Omeprazole Has a Dual Mechanism of Action: It Inhibits Both $H^+K^+$ ATPase and Gastric Mucosa Carbonic Anhydrase Enzyme in Humans (In Vitro and In Vivo Experiments)

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ABSTRACT
In this study our experiments followed in vitro and in vivo the effect of omeprazole on purified and erythrocyte carbonic anhydrase (CA) I and II isozymes, as well as on gastric mucosa CA IV in humans. Our in vitro results show that omeprazole-induced inhibition of purified CA I and CA II and gastric mucosa CA IV is dose- and pH-dependent. In vivo, the i.v. administration of omeprazole in humans in therapeutic doses produced a decrease in erythrocyte CA I and CA II activity, as well as in gastric mucosa CA I, II, and IV. Regarding CA IV, the results lead to the conclusion that omeprazole selectively inhibits gastric mucosa CA IV and does not modify the activity of the same isozyme from the kidney and lung, indicating organ specificity. Our results strongly suggest that omeprazole has a dual mechanism of action: $H^+K^+$ ATPase inhibition and gastric mucosa CA inhibition, and that these enzymes may be functionally coupled. This 2-fold mechanism of action could explain the greater effectiveness of substituted benzimidazoles as compared with other therapies.

Substituted benzimidazoles such as omeprazole, lansoprazole, and pantoprazole are today among the most effective antiulcer therapies worldwide (Hersey and Sachs, 1995). Research started in 1986 regarding the gastric mucosa $H^+K^+$ ATPase inhibitors finally led to the discovery of omeprazole in 1979 (Lindberg et al., 1990).

Concerning the mechanism of action of omeprazole in decreasing gastric acid secretion, it blocks changes $H^+ - K^+$ within the parietal cells. Omeprazole is a weak base, which is inactive at pH 7 in the parietal cell cytoplasm. However, when it passes into the luminal secretory canaliculi, where the acidic pH may reach a value of 1.0, omeprazole gains a proton and concentrates itself in this form in the luminal canaliculi (Wallmark et al., 1983; Lorentzon et al., 1987).

Omeprazole, by gaining a proton in acidic conditions, changes into the sulfenamide, a form that reacts with the sulphydryl group of cysteines located in the lumen of the $\alpha$ subunit of $H^+K^+$ ATPase (Lorentzon et al., 1987; Hersey and Sachs, 1995) linking to the cysteine 813, which is responsible for acid inhibition (Besancon et al., 1993). As a consequence of this irreversible coupling, ion channels involved in expulsion of $H^+$ from the cell and uptake of $K^+$ are blocked (Hersey and Sachs, 1995). The inhibition lasts 16 to 18 h, and it returns gradually to the normal values after 4 days from the interruption of the treatment (Hersey and Sachs, 1995).

Other research started in 1952 by Janowitz, Colcher, and Hollander studied the involvement of another enzyme, carbonic anhydrase (CA), in gastric acid secretion. They proved that acetazolamide, a specific inhibitor of CA, administered in acute experiments in the dog inhibits HCl output up to 97% (Janowitz et al., 1952).

Our research, which started in 1971 (Puscas, 1971), has proved that gastric acid secretion is inhibited in humans after oral administration of acetazolamide in therapeutic doses of 25 mg/kg body weight. Our findings show that the decrease of gastric acid secretion does not occur immediately after the oral acetazolamide administration, but only after 2 to 4 days of treatment. Gastric acid secretion is progressively inhibited as treatment continues. After 5 days of acetazolamide treatment the basal acid output is inhibited by 81% and maximal acid output by 64%. After 10 days of acetazolamide treatment inhibition reaches 98 and 84%, respectively. After stopping the acetazolamide treatment, the inhibition lasts for 5 to 7 days, with a progressive return to initial values (Puscas, 1971). Thus, we started using for the first time the CA inhibitors in the treatment of gastroduodenal ulcer in humans (Puscas, 1984, 1987, 1990a). The effectiveness of this therapy results both from the antisecretory activity and from a vasculary improvement of gastric microcirculation (Puscas and Buzas, 1986; Puscas, 1990b).

Concerning the physiological role of CA I, our research has proved that the vasodilating substances inhibit CA I and the

ABBREVIATIONS: CA, carbonic anhydrase; r.t., room temperature.
vasoconstrictive ones activate this isozyme. Thus, organic nitrates, nitroglycerin, and sodium nitroprusside reduce CA I activity concomitantly with a decrease in blood pressure (Puscas et al., 1997). The same effect is also induced by vasodilating prostaglandins and nitric oxide (Puscas and Coltau, 1995a,b). In contrast, vasoconstrictive substances like vasoconstrictive prostaglandins, leukotrienes, and thromboxanes (Puscas and Coltau, 1995a) increase CA I activity in parallel with the increase in blood pressure values.

The same research showed that CA II is involved in the gastric secretory changes, its activation being accompanied by an increase in gastric acid secretion, and its inhibition being followed by a reduction in HCl production (Puscas, 1994). Starting in 1978 we provided evidence that histamine is a direct activator of gastric mucosa CA (Puscas et al., 1978). Subsequently, we showed that both in vitro and in vivo CA II is activated not only by histamine but also by gastrin, acetylcholine, cysteamine (Puscas, 1994), and nonsteroidal anti-inflammatory drugs (Puscas et al., 1996).

Our studies have also shown that CA IV, known as a membrane-bound isozyme (Wistrand, 1984) located in the gastric parietal cells, and renal and pulmonary membranes, has an organ specificity (Puscas, 1994). According to this hypothesis, the major gastric acid secretion stimuli like histamine, gastrin, and acetylcholine activate gastric parietal cell membrane CA IV but do not modify the activity of the same isozyme from kidney and lung (Puscas, 1994). Somatostatin and calcitonin decrease gastric CA IV activity (Puscas, 1994) and do not modify the renal and pulmonary ones (Puscas, 1998).

**Materials and Methods**

**In Vitro.** We followed the effect of omeprazole at concentrations between 10^-8 and 10^-4 M on purified CA I and CA II, as well as on pig isolated gastric mucosa CA IV and renal and pulmonary CA IV activity. The effects of omeprazole on CA isozymes using different pH values from 5 to 1 were also monitored. In parallel, kinetic studies were conducted to establish the mechanism of action of H+K+ATPase inhibitors upon CA isozymes.

**In Vivo.** The experiments were approved by the local human ethics committee and informed consent was obtained from each patient.

Starting from literature data that show that there are no gender-related differences in response to omeprazole therapy, we selected by randomization a group of 19 healthy male volunteers, aged between 25 and 50, having a body weight between 65 and 82 kg. In this group we studied the effect of omeprazole on gastric CA I, II, and IV, as well as on erythrocyte CA I and CA II. Omeprazole (Losec, Astra, Sweden) was administered in a single i.v. infusion of 40 mg. Blood was collected from all patients before omeprazole administration, to assess red blood cell CA I and CA II activity. At the same time, under videoendoscopic control, we collected gastric mucosa biopsies from the parietal cell area to determine the activity of CA I, II, and IV. We repeated in all patients the blood and gastric mucosa biopsies collection 2 h after omeprazole administration to measure the activity of the same CA isozymes determined before treatment.

Human gastric mucosa parietal cell isolation was performed using the collagenase method described by Lewin (1982). The CA IV separation from the human and pig gastric parietal cell membrane, as well as for renal and pulmonary CA IV was performed following the method described by Maren et al. (1990). The differentiation between CA I and CA II activity from red blood cells and from human gastric mucosa was performed using the test with nicotinates. This test relies on the selective inhibitory effect of nicotinic acid methyl ester, nicotinic acid ethyl ester, and nicotinic acid propyl ester, respectively, on CA I (Puscas et al., 1999).

The CA isozyme activity was assessed using the stopped-flow method (Khalifah, 1971). This method consists in measuring the enzymatic activity of CO2 hydration and relies on the colorimetric method of changing pH. The time in which the pH of the reagent mixture decreases from its initial value of 7.5 to its final value of 6.5 is measured. Follow-up of the reaction is achieved spectrophotometrically at 400 nm, using a rapid kinetic spectrophotometer HIT-TECH SF-51 MX (Hi-Tech Co., Salisbury, UK), equipped with a mixing unit and a system of two syringes that supply the reagents. The signal transmitted by a photomultiplier from the mixing chamber is received and visualized by a computer equipped with a mathematico coprocessor and a kinetic software package, RKBIN IS-1 (Hi-Tech Co.).

The reagents used are:

- p-nitrophenol, as color indicator, is used at a concentration of 0.2 mM, pH = 7.5, temperature (room temperature; r.t.) = 20–25°C;
- HEPES buffer at a concentration of 20 mM, pH = 7.5, r.t. = 20–25°C;
- stock solution of purified CA I and II at a concentration of 3.44 × 10^-6 M, pH = 7.5, r.t. = 20–25°C;
- CO2 solution at a concentration of 15 mM (as substrate), which is obtained by bubbling CO2 in bidistilled water to saturation; and Na2SO4 at a concentration of 0.1 M is used to keep a constant ionic strength.

Activity of carbonic anhydrase is obtained by the formula:

\[ A = \frac{T_0 - T}{T} \text{[EU/ml]} \]

where \(T_0\) represents the uncatalyzed reaction time, and \(T\) represents the catalyzed reaction time (in the presence of purified CA I, CA II, and of red blood cell or gastric mucosa CA activity). Processing of kinetic data was achieved by the Michaelis-Menten equation using Lineweaver-Burk linearization.

Values were expressed as mean ± S.E. Statistical analysis was performed by using Student’s test and Dunnett’s test (the SAS T-TEST procedure and the SAS GLM procedure; SAS institute Inc., Cary, NC). A level of \(p < .05\) was considered a significant difference.

Purified CA I, CA II, methyl nicotinate, HEPES buffer, \(p\)-nitrophenol, and collagenase were obtained from Sigma (Dienzenhofen, Germany). Omeprazole for i.v. infusion (Losec) was made by Astra (Södertälje, Sweden). The kit for assessment of CO2 concentration was obtained from Gilford Systems (Oberlin, OH) and the Olympus Evis 100 gastroduodenal video-endoscope was provided by Olympus GmbH (Hamburg, Germany).

**Results**

**In Vitro.** Omeprazole inhibits purified CA I and CA II. The inhibitory effect of omeprazole occurs at 10^-8 M at a pH value of 5.0. At a concentration of 10^-4 M and a pH of 5.0, the CA I inhibition reaches 19%, and that of CA II reaches 24%. At a concentration of 10^-4 M and a pH of 1.0, the CA I inhibition is 51% and that of CA II is 69%. (Tables 1 and 2).

Pig gastric mucosa CA IV activity is inhibited by omeprazole. Thus, at a concentration of 10^-6 M and a pH of 5.0, the CA IV inhibition is 20%, and at 10^-4 M concentration and a pH of 1.0, gastric CA IV inhibition is 68% (Table 3). Omeprazole does not significantly inhibit the renal and pulmonary CA IV activity, irrespective of the concentration or the pH used (Table 4). The kinetic data demonstrate a noncompetitive mechanism of action of omeprazole upon purified CA I.
In vivo omeprazole decreases purified CA I and CA II activity in a dose-dependent manner and is proportional as the pH decreases. The same inhibitory effect is also induced by omeprazole on pig gastric mucosa parietal cell membrane CA IV. The increase in activity of omeprazole to inhibit CA I, II, and IV is proportional as the pH decreases. The lack of the omeprazole inhibitory effect on renal and pulmonary CA IV amide when the medium is acidified. The lack of the reduction in pH. The explanation for this observation may be the formation of a large quantity of sulfenic acid, which is then converted to the sulfinic acid form and finally to the sulfonic acid form, which is not inhibitory to CA.

Discussion

The results of this study show that in vitro, omeprazole decreases purified CA I and CA II activity in a dose-dependent manner and is proportional as the pH decreases. The same inhibitory effect is also induced by omeprazole on pig gastric mucosa parietal cell membrane CA IV. The increase in activity of omeprazole to inhibit CA I, II, and IV is proportional to the reduction in pH. The explanation for this observation may be the formation of a large quantity of sulfenic acid when the medium is acidified. The lack of the omeprazole inhibitory effect on renal and pulmonary CA IV.
when the pH is reduced suggests that sulfenamide, as the active form of omeprazole, has a higher affinity for gastric parietal cell membrane CA IV because the secretory canaliculi are rich in membrane-bound CA. The absence of sulfenamide-induced inhibition on renal CA IV (an isozyme involved in diuresis) could explain the lack of diuretic effects after treatment with omeprazole.

Our in vivo results prove that, in humans, i.v. omeprazole administration reduces erythrocyte CA I and CA II activity. Erythrocyte CA I and CA II inhibition indicates that sulfenamide diffuses into red blood cells. This inhibition also proves that omeprazole protonation in the lumen of secretory canaliculi results from sulfenamide formation and this does not prevent its crossing the cell membrane as others have suggested to date.

In human, i.v. administration of omeprazole in therapeutic doses also inhibits gastric mucosa CA I, II, and IV as demonstrated by the activity of these isozymes measured before and after treatment. Correlating in vivo results with the data obtained in vitro suggests that gastric mucosa CA I, II, and IV inhibition is induced by sulfenamide, the active form of omeprazole.

CA I, II, and IV activity is directly linked to pH (Maren, 1967). For example, in the secretory canaliculi where the pH is near 1.0, the basal activity of these CA isozymes is 2 to 3 times greater than the basal activity of these isozymes at neutral pH. Our research has demonstrated that omeprazole in its active form reduces this high activity to its initial values.

The involvement of CA I and CA IV in gastric acid secretion, the positive effect of CA inhibitors in reducing HCl secretion, and their healing effect on gastric and duodenal ulcers is well documented (Puscas, 1984, 1987). Our in vivo results after i.v. omeprazole administration in humans show that parietal cell CA II and CA IV inhibition could be responsible for the increase in antisecretory effects of H+K+ATPase inhibitors.

These data could also explain the effectiveness of substituted benzimidazoles such as omeprazole, lansoprazole, and pantoprazole in reducing gastric acid secretion as compared with other therapies. Our in vivo results, performed in humans, show that omeprazole inhibits not only H+K+ATPase, but also CA II and CA IV, isozymes present in large quantities in the cytosol, in the walls of the secretory canaliculi, and in the parietal cell membrane. Concerning the relationship between CA and the proton pump, our results suggest the existence of a functional coupling between these two enzymes, a relationship which requires further data to support.

As mentioned above, CA I inhibition is correlated with vasodilating effects (Puscas et al., 1997) as demonstrated by the cytoprotective effects of acetazolamide (Robert et al., 1982; Konturek et al., 1983) and the cerebral vasodilating effects of other CA inhibitors (Vorstrup et al., 1984; Wang, 1993).

Our results demonstrate that both the cytoprotective effects of acetazolamide and the vasodilating effects (e.g., the increase in gastric mucosal blood flow) are dependent on CA I inhibition (Puscas, 1994). As results from this study ome-
prazole inhibits gastric mucosa CA I like acetazolamide does; consequently, our findings suggest that omeprazole increases gastric mucosal blood flow similarly to acetazolamides by a mechanism dependent on CA I.

These data suggest that H⁺K⁺-ATPase inhibitors have a dual mechanism of action in antiulcer therapy: antisecretory effects and vascular effects, resulting in an increase in gastric mucosal blood flow. These two effects could explain both the high rate and the short period of healing induced by the proton pump inhibitors as compared with other therapies.

Our results suggest that the antisecretory mechanism of omeprazole involves inhibition of gastric mucosa CA II and CA IV, which are located in abundance in the parietal cell and in its secretory canaliculi walls. This inhibition potentiates the inhibitory effect of omeprazole on the proton pump. Additionally, the vascular effects responsible for the increase in microcirculation and the cytoprotective effects induced by omeprazole are mediated by gastric mucosa CA I inhibition.

The combination of these two mechanisms of action (anti-secretory and vasodilating), which do not occur with other antiulcer therapies, would confer a therapeutic superiority to the substituted benzimidazoles used in the treatment of gastric and duodenal ulcers, Zollinger-Ellison syndrome, reflux esophagitis, or in other related diseases.

Our results strongly suggest that omeprazole has a dual mechanism of action: H⁺K⁺-ATPase inhibition and, gastric mucosa CA inhibition and that these enzymes may be functionally coupled. This 2-fold mechanism of action could explain the greater effectiveness of substituted benzimidazoles as compared with other therapies.

References

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