cells at the bottom of a gland in an omeprazole-treated guinea pig. Note extensive vacuolization (asterisks) in the parietal cells. Bar = 1 μm.

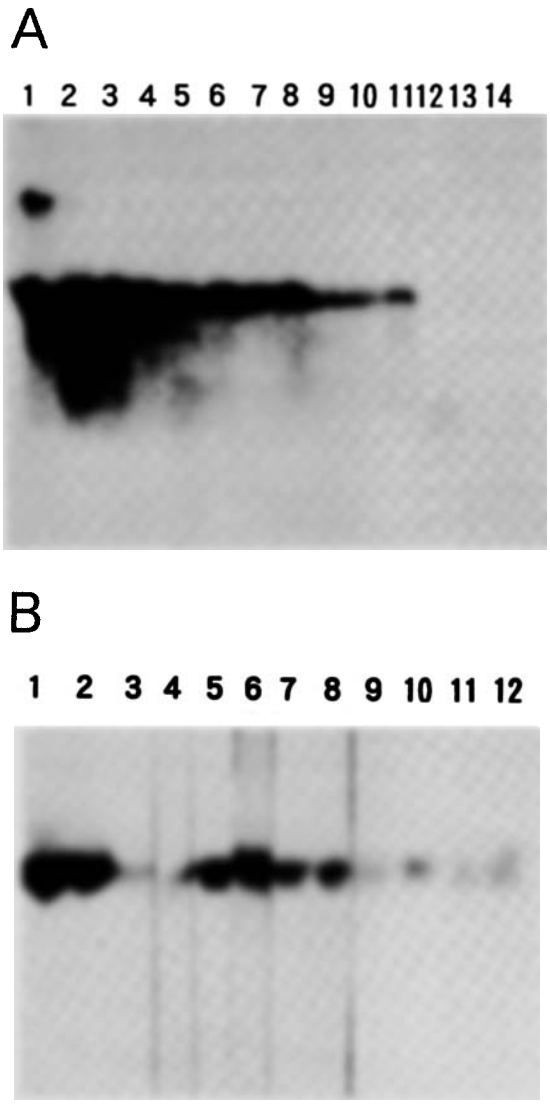

Figure 6. A, Northern blot hybridization of total RNA (10 μg lane^{-1}) from control (lanes: 1 and 2) and omeprazole-treated guinea pig stomach (3 and 4, 0·1 mg kg^{-1} B.W. day^{-1}; 5 and 6, 2·5 mg kg^{-1} B.W. day^{-1}; 7 and 8, 5·0 mg kg^{-1} B.W. day^{-1}; 9, 10 and 11, 12·5 mg kg^{-1} B.W. day^{-1}; 12, 13, 14, 25 mg kg^{-1} B.W. day^{-1}) with pepsinogen cDNA as a probe. Animals were treated with the various doses of omeprazole for five days. B, effect of omeprazole on pepsinogen mRNA level in the gastroduodenal mucosa (lanes: 1–4, fundic mucosa; 5–8, pyloric mucosa; 9-12, duodenal mucosa). Lanes 1, 2, 5, 6, 9 and 10 are from normal controls and lanes 3, 4, 7, 8, 11 and 12 are from animals treated with omeprazole (25 mg kg^{-1} B.W. day^{-1}) for five days.

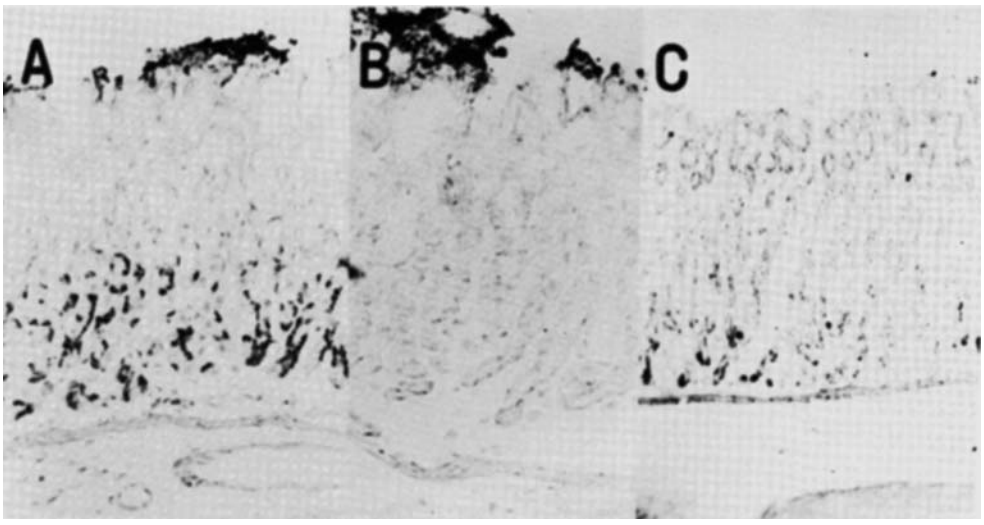

Figure 7. *In situ* localization of pepsinogen mRNA in guinea pig stomach mucosa. Frozen sections of stomach mucosa were hybridized *in situ* with T-T dimerized antisense pepsinogen oligo DNA (A and C) or T-T dimerized sense pepsinogen oligo DNA (B). A and B are from control animals and C is from an omeprazole-treated animal.

dose-dependent manner. This observation is consistent with the previous *in vivo* studies in rats and humans.[2,3] Under the same experimental conditions, omeprazole also induced a dose-dependent reduction in pepsinogen mRNA expression. The mucosal pepsinogen content increased with the reduction in the secretion. Light and electron microscopic findings also confirmed this, revealing an increase in the intracellular store of pepsinogen in the producing cells. As expected from the reduction in the mRNA level, this increase in the intracellular pepsinogen store was not induced by increased protein synthesis; the ultrastructure of these cells revealed a poorly developed endoplasmic reticulum and increased number of free ribosomes, a finding indicating a reduction in protein synthesis. The time course of these omeprazole-induced changes revealed that reduction in the secretion precedes the reduction in the mRNA level and increase in the mucosal pepsinogen level. Thus, taking together all these findings, it appears that omeprazole inhibits the secretion of pepsinogen first, increasing the intracellular store, which in turn leads to the reduction in protein synthesis and gene expression, probably by a feedback mechanism. The mechanism by which omeprazole inhibits pepsinogen secretion is unknown. Incomplete inhibition of pepsinogen secretion by omeprazole, although this may be caused by an overflow secretion of pepsinogen excessively stored in the cell,[18] may suggest the possibility that it is caused by an indirect effect of the agent. Indeed, Fryklund *et al.* reported that omeprazole had no effect on pepsinogen release induced by secretagogues in isolated pepsinogen-producing cells from rabbit gastric mucosa, whereas the acid secretion in isolated parietal cells was strongly inhibited by omeprazole.[4] Therefore, it may be that omeprazole has no direct effect on the pepsinogen-producing cells and that the observed effects on these cells are phenomena limited to the *in vivo* system. Previous studies have indicated that there is a close correlation between acid and pepsin secretion with stimulation by most gastric secretagogues[19] and that the luminal application of acid stimulates pepsinogen secretion by local cholinergic reflexes.[20] Thus, the inhibition of pepsinogen secretion by omeprazole could be caused by omeprazole-induced reduction in the luminal acid. In this context, the fact that the reduction in pepsinogen mRNA level was greater in the fundic gland, where pepsinogen-producing cells coexist with parietal cells than that in the pyloric glands where no parietal cells exist, is interesting. In Brunner's gland where bicarbonate is also excreted and the luminal pH is elevated, no reduction was observed. These results give further support to the possibility that the observed omeprazole-

induced changes in the functions of the pepsinogen-producing cells are phenomena linked with the inhibition of acid secretion, but the exact mechanisms of the omeprazole action remain to be elucidated.

In the present study, we have investigated the effect of omeprazole on the processes leading from pepsinogen gene expression to the secretion in guinea pig stomach. Our results demonstrated that omeprazole, a proton pump inhibitor, potently inhibited pepsinogen mRNA expression and protein synthesis, probably through a feedback mechanism by increased intracellular pepsinogen store due to reduction in the secretion. The present results strongly suggest that the gene regulation, synthesis and secretion of pepsinogen is regulated by the typical pathway of secretory proteins. The fine elucidation of the pathway is a problem for future investigation.

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