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# IRREVERSIBLE INACTIVATION OF RAT GASTRIC ($H^+$-$K^+$)-ATPASE IN VIVO BY OMEPRAZOLE

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Subcutaneous administration of omeprazole, a gastric antisecretory agent belonging to the family of substituted benzimidazoles, brought about a dose-dependent decrease in gastric mucosal ($H^+$-$K^+$)-ATPase activity in the rat. The dose which inhibited 50% of the enzyme activity was 1 mg/kg from dose-response profiles obtained 3 h after the drug dosing. Duration profiles of the drug at 10 mg/kg showed that its ATPase-inhibitory effect reached the maximum in 2 h with 80% reduction of the enzyme activity. The gastric mucosal level of the ATPase activity remained to be maximally inhibited for 12 h and returned to a normal level with a half time of about 20 h. The return of the enzyme activity, however, was blocked by treatment with cycloheximide, an inhibitor of protein synthesis. These observations indicate that omeprazole irreversibly inactivates gastric ($H^+$-$K^+$)-ATPase in vivo. 

Gastric ($H^+$-$K^+$)-ATPase is an electroneutral $H^+/K^+$ exchange pump embedded in the lumenal membranes or endoplasmic tubulovesioles of gastric parietal cells (1-4). A variety chemicals inhibits the gastric ATPase in vitro by modifying its sulfhydryl, amino or carboxylate groups (2-9). Among these inhibitors, substituted benzimidazoles are unique in that they are clinically effective and long-acting gastric antisecretory agents (10-12). The clinical efficacy of the benzimidazoles has been ascribed to their specificity toward the ATPase in vivo and covalent modification of the enzyme (9,12). Nevertheless, the mode of interaction(s) between the drugs and the ATPase are not fully understood. In this communication, we will report the inhibitory effect of omeprazole, a substituted benzimidazole, on rat gastric ($H^+$-$K^+$)-ATPase in vivo and show that duration of the omeprazole effect was prolonged with treatment with cycloheximide, an inhibitor of protein synthesis.

Abbreviation; EGTA, Ethyleneglycol-bis-(β-aminoethyl ether)N,N'-tetracetic acid. Hepes, (N-2-Hydroxyethylpiperazine-N-2-ethanesulfonic acid. Pipes, (Piperazine-N,N'-bis[2-ethanesulfonic acid]).

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## MATERIALS AND METHODS

Male Sprague-Dawley rats weighing about 230 g were fasted for 16 h. Various amounts of omeprazole dissolved in 1 ml of 0.9% saline containing 25% ethanol were injected subcutaneously. In some experiments, a dose of cycloheximide, 1 mg/kg, dissolved in 0.9% saline was given intraperitoneally 3 h after administration of omeprazole and followed by the same dose every 10 or 12 h as indicated. The rats were fed ad libitum, unless they were sacrificed within 3 h of the omeprazole treatment. The animals were sacrificed by cervical dislocation.

The fundic region of the rat stomachs was scraped with a glass slide. Typically, the gastric mucosal tissues scraped from 4 rat stomachs were suspended in 25 ml of ice-cold buffer 1 containing 250 mM sucrose, 2 mM $MgCl_2$, 1 mM EGTA and 2 mM Hepes/Tris, pH 7.4. The tissues were homogenized in a Sorvall Omni-Mixer at the maximum speed for three minutes at 4°C. The homogenates were centrifuged at 20,000 x g for 15 min. The supernatants were centrifuged at 170,000 x g for 35 min. The microsomal pellets thus obtained were resuspended in 2 ml of buffer 1. Assay for $(H^+-K^+)$-ATPase activity in various fractions was carried out in the presence of 0.56 µM dicyclohexylcarbodiimide and other experimental conditions were the same as described previously (14). The microsomal fractions usually showed a three-fold enrichment of $(H^+-K^+)$-ATPase activity as compared to homogenates and also their ATPase level closely reflected the changes in that of the mucosal homogenates due to omeprazole or cycloheximide treatments. Therefore, in this study we have employed the microsomal fractions for comparison of the gastric ATPase levels. All the data presented in this report represent a mean ± standard deviation from three experiments. Protein was determined by the Lowry method (15).

A sample of omeprazole used in this study was prepared by Dr. John C. Sih in The Upjohn Company. Cycloheximide was purchased from Sigma.

## RESULTS AND DISCUSSION

Subcutaneous administration of omeprazole at the dose of 10 mg/kg led to a measurable decrease in gastric mucosal $(H^+-K^+)$-ATPase activity in 30 min and reached its maximum effect in 2 h with 80% reduction of the enzyme activity (Figure 1). The maximal effect was maintained for 12 h. Then the animals recovered the ATPase activity with a half time of about 20 h. Such a long duration of the omeprazole effect on the gastric ATPase is comparable to that of the drug effect on gastric acid secretion in the rat as reported by Larsson et. al. (16). These results further suggest covalent modification of gastric $(H^+-K^+)$-ATPase by omeprazole in vivo as already reported in vitro by Wallmark et. al. (9).

Dose-dependence of the inhibitory effect of omeprazole on the gastric ATPase is shown in Figure 2. The profile was obtained 3 h after administration of the drug and shows that the dose of 1 mg/kg inhibited 50% of

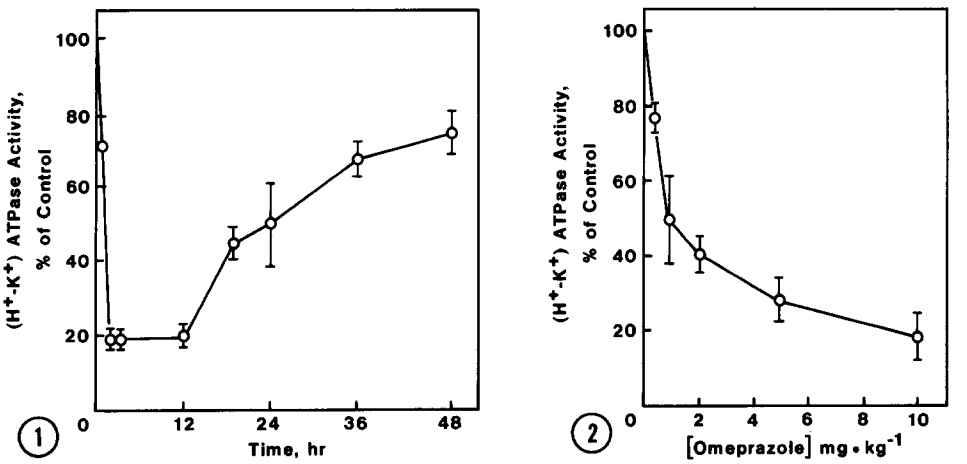


Fig. 1. Plots showing duration of the ATPase-inhibitory effect of omeprazole. The rats were fasted overnight and injected subcutaneously with omeprazole at 10 mg/kg. The rats (4 in each group) were sacrificed at 1, 2, 3, 12, 18, 24, 36 and 48 h after the drug treatment. The level of $(H^+-K^+)$-ATPase activity in the gastric mucosal microsomes was measured as described.

Fig. 2. Dose-response profile for inhibition of rat gastric $(H^+-K^+)$-ATPase *in vivo* by omeprazole. The rats were fasted overnight. Omeprazole was injected subcutaneously at the indicated dose. The control animals received the same volume of vehicle (saline-25% ethanol). They were sacrificed 3 h after the injections. The gastric mucosal microsomes were prepared as described under Methods section. The specific activity of the gastric ATPase in the microsomes from the control was typically 25 μmol pi/h. mg protein.

the enzyme activity. This dose-dependent and long acting effect of omeprazole could be due to irreversible inactivation of gastric $(H^+-K^+)$-ATPase and consequently the recovery of the enzyme activity may involve synthesis of new enzymes. This possibility led us to test the effect of cycloheximide, an inhibitor of protein synthesis, alone or in combination with omeprazole on the gastric ATPase activity.

As shown in Table I, treatment with cycloheximide alone diminished total enzyme activity in gastric mucosal homogenates as a function of the treatment periods. The half life of gastric $(H^+-K^+)$-ATPase was about 72 h as obtained by extrapolation of the data assuming first-order kinetics under our experimental conditions. Interestingly, the specific activity of the ATPase in the homogenates and microsomes was not significantly affected during cycloheximide treatments. These observations suggest that a majority of gastric mucosal proteins has a half life similar to that of $(H^+-K^+)$-ATPase under cycloheximide poisoning.

Table I

Effect of cycloheximide on the activity of $(H^{\pm}-K^{\pm})$-ATPase in rat gastric mucosa

| Treatments | $(H^+-K^+)$-ATPase Activity | | |
|---|---|---|---|
| | Specific Activity | | Total Activity in Homogenates |
| | Homogenates | Microsomes | |
| | µmol pi/h·mg protein | | % of control |
| Control | 11.5 ± 2.0 | 27.5 ± 4.5 | 100 ± 9 |
| Cycloheximide 3 h | 12.5 ± 0.5 | 31.3 ± 2.3 | 95 ± 3 |
| Cycloheximide 24 h | 12. 8 ± 0.6 | 27.8 ± 3.2 | 79 ± 6 |
| Cycloheximide 48 h | 14.1 ± 1.0 | 30.1 ± 2.3 | 65 ± 6 |

The rats were injected intraperitoneally with cycloheximide at the dose of 1 mg/kg at 0, 12, 23, 36 and 47 h. Each group consists of 4 rats. The rats were sacrificed by cervical disolocation at the indicated time. Experimental conditions for preparation of gastric mucosal homogenates and microsomes and for assay of $(H^+-K^+)$-ATPase activity were given under "Materials and Methods" section.

Administration of cycloheximide twice daily following the omeprazole treatment blocked the return of the ATPase activity (Table II). This results indicate that synthesis of new enzymes is required for recovery from the omeprazole effect. It should be noted that the synthetic rate of $(H^+-K^+)$-ATPase observed upon omeprazole treatment is apparently much faster than the

Table II

Effect of cycloheximide on duration of the inhibitory activity of omeprazole on rat gastric $(H^{\pm}-K^{\pm})$-ATPase

| Treatments | Specific Activity of $(H^+-K^+)$-ATPase[a] | | |
|---|---|---|---|
| | 3h | 24h | 48h |
| | µmol pi/h·mg protein | | |
| Omeprazole | 6.4 ± 0.5 | 13.4 ± 0.7 | 17.6 ± 2.6 |
| Omeprazole & cycloheximide | | 10.6 ± 1.8 | 9.4 ± 0.6 |

The rats were fasted overnight and treated with omeprazole at a subcutaneous dose of 10 mg/kg or the vehicle (saline-25% ethanol). Cycloheximide at the dose of 1 mg/kg was injected intraperitoneally at 3, 12, 23, 36 and 47 h after the omeprazole treatment. The rats were fed *ad libitum* immediately after injection of omeprazole until sacrifice at the indicated time. Experimental conditions for preparation of gastric microsomes and for assay of $(H^+-K^+)$-ATPase activity were given under "Materials and Methods" section.

[a]In rat gastric microsomes

rate of enzyme disapperance under cycloheximide poisoning. Conceivably, the enzyme inactivation by omeprazole may accelerate synthesis of new enzyme above the normal rate. In short, we have established that omeprazole irreversibly inhibits gastric ($H^+$-$K^+$)-ATPase *in vivo*.

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