Effects of Topical Application of Acidified Omeprazole on Acid Secretion and Transmucosal Potential Difference in Anesthetized Rat Stomachs

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Accepted May 6, 1988

Abstract—Effects of topical application of omeprazole on transmucosal potential difference (PD), luminal pH and histamine-stimulated acid secretion were examined in anesthetized rat stomachs, and they were compared with those of systemic administration. Omeprazole was suspended in 1% CMC with NaHCO₃ (pH 9.0) or dissolved in 0.1 N HCl (pH 1.0). Both omeprazole (30 mg/kg, pH 9.0) and cimetidine (100 mg/kg), given i.d., increased the pH and inhibited acid secretion induced by histamine (8 mg/kg/hr, i.v.), while basal gastric PD was markedly elevated only by the former. Similar responses in PD, pH and acid output were obtained dose-dependently after brief exposure of the stomach (10 min) to omeprazole (0.3-30 mg/kg), even in acidic conditions, but the effects of acidified omeprazole disappeared depending upon the latency period in 0.1 N HCl; there was no effect when applied at more than 30 min after dissolution. Of interest, subsequent exposure of the stomach to a mercaptane compound (cysteine, 100 mg/kg) for 30 min significantly reversed the antisecretory effect of omeprazole (both i.d. and i.g.) but not of cimetidine. These results suggest that omeprazole has a local antisecretory action even in acidic stomachs, probably through an inhibition of the H⁺/K⁺ ATPase activity, and the increase of PD caused by omeprazole may be a characteristic phenomenon seen after the blockade of H⁺/K⁺ ATPase, but is not associated with acid inhibition itself.

Substituted benzimidazoles are potent inhibitors of gastric acid secretion in experimental animals and in man (1-3). Studies with the most potent compound, omeprazole, provided strong evidence that this class of drugs inhibits the activity of the H⁺/K⁺ adenosine triphosphatase (ATPase), an enzyme unique for the gastric mucosa and located on the microvilli of the secretory canaliculi of the parietal cell (4-7). Upon various routes of administration, it may accumulate in the acid compartment of gastric glands, resulting in the inhibition of acid secretion (7). However, Konturek et al. (8) showed that topical application of omeprazole (with NaHCO₃) to Heidenhain pouch dogs inhibited histamine-stimulated acid secretion with no alteration in acid output from the main stomach, suggesting a local action of the drug on the oxyntic glands. Although the mechanism of its local action remains unknown, it may be possible to assume that this action is responsible, at least partly, for a potent and sustained suppression of gastric acid secretion observed after its oral administration, provided that acidified omeprazole exerts similar local antisecretory effects.

In the present study, the effects of topical application of acidified omeprazole on basal and histamine-stimulated acid secretion were examined in anesthetized rats, and they were compared with those of systemic administration of omeprazole and cimetidine. Since recent studies (9, 10) showed that benzimidazoles have characteristic effects on transmucosal potential difference (PD) in the stomach, we also measured the PD responses under various conditions in the presence of
omeprazole.

Materials and Methods

Male Sprague Dawley rats (230–250 g), kept in individual cages with raised mesh bottoms, were deprived of food but allowed free access to tap water for 24 hr prior to the experiments. All studies were carried out using 4 to 6 rats per group, under anesthetized conditions induced by intraperitoneal administration of urethane (Nakarai, 1.25 g/kg).

Determination of PD and pH: Simultaneous measurement of PD and pH was performed according to a previously published method (11). Briefly, the stomach was perfused at a flow rate of 1 ml/min with saline (154 mM NaCl) that was gassed with 100% O₂, heated at 37°C, and kept in a reservoir. The pH of fluid emerging from the stomach was measured using a pH glass electrode of the flow type (Horiba Model 6901-25T), and the PD was determined using two agar bridges, one positioned in the stomach and the other in the abdominal cavity. Changes in both PD and pH were continuously monitored on a Hitachi two channel recorder (Model 056). Approximately 1 hr after both PD and pH had stabilized, the perfusion system was interrupted, and the solution in the stomach was withdrawn. The stomach was then exposed for 10 min to 1 ml of omeprazole (Hassle, 0.3–30 mg/kg), either suspended in 1% carboxymethylcellulose (CMC) and adjusted to pH 9.0 with addition of NaHCO₃ or dissolved in 0.1 N HCl (pH 1.0). In a preliminary study, we examined the different exposure time on basal acid secretion (5, 10, 20 and 30 min) and found that the degree of inhibition in acid output reached the maximum after exposure of the stomach for 10 min to omeprazole (pH 9.0). Thus, we selected 10 min as the exposure time period in the present study. In the case of acidified omeprazole, the stomach was exposed for 10 min to this drug immediately or at 15 min, 30 min, 45 min or 60 min after dissolution of omeprazole in 0.1 N HCl. After application, the stomach was rinsed with saline, another 2 ml of saline was instilled, and the perfusion was resumed. Monitoring of pH was also interrupted for 10 min while the stomach was exposed to omeprazole, whereas the PD was continuously measured throughout a 2 hr test period. In some cases, omeprazole (30 mg/kg, pH 9.0) or cimetidine (Sigma, 100 mg/kg), suspended in 1% CMC, was given intraduodenally, and both PD and pH were measured for 1 hr thereafter, under basal and histamine (8 mg/kg/hr, i.v.)-stimulated conditions.

Determination of acid secretion: Since acid secretion in anesthetized rats showed a spontaneous decrease with time, the effects of omeprazole on acid secretion were studied in rats given histamine 2HCl (Nakarai) by continuous intravenous infusion, according to a previous paper (12). The operative procedures were similar in the case of determination of PD and pH. Briefly, the stomach was perfused at a flow rate of 1 ml/min with saline (pH 7.4), and acid secretion was measured at pH 7.4 using a pH-stat method (Hiranuma Comtite-7) and by adding 100 mM NaOH to the reservoir. A fine polyethylene tube was inserted into the tail vein for intravenous infusion of histamine (8 mg/kg/hr) at a rate of 1.2 ml/hr using a peristaltic pump (Harvard Apparatus, Model 931-D). Approximately 1 hr after acid secretion had stabilized, the perfusion was interrupted, and the stomach was exposed for 10 min to omeprazole (30 mg/kg, pH 9.0 and 1.0). After the exposure, the stomach was rinsed with saline, and acid secretion was measured for 2 hr thereafter. When the luminal pH was raised above 7.4 by complete inhibition of acid secretion, we measured alkaline secretion by adding 10 mM HCl to the reservoir. In some cases, omeprazole was applied for 10 min to the stomach from immediately or at 15 min or 60 min after being suspended in 1% CMC (pH 9.0) or dissolved in 0.1 N HCl (pH 1.0). After other, omeprazole (pH 9.0) was given intraduodenally, and acid secretion was measured for 2 hr thereafter. In separate experiments, the effect of cysteine (Sigma), a SH protector (13), was examined on the antisecretory action of omeprazole. After acid secretion had stabilized under histamine infusion, omeprazole (30 mg/kg) was given intraduodenally (pH 9.0) or applied for 10 min to the stomach (pH 1.0); and in both cases, cysteine was subsequently applied to the stomach for 30 min in a dose of 100 mg/kg from 60 min after treatment with omeprazole. The effect of
cysteine was examined in some cases on the antisecretory action of intraduodenally administered cimetidine (100 mg/kg).

Statistics: Data are presented as the mean±S.E. from 4 to 6 rats per group. Statistical analysis was performed using a two-tailed Dunnett's multiple comparison test (14), and values of P<0.05 were regarded as significant.

Results

Effects of omeprazole and cimetidine on gastric PD and pH: The saline-perfused stomach maintained a stable PD (-35 mV to -40 mV, mucosa negative) and pH (2.9–3.4) throughout the test period (2 hr). After intraduodenal administration of omeprazole (pH 9.0, 30 mg/kg) and cimetidine (100 mg/kg), the pH started to elevate from 15 min and reached the plateau levels 40–60 min later (Fig. 1), the maximal pH obtained being 5.2±0.3 and 4.5±0.1 in cases of omeprazole and cimetidine, respectively. The PD markedly increased in association with elevation of the pH after omeprazole treatment, while there was little or no change in the PD after administration of cimetidine (Figs. 1 and 2). Similar increases of pH and PD were observed in the stomach after exposure for 10 min to omeprazole (pH 9.0); the maximal pH obtained was not significantly different from that seen after intraduodenal administration.

Fig. 1. Effects of omeprazole (i.d. and i.g.) and cimetidine (i.d.) on pH and PD of the stomach in anesthetized rats. Omeprazole (30 mg/kg), suspended in 1% CMC and adjusted to pH 9.0 with addition of NaHCO₃, was given intraduodenally or by topical application to the stomach for 10 min, and cimetidine (100 mg/kg), suspended in 1% CMC, was given intraduodenally. Data are presented as the mean±S.E. of values determined every 10 min from 5 rats. *Statistically significant difference from controls, at P<0.05.
of this agent. On the other hand, both PD and pH were decreased in response to histamine (8 mg/kg/hr, i.v.), and either omeprazole or cimetidine reversed the decreased pH and PD responses caused by histamine (Fig. 3).

Effects of intragastric application of acidified omeprazole on gastric PD and pH: When omeprazole was dissolved in 0.1 N HCl and immediately applied to the stomach for 10 min, both pH and PD were elevated in a dose-related manner (0.3–30 mg/kg) (Fig. 4). The significant effect on pH and PD was observed even at 0.3 mg/kg, and the maximal pH (5.8±0.3) was obtained at 30 mg/kg. In these cases, the increases of PD were seen in association with those of pH. Exposure of the stomach for 10 min to 1 ml of 0.1 N HCl did not significantly affect both pH and PD. However, when acidified omeprazole was applied to the stomach from various times after dissolution of this drug in 0.1 N HCl, the effects on pH and PD disappeared in a time-dependent manner (Fig. 5). The maximal pH seen after application of acidified omeprazole (30 mg/kg) was 5.9±0.2, 5.6±0.3, 4.8±0.3, 4.0±0.2 and 3.7±0.3, respectively, in cases of immediately, 15 min, 30 min, 45 min and 60 min, and the significant effect was observed in the former three cases as compared to the control. The increased responses of PD also corresponded to the latency period of this drug in 0.1 N HCl, similar to those of pH seen after intragastric application of acidified omeprazole.

Effects of omeprazole on acid secretion induced by histamine: Acid secretion in anesthetized rats was increased from 5–6 μEq/10 min to 26–34 μEq/10 min within 50 min in response to intravenous infusion of histamine (8 mg/kg/hr), and it remained elevated during a 2 hr test period without any tachyphylaxis with time (not shown). Intraduodenal administration of omeprazole (pH 9.0, 30 mg/kg) produced a significant inhibition of histamine-stimulated acid secretion from 20 min after administration, and the maximal inhibition was obtained 1 hr later (Fig. 6). In some cases, acid secretion ceased
completely, and a significant amount (0.3–0.5 μEq/10 min) of \( \text{HCO}_3^- \) was detected in the lumen. The potent inhibition of acid secretion induced by histamine was similarly observed after intraluminal application of omeprazole (30 mg/kg) to the stomach for 10 min, the magnitude of inhibition being similar irrespective of whether the stomach was exposed to omeprazole, either adjusted to pH 9.0 or 1.0. In contrast to intraduodenal administration, the onset of the antisecretory action of omeprazole was very rapid after topical application, and about 70% inhibition was obtained immediately after exposure of the stomach for 10 min to this agent. The antisecretory action of omeprazole, when dissolved in 0.1 N HCl and applied for 10 min to the stomach, was time-dependent as observed in case of pH and PD. The potency of the antisecretory effect diminished depending upon the latency period of this drug in 0.1 N HCl; the effect disappeared totally when omeprazole was applied for 10 min to the stomach from 1 hr after dissolution in 0.1 N HCl (Fig. 7). The maximal inhibition against histamine-induced acid output was 87.3%, 68.2% and 3.6%, in cases of immediately, 15 min and 60 min, respectively. On the other hand, the antisecretory effect of intragastric application of omeprazole (pH 9.0) remained unchanged in potency, regardless of whether this drug was applied to the stomach immediately or 1 hr after suspension in 1% CMC (pH 9.0).

Effects of cysteine on antisecretory action of omeprazole and cimetidine: Histamine-stimulated acid secretion was significantly inhibited by intraduodenally administered omeprazole or by exposure of the stomach for 10 min to acidified omeprazole (30 mg/kg) (Fig. 8, A and B). In the former, acid secretion was completely abolished and small amounts of \( \text{HCO}_3^- \) appeared in the lumen, while in the latter, low rates of acid secretion (3–4 μEq/10 min) persisted during a 2 hr test period. However, when the stomach was exposed subsequently for 30 min to cysteine (100 mg/
kg), the inhibited or reduced acid secretion was restored to the levels that were significantly different from those without cysteine treatment. The net change of acid secretion was 3-4 μEq/10 min and 6-7 μEq/10 min in the cases of intraduodenal and intragastric administration of omeprazole, respectively. The treatment of the stomach with cysteine alone had no effect on acid secretion induced by histamine, and it did not affect the antisecretory effect of cimetidine (100 mg/kg, i.d.) (not shown).

Discussion
The present findings in rats confirmed the previous observation that omeprazole potently inhibited both basal and histamine-stimulated acid secretion in experimental animals and humans (2, 3, 15). The antisecretory action of omeprazole was achieved by either systemic (intraduodenal) administration or intragastric application of this agent. Especially, since a potent inhibition of acid output was observed after a brief exposure of the pylorus-ligated stomach to omeprazole, it may be likely that a local effect of this agent contributes at least partly to the potent and long-lasting inhibition of acid output seen after oral administration of omeprazole in rats as reported by others (3, 15).

It is generally known that substituted benzimidazoles are unstable in acidic milieu and easily degrade into products which are devoid of antisecretory property (3). On the other hand, these agents have to be transformed to the active inhibitor through their protonation in the acid compartment of the parietal cell before acting on the H⁺/K⁺ ATPase (16, 17).
Thus, it may be possible to assume that topically applied omeprazole has similar inhibitory effects on the H⁺/K⁺ ATPase, provided that the protonated omeprazole in the acidic stomach could move into the secretory canaliculi of the parietal cell directly from the lumen. Konturek et al. (8), in fact, showed a local effect of omeprazole (with addition of NaHCO₃) on histamine-stimulated acid secretion in the Heidenhain pouch dog. We confirmed the above finding in the rat stomach and further showed that omeprazole in acidic conditions dose-dependently inhibited acid secretion when this agent was applied to the stomach within 15 min of a latency period in 0.1 N HCl (pH 1.0). The antisecretory action of omeprazole reached the maximal level (>80%) when the stomach was exposed to this agent for 10 min, suggesting a rapid movement of omeprazole from the lumen to the secretory canaliculi of the parietal cell. Since the PD was increased in response to topically applied omeprazole and since acid secretion was determined at luminal pH 7.4, it is unlikely that omeprazole may reduce acid output through H⁺ back-diffusion due to disruption of the gastric mucosal barrier. In the present study, topically applied drug to the pylorus-ligated stomach could not move into the intestine, suggesting no possibility of intestinal absorption of the drug. Moreover, it is generally known that it is difficult for the drug which is ionized or protonated to cross the cell membrane. Since the pKa of omeprazole is about 4.0, acidified omeprazole (pH 1.0) is mostly protonated and rarely...
absorbed in the stomach through the gastric mucosa. Thus, the observed effects of topically applied omeprazole may be largely ascribed to its local action but not due to the systemic one.

The mechanism of the antisecretory action of topically applied omeprazole seems to be due to inhibition of the H⁺/K⁺ ATPase activity, similar to the case of systemic administration. Cysteine and other mercaptane compounds have been shown to prevent and reverse the inhibition induced by omeprazole both in the gastric gland preparations and on the purified H⁺/K⁺ ATPase (13, 18, 19). This reversal is specific for the benzimidazole group of compounds, since no reversal occurred when the inhibition was induced by cimetidine or SCN⁻ (20). As expected, the inhibition of acid secretion induced by omeprazole, given either i.d. (pH 9.0) or i.g. (pH 1.0), was partly but significantly reversed by later exposure of the stomach to cysteine, which had no effect on cimetidine-induced suppression of acid secretion. Based on these findings, topically applied omeprazole even in acidic stomachs may move into the secretory canaliculi of the parietal cell directly to inhibit the H⁺/K⁺ ATPase activity, if the latency period is less than 15 min in acidic conditions. Rackur et al. (21) recently demonstrated a reaction pathway of benzimidazoles in the parietal cell, and showed that a product in the reaction pathway from the mother compound to the corresponding sulfide and the tetracyclic compound is formed in the intact parietal cell and acts as inhibitor of acid secretion. Since the sulfide inhibits acid secretion without influencing the H⁺/K⁺ ATPase (18–20), and since the tetracyclic compound shows potent antisecretory effect through an inhibition of the H⁺/K⁺ ATPase by non-specific and mercaptane-insensitive manners (22), the inhibition of acid secretion caused by acidified omeprazole may be partly due to the antisecretory action afforded by these degradation products.

Fig. 6. Effects of omeprazole on histamine-stimulated acid secretion in anesthetized rats. Acid secretion was stimulated by intravenous infusion of histamine (8 mg/kg/hr) during a test period. Omeprazole (30 mg/kg) was given intraduodenally (pH 9.0) or applied topically to the stomach for 10 min immediately after it was suspended in 1% CMC (pH 9.0) or dissolved in 0.1 N HCl (pH 1.0). Data are presented as the mean+S.E. of values determined every 10 min from 4 rats. *Statistically significant difference from controls, at P<0.05.
Another interesting finding is that omeprazole, but not cimetidine, increased gastric PD in association with acid inhibition under both basal and histamine-stimulated conditions. An increase of gastric PD in response to substituted benzimidazoles has been reported by several investigators in both in vitro amphibian stomachs and in vivo rat stomachs under histamine stimulated conditions (9, 10, 23). Starlinger et al. (9) found that the increased PD responses caused by omeprazole were completely abolished by pre- and post-treatment with metiamide, suggesting that the increase of PD may be a characteristic phenomenon observed after the blockade of H⁺/K⁺ ATPase to unmask the net Cl⁻ transport in the parietal cell, but is not directly associated with acid inhibition itself. This speculation is supported by the present finding that cimetidine at the dose (100 mg/kg) which completely abolished histamine-induced acid secretion failed to produce an apparent rise of basal gastric PD. In contrast, Yano et al. (10) showed in rats that timoprazole markedly elevated gastric PD under histamine-stimulated but not basal conditions, while cimetidine produced a rise of the PD in the absence or presence of histamine. Although the reasons for these different results remain unknown, they may be partly ascribed to the different types of benzimidazoles used and the different degrees of basal and histamine stimulated acid secretion. The decreased PD responses caused by histamine may be associated with the increase of ionic conductance coupled with acid secretion (Cl⁻, K⁺, Na⁺). Thus, the increase of PD seen after the treatment with these benzimidazoles and cimetidine in the presence of histamine may be largely implicated as the reverse of the decreased PD responses caused by stimulation of acid secretion.

There are species differences in the origin of gastric PD: under resting conditions, short circuit current (Isc) is approximately equivalent to the net Cl⁻ transport in the amphibian
fundic mucosa, while in the rat fundic mucosa, \( I_{\text{sc}} \) mostly reflects the net Na\(^+\) transport across the mucosa (24, 25). Thus, a definite conclusion about the mechanism of the PD responses caused by omeprazole must await further studies including the measurement of ionic fluxes in both the parietal and epithelial cells in the absence or presence of this agent.

The present study clearly showed that topical application of omeprazole has a potent antisecretory action, even in acidic stomachs, probably through a local action rather than a systemic one. Inasmuch as the high selectivity of substituted benzimidazoles depends on the protonation process in the acid space of the parietal cell, acidified omeprazole may lose its selectivity to cause unexpected actions. However, this is unlikely because the tissue membrane may be highly impermeable to the protonated form of the drug. Moreover,

**Fig. 8.** Effects of cysteine on antisecretory action of omeprazole in anesthetized rats. Acid secretion was stimulated by intravenous infusion of histamine (8 mg/kg/hr). Omeprazole (30 mg/kg) was given intraduodenally (pH 9.0) (B) or by topical application to the stomach for 10 min (pH 1.0) (A) immediately after dissolution in 0.1 N HCl. Cysteine (100 mg/kg) was given by topical application to the stomach for 30 min from 1 hr after treatment with omeprazole. Data are presented as the mean±S.E. of values determined every 10 min from 6 rats. *Statistically significant difference from controls, at P<0.05.
since omeprazole is given as an enteric coated capsule in humans, and thus the compound is released in the intestine, its local effect can be ignored under clinical conditions. Our findings are, nevertheless, important on the basis of pharmacology and suggest the minimum criteria required for compounds that inhibit acid secretion by acting as specific H+K+ ATPase inhibitors under in vivo conditions: (a) they should inhibit both basal and stimulated acid secretion, (b) their antisecretory effects are counteracted by SH protectors such as cysteine and (c) they produce a definite rise in gastric PD in association with acid inhibition. The fulfillment of these criteria may be used for screening of H+K+ ATPase inhibitors under in vivo conditions.

References

1. Fellenius, E., Elander, B., Wallmark, B., Helander, H. and Berglindh, T.: Inhibition of acid secretion in isolated gastric glands by substituted benzimidazoles. Am. J. Physiol. 243, G505-G510 (1982)

2. Olbe, L., Haglund, U., Leth, R., Lind, T., Cederberg, C., Ekenved, G., Elander, B., Fellenius, E., Lundberg, P. and Wallmark, B.: Effect of substituted benzimidazole (H 149/94) on gastric acid secretion in humans. Gastroenterology 85, 193–198 (1982)

3. Larsson, H., Carlsson, E., Junggren, U., Olbe, L., Sjostrand, S.E., Skanberg, I. and Sundell, G.: Inhibition of gastric acid secretion by omeprazole in the dog and rat. Gastroenterology 85, 900–907 (1983)

4. Ganser, A.L. and Forte, J.: K+-stimulated ATPase in purified microsomes of bullfrog oxyntic cells. Biochim. Biophys. Acta 307, 169–180 (1973)

5. Fellenius, E., Berglindh, T., Brandstrom, A., Elander, B., Henlander, H.F., Olbe, L., Sachs, G., Sjostrand, S.E. and Wallmark, B.: The inhibitory action of substituted benzimidazoles on isolated oxyntic glands and H+K+ ATPase. In Hydrogen Ion Transport in Epithelia, Edited by Schultz, I., Sachs, G., Forte, G. and Ullrich, K.J., p. 193–202. Elsevier, Amsterdam (1980)

6. Fellenius, E., Berglindh, T., Sachs, G., Olbe, L., Elander, B., Sjostrand, S.E. and Wallmark, B.: Substituted benzimidazoles inhibit acid secretion by blocking the H+K+ ATPase. Nature 290, 159–161 (1981)

7. Carlsson, E., Karlsson, A., Larsson, H., Loeborg, I., Sundell, G. and Skanberg, I.: Concentration of omeprazole in blood and gastric mucosa and the relation to inhibition of gastric acid secretion. Gastroenterology (abstract) 86, 1041 (1984)

8. Konturek, S.J., Cieszkowski, M., Kwiecien, N., Konturek, J., Tasler, J. and Bilski, J.: Effects of omeprazole, a substituted benzimidazole, on gastrointestinal secretion, serum gastrin, and gastric mucosal blood flow in dogs. Gastroenterology 86, 71–77 (1984)

9. Starlinger, M.J., Hollands, M.J., Rowe, P.H., Matthews, J.B. and Silen, W.: Chloride transport of frog gastric fundus: effects of omeprazole. Am. J. Physiol. 250, G118–G128 (1986)

10. Yano, S., Katsuyama, Y. and Watanabe, K.: Some profiles of transmucosal potential differences in rat stomach in situ with special reference to effect of timoprazole, a H+/K+ ATPase inhibitor. Japan. J. Pharmacol. 42, 209–216 (1986)

11. Takeuchi, K. and Okabe, S.: Role of luminal alkalinization in repair process of ethanol-induced mucosal damage in rat stomachs. Dig. Dis. Sci. 28, 993–1000 (1983)

12. Takeuchi, K., Furukawa, O., Tanaka, H. and Okabe, S.: A new model of duodenal ulcers induced in rats by indomethacin plus histamine. Gastroenterology 90, 636–645 (1986)

13. Im, W.B., Blackman, D.P. and Sachs, G.: Reversal of anti-secretory activity of omeprazole by sulfhydryl compounds in isolated rabbit gastric glands. Biochim. Biophys. Acta 845, 54–59 (1985)

14. Dunnett, C.W.: A multiple comparison procedure for comparing several treatments with a control. J. Am. Stat. Assoc. 50, 1086–1121 (1955)

15. Yamamoto, O., Okada, Y. and Okabe, S.: Effects of a proton pump inhibitor, omeprazole, on gastric secretion and gastric and duodenal ulcers or erosions in rats. Dig. Dis. Sci. 29, 394–401 (1984)

16. Wallmark, B., Brandstrom, A. and Larsson, H.: Evidence for acid-induced transformation of omeprazole into an active inhibitor of H+/K+ ATPase within the parietal cell. Biochim. Biophys. Acta 778, 549–558 (1984)

17. Lindberg, P., Nordberg, P., Alminner, T., Brandstrom, A. and Wallmark, B.: The mechanism of action of the gastric acid secretion inhibitor, omeprazole. J. Med. Chem. 29, 1327–1329 (1986)

18. Fryklund, J. and Wallmark, B.: Sulfide and sulfoxide derivatives of substituted benzimidazoles inhibit acid formation in isolated gastric glands by different mechanisms. J. Pharmacol. Exp. Ther. 236, 248–253 (1986)

19. Beil, W., Eltze, M., Heintze, K., Klemm, K., Riedel, R., Schulte, C., Sewing, K.-Fr. and Simon, A.: The sulfoxide moiety of substituted benzimi-
dazoles is essential for inhibition of parietal cell H+/K+ ATPase. Br. J. Pharmacol. 88, 389-395 (1986)

20 Wallmark, B., Jaresten, B.M., Larsson, H., Ryberg, B., Brandstrom, A. and Fellenius, E.: Differentiation among inhibitory actions of omeprazole, cimetidine, and SCN− on gastric acid secretion. Am. J. Physiol. 245, G64-G71 (1983)

21 Rackur, G.M., Bickel, H.W., Fehlhaber, A., Herling, V., Hitzel, H.J., Rosner, L.M. and Weyer, R.: 2-(2-Pyridyl-methyl sulfinyl) benzimidazoles—acid sensitive suicide inhibitors of the proton transport system in the parietal cell. Biochim. Biophys. Res. Commun. 128, 477-483 (1985)

22 Beil, W., Hannermann, H., Madge, S. and Sweing, K.-Fr.: Inhibition of gastric H+/K+ ATPase by acid-sensitive 2-(2-pyridyl methyl sulfinyl) benzimidazole products. Eur. J. Pharmacol. 133, 37-45 (1987)

23 Curci, S., Schettino, T. and Fromter, E.: Histamine reduces Cl− activity in surface epithelial cells of frog gastric mucosa. Suggestive evidence for ionic coupling between surface epithelia and oxyntic cells. Pflügers Arch. 406, 204-211 (1988)

24 Heintz, E. and Durbin, R.P.: Studies of the chloride transport in the gastric mucosa of the frog. J. Gen. Physiol. 41, 101-107 (1957)

25 Cummins, J.T. and Vaughan, B.E.: Ionic relationships of the bioelectrogenic mechanism in isolated rat stomach. Biochim. Biophys. Acta 94, 280-292 (1965)