Antisecretory Effect of Leminoprazole on Histamine-Stimulated Gastric Acid Secretion in Dogs: Potent Local Effect

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ABSTRACT—Leminoprazole, an acid pump inhibitor, significantly reduces basal and stimulated gastric acid secretion in rats when administered via the systemic or local route. Our aim here was to characterize the antisecretory effect of leminoprazole on gastric acid secretion in conscious dogs. Gastric acid secretion by dogs with a vagally denervated Heidenhain pouch was stimulated by intravenous histamine infusion. Leminoprazole or omeprazole (as a reference drug) was administered either intravenously or locally into the pouch before or after histamine infusion. A bolus intravenous administration of leminoprazole and omeprazole, respectively, significantly and dose-relatedly inhibited the stimulated gastric acid secretion for >26 hr. Local application of leminoprazole, but not omeprazole, significantly inhibited the acid secretion when applied for 15 to 30 min. The duration of the local antisecretory effect observed after 30 min application was around 8 – 10 hr. The acid-degraded products of leminoprazole had no effect when applied to the pouch. The blood concentration of leminoprazole was very low at 1 hr after local application. These results indicate that leminoprazole suppresses the secretory function of the parietal cells of dogs, via both the intravenous and local routes. It remains unknown whether or not locally applied leminoprazole produced the acid inhibition by inhibiting the acid pump.

Keywords: Leminoprazole, Omeprazole, Acid pump inhibitor, Heidenhain pouch dog, Local effect

As recently reviewed by Hirshowitz et al. (1), the mechanism of action of acid pump inhibitors such as omeprazole or lansoprazole is now clearly delineated. Leminoprazole is a novel acid pump (H+K+-ATPase) inhibitor (2, 3). Its chemical structure has a substituted aniline ring instead of the pyridine ring found in omeprazole and lansoprazole (Fig. 1). As we already reported (2, 4, 5), the drug inhibited both basal and secretagogue-stimulated gastric secretion in rats. Therefore, the present study was carried out to determine whether or not intravenous (i.v.) administration of leminoprazole also exerts an antisecretory effect in conscious dogs.

Konturek et al. (6) reported that omeprazole topically applied to the denervated pouch of dogs significantly inhibited the gastric acid response to histamine, suggesting local antisecretory activity. Therefore, we also examined the possible local effect of leminoprazole on gastric acid secretion by applying it to the mucosa of the denervated pouch. Omeprazole was used as the reference drug.

MATERIALS AND METHODS

Study of gastric secretion

Fourteen beagles (10–15 kg), of both sexes, with a cannulated Heidenhain pouch were used, at least 2 months after the operation. The animals were trained in a Pavlov stand. The interval between experiments was at least 5 days. Food was withheld for 18 hr before each experiment, but water was given ad libitum. The pouch of each animal was then washed out with 15 ml of warm saline several times until the washings became clear. Gastric juice samples were then collected every 15 min by gravity drainage throughout the experiments. After collecting the gastric acid secretion (basal secretion) in the initial 30 min, histamine·2HCl (Nacalai Tesque, Kyoto) was continuously infused at the dose of 160 µg/kg/hr (as the base) in the volume of 10 ml/hr via a catheter inserted in a leg vein for 2 hr. The dose of histamine used gave nearly maximal acid secretion in our dogs. Leminoprazole or omeprazole was administered by rapid i.v. injection (about 60 sec) into the leg vein 1 hr after histamine infusion. In the case of local application, the drugs were...
applied to the mucosa of the denervated pouch for either 5, 15 or 30 min at 1 hr after or for 30 min immediately before starting histamine infusion. Control experiments were also carried out, the same volume of the vehicle alone being applied instead of a drug. During the application of drugs, histamine infusion was continued at the same rate. After a certain time, the test drug was removed, and the pouch was washed out with saline three times. Thereafter, gastric juice samples were continuously collected every 15 min and analyzed as to volume and acid output. In some experiments, the duration of the antisecretory effect of leminoprazole administered i.v. (6 mg/kg, submaximal inhibitory dose) and locally (160 mg/pouch) was determined for up to 50 and 10 hr, respectively. In these experiments, the animals were given standard dog food for 1 hr after each experiment was finished and fasted thereafter. Omeprazole (0.2 mg/kg, i.v., submaximal inhibitory dose) was also studied as to the duration of the antisecretory activity. Total acidity was determined by automatic titration of the gastric juice against 0.1 N NaOH to pH 7.0 (Radiometer, Copenhagen, Denmark), and the acid output was expressed as mEq/15 min. Omeprazole is protonated in acidic conditions at pH 5 and below and transformed to its active inhibitor, but it is quickly degraded into inactive compounds at pH 1 (7–9). Leminoprazole is also unstable, as evidenced by the fact that the drug (240 mg), mixed in the acidic juice (pH 1.2) for 20 min at 25°C, was degraded into its sulfide (127 mg), 2-mercaptopobenzimidazol (MBI, 13.7 mg), benzimidazol (BI, 28.2 mg) and 2-(isobutylmethylamino)-benzylalcohol (2-MBIZ, 65.9 mg) (Fig. 1). Accordingly, both leminoprazole and omeprazole were administered together with 1% NaHCO₃ (pH 8.3) in the case of local application. The pH value of the solution recovered from the pouch at 5, 15 and 30 min after instillation was 8.1 ± 0.2, 7.3 ± 0.1 and 2.2 ± 0.01 in the leminoprazole group (160 mg/pouch) vs 8.1 ± 0.3, 7.3 ± 0.1 and 2.1 ± 0.1 (n = 4) in the control group, respectively. Thus, leminoprazole appears to be stable for 15 min at least in the pouch. The possible antisecretory effect of each component of acid-degraded leminoprazole (no inhibitory effect on acid pump; S. Okabe et al., unpublished data) and acidified leminoprazole (obtained by mixing with 0.1 N HCl for 1 hr) was determined after local application. Control animals received the vehicle alone.

**Determination of the serum concentration of leminoprazole**

To determine whether or not leminoprazole applied to the pouch before or after histamine infusion was absorbed, the concentration of the unchanged drug in the serum of dogs with a Heidenhain pouch was determined. Leminoprazole (160 mg/pouch) was locally applied to the pouch for 30 min immediately before or 1 hr after histamine infusion. In the case of local application before histamine infusion, blood samples were taken immediately before, and then immediately, 30 and 60 min after application of the drug. In the case of local application after

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**Fig. 1.** Chemical structures of leminoprazole and its acid-degraded products.
histamine infusion, blood samples were taken immediately, 30, 60 and 90 min after drug application. In addition, the serum concentration of leminoprazole was determined 15, 30, 60 and 120 min after i.v. administration of it (1 mg/kg) to dogs with a Heidenhain pouch. This time, histamine infusion was not performed. Blood samples were centrifuged at 3,000 rpm for 15 min, and the serum was kept at -20°C until analysis. The concentration of leminoprazole was determined by HPLC (Hitachi, Ibaragi) at the Department of Chemistry, Nippon Chemipharm (Misato).

Drugs

Leminoprazole and omeprazole (provided by Nippon Chemipharm, Tokyo) were suspended in 0.5% carboxymethylcellulose (CMC, Nacalai Tesque) for local administration or dissolved in dimethyl sulfoxide (Wako, Osaka) for i.v. administration, respectively. Each drug was prepared immediately before administration. The volumes for i.v. and local application to the pouch were 2 ml/dog and 15 ml/pouch, respectively.

Data analyses

The results are presented as means±S.E. Student's t-test or Dunnett's multicomparison test was used to determine the statistical significance of the data at the level of P<0.05. ED50 values (doses that inhibit acid secretion by 50%) and 95% confidence limits were calculated 1 hr after drug treatment by the Litchfield-Wilcoxon method.

RESULTS

Effect of intravenously administered leminoprazole or omeprazole on gastric acid secretion

Continuous i.v. infusion of histamine at 160 μg/kg/hr caused nearly maximal stimulation of gastric acid secretion, and the plateau level was maintained for 2 hr. Leminoprazole administered i.v. significantly inhibited the acid output in response to histamine in a dose-related manner (Fig. 2). Inhibition of the volume output was correlated to the inhibition of acid output (data not shown). With 10 mg/kg, the inhibitory effect was immediate and most pronounced in the first hour, and the acid output was 0.09±0.02 mEq/15 min (n=5) vs...
Fig. 3. Duration of the antisecretory effect of i.v. leminoprazole (6 mg/kg) on histamine-stimulated gastric acid secretion in Heidenhain pouch dogs. Gastric acid secretion was apparently inhibited for >26 hr. ○ Control, ● 6 mg/kg. Data are means ± S.E. for 4 experiments. *Significantly different from the corresponding control groups, at P < 0.05.

Fig. 4. Effect of omeprazole administered i.v. or locally to the pouch on gastric acid secretion stimulated by histamine infusion in Heidenhain pouch dogs. The drug was administered i.v. or locally (for 30 min) 60 min after starting histamine infusion. ○ Control, ● 0.1 mg/kg, ▲ 0.2 mg/kg, ■ 0.3 mg/kg, △ 160 mg/pouch. Data are means ± S.E. for 4–5 experiments. *Significantly different from the corresponding control groups, at P < 0.05.
Fig. 5. Duration of the antisecretory effect of i.v. omeprazole (0.2 mg/kg) on histamine-stimulated gastric acid secretion in Heidenhain pouch dogs. Gastric acid secretion was apparently inhibited for >26 hr. ○ Control, ● 0.2 mg/kg. Data are means±1 S.E. for 4 experiments. *Significantly different from the corresponding control groups, at P<0.05.

Local effect of leminoprazole or omeprazole on gastric acid secretion

The local application of 0.5% CMC alone for 30 min had no or only little effect on the volume output in response to histamine infusion. The acid output was transiently reduced for 15 min after removal of the solution but returned to the stimulated level thereafter. When leminoprazole was applied to the pouch for 30 min 1 hr after histamine infusion, it caused significant and persistent inhibition of the acid output in a dose-related manner (Fig. 6). The rates of inhibition caused by 40, 80 and 160 mg/pouch 1 hr after application were 25.0%, 56.1% and 79.8%, respectively. The ED$_{50}$ value was 139.0 mg/pouch. The volume output was also inhibited in parallel with inhibition of the acid output. However, leminoprazole (160 mg/pouch) only slightly inhibited the acid secretion when it was applied immediately before histamine infusion (Fig. 6). Of note was that even 15 min application of the drug at 1 hr after starting histamine infusion also significantly inhibited the acid secretion (Fig. 7). Even after 5 min application, there was a tendency for acid inhibition. The duration of the local antisecretory effect of leminoprazole (160 mg/pouch, 30 min) was >7 hr, but its effect had diminished when examined 9–12 hr later (Fig. 8).

Local application of omeprazole at 160 mg/pouch for 30 min 1 hr after histamine infusion had no effect on the gastric acid secretion, except for the transient inhibition observed 15 min after the removal of the drug (Fig. 4). Similarly, locally applied omeprazole (160 mg/pouch, 30 min) immediately before histamine infusion had no effect on the acid secretion (data not shown).

Local effect of degraded products of leminoprazole on gastric acid secretion

Leminoprazole (240 mg/pouch) locally applied for 30 min 1 hr after histamine infusion markedly inhibited the acid secretion (Fig. 9). However, leminoprazole (240 mg/pouch), acidified immediately before local
Fig. 6. Effect of leminoprazole administered locally to the pouch on histamine-stimulated gastric acid secretion in Heidenhain pouch dogs. The drug was applied for 30 min at 60 min after or immediately before starting histamine infusion. Note that the acid output was markedly reduced by the drug (160 mg/pouch) only when applied after histamine infusion. ○ Control, ● 40 mg/pouch, ▲ 80 mg/pouch, ■ 160 mg/pouch. Data are means ± 1 S.E. for 6 or 4 experiments. *Significantly different from the corresponding control groups, at P < 0.05.

Fig. 7. Influence of the time of application of leminoprazole to the pouch on histamine-stimulated gastric acid secretion in Heidenhain pouch dogs. The drug was administered to the pouch for 5, 15 or 30 min at 60 min after starting histamine infusion. Note that even 15 min application of the drug significantly inhibited the acid secretion. ○ Control, ● 160 mg/pouch. Data are means ± 1 S.E. for 4 experiments. *Significantly different from the corresponding control groups, at P < 0.05.
Fig. 8. Duration of the antisecretory effect of locally applied leminoprazole (160 mg/pouch) on histamine-stimulated gastric acid secretion in Heidenhain pouch dogs. The drug was applied for 30 min at 60 min after starting histamine infusion. The antisecretory effect persisted for around 10 hr. ○ Control, ● 160 mg/pouch. Data are means ± 1 S.E. for 5 experiments. *Significantly different from the corresponding control groups, at P<0.05.

Fig. 9. Effect of acidified leminoprazole or degraded products of leminoprazole on histamine-stimulated gastric acid secretion in vagally denervated Heidenhain pouch dogs. The compounds were locally applied to the pouch for 30 min at 1 hr after starting histamine infusion. A: ○ Control, ● leminoprazole (240 mg/pouch), ▲ acidified leminoprazole (240 mg/pouch). B: ○ Control, ● 2-MBlZ (65.9 mg/pouch), ▲ sulfide (127.0 mg/pouch). C: ○ Control, ● MBl (13.7 mg/pouch), ▲ BI (28.2 mg/pouch). None of the compounds had an inhibitory effect on acid secretion. Data are means ± 1 S.E. for 5 to 6 experiments. *Significantly different from the corresponding control group, at P<0.05.
application for 30 min, had no effect on the gastric acid secretion. In addition, neither leminoprazole sulfide (127 mg/pouch), 2-MBIZ (65.9 mg/pouch), BI (28.2 mg/pouch) nor MBI (13.7 mg/pouch) applied for 30 min had any effect on the histamine-stimulated gastric acid secretion.

**Serum concentration of leminoprazole**

Leminoprazole (160 mg/pouch) was administered into the pouch for 30 min immediately before or 60 min after starting the histamine infusion. Its serum concentration determined immediately, 30, 60 or 90 min after removal of the drug from the pouch was low (about 3 - 6 ng/ml) (Fig. 10). In contrast, the concentrations of leminoprazole (1 mg/kg) after i.v. administration were 541.0±60.5, 280.6±27.4, 144.4±16.7 and 64.6±9.1 ng/ml at 15, 30, 60 and 120 min later, respectively.

**DISCUSSION**

These results clearly indicate that leminoprazole dose-relatedly and persistently inhibited the gastric acid secretion when administered i.v. or locally to the gastric mucosa. In contrast, omeprazole extensively inhibited the gastric acid secretion through the i.v. route, but did not inhibit it when it was locally applied. The following is an explanation for the different results.

Both leminoprazole and omeprazole inhibited the histamine-stimulated acid secretion through the i.v. route with nearly the same efficacy. Based upon the ED50 values, the antisecretory effect of leminoprazole was about 50 times less potent compared to that of omeprazole. However, the duration of the antisecretory effect of leminoprazole and omeprazole, respectively, after a bolus i.v. injection was about 26 hr, suggesting covalent binding to the acid pump.

The gastric acid pump is located in the secretory membrane when parietal cells are activated by a secretagogue (10, 11). Therefore, the pump can react with omeprazole-like drugs at a luminaly accessible thiol group. Accordingly, an acid pump inhibitor in an activated form seems to directly inactivate the pump from the luminal surface, thereby inhibiting gastric acid secretion. As a matter of fact, omeprazole continuously infused in graded doses (1.25 to 5.0 μmol/ml) into Heidenhain pouch dogs significantly inhibited the gastric acid secretion in response to histamine infusion (6). Of interest is that such inhibition of gastric acid secretion was observed without absorption of the drug, i.e., the plasma concentration of omeprazole was zero after local application. These results suggest that omeprazole might inhibit the parietal cells without entering the circulation. In contrast, we could not confirm their results despite applying a much higher dose (31.1 μmol/ml) to the pouch. In our experiment in
Locally applied leminoprazole clearly inhibited the histamine-stimulated gastric acid secretion in a dose-related manner. Even after a 15 min application, there was significant inhibition of gastric acid secretion, which persisted for >2 hr. The pH value of the solution recovered, containing leminoprazole or vehicle, was around 7.3 after 15 min instillation. These results suggest that leminoprazole is able to reach the parietal cells under an acid secreting condition, more rapidly than its breakdown. Acid-degraded products, such as leminoprazole sulfide, MBI, BI and 2-MBIZ or acidified leminoprazole, had no effect on acid secretion when administered into the pouch. These findings constitute evidence that leminoprazole, but not its degraded products, applied to the mucosal surface is the crucial substance for acid inhibition. The efficacy of the drug after local application was slightly weaker as observed after i.v. administration; i.e., the inhibition (1 hr after treatment) was approximately 80% vs 90% in the i.v. injection group. However, it was clear that the function of parietal cells can be markedly inhibited even from the mucosal surface. De Graef and Woussen-Colle (12) reported that i.v. administered omeprazole in dogs with a fistula is much more active than that observed after i.v. injection, with which the effect persisted for >26 hr. This phenomenon raises the question of whether or not the mechanism underlying the local action of leminoprazole is primarily related to the inhibition of the acid pump through tight binding to the pump. Alternatively, the shortness of the antisecretory effect of locally applied leminoprazole may be due to a lesser amount of drug reaching the parietal cells, the amount being insufficient to sustain a longer acid inhibition.

It is possible that the local antisecretory effect of leminoprazole is due to the drug absorbed from the pouch. However, the amount of serum leminoprazole after local application for 30 min was negligible. The serum concentration after i.v. administration of leminoprazole (1 mg/kg, non-antisecretory dose) was >500 ng/ml 15 min later. Therefore, the idea that leminoprazole absorbed from the mucosa inhibited the parietal cell function via the basolateral membrane could be ruled out. Certainly, it is possible that locally absorbed leminoprazole, without entering the circulation, inhibited the function of parietal cells directly.

There is a possibility that the undegraded leminoprazole injures the mucosa, resulting in a reduction in acid secretion. However, we found that leminoprazole had no injurious effect on the gastric mucosa when the rate of acid back diffusion through the pouch was determined (unpublished data). Therefore, the possibility that the reduction of acid secretion by locally applied leminoprazole was due to an injured mucosa was ruled out. If leminoprazole is injurious to the gastric mucosa, then it should inhibit the acid secretion even when administered before histamine infusion.

Recently, we found that leminoprazole could inhibit the maximal acid secretion response to carbachol and pentagastrin (unpublished data).

We conclude that leminoprazole suppresses the secretory function of the parietal cells of dogs, via both the intravenous and local routes. It remains unknown whether or not the antisecretory effect of leminoprazole administered either intravenously or locally involves a common mechanism, i.e., the inhibition of the acid pump.

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