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The effects of oral administration of clarithromycin (CLR), amoxicillin (AMX), and lansoprazole (LPZ) on gastric emptying in rats were investigated by a glass powder method and a phenol red method. By both test methods, no significant effects on gastric emptying were observed when CLR, AMX, or LPZ was administered alone or when the three drugs were administered concomitantly. The levels of gastrointestinal absorption of [14C]CLR and [14C]AMX were measured. Four hours after injection of [14C]CLR or [14C]AMX into the stomach and duodenum loops of rats, 86.63 and 1.27% of the original amount of [14C]CLR administered were recovered in the contents of the stomach and duodenum loops, respectively, and 80.01 and 55.88% of the original amount of [14C]AMX administered were recovered in the contents of the stomach and duodenum loops, respectively.

Triple therapy, consisting of clarithromycin (CLR), amoxicillin (AMX), and lansoprazole (LPZ), is widely used as eradication therapy for patients with peptic ulcer disease and Helicobacter pylori infection (9). H. pylori mainly colonizes both the apical surface of the mucosal layer and the mucous gel layer (8). A previous paper demonstrated that the levels of [14C]CLR in gastric tissue after oral administration to rats were enhanced by coadministration of LPZ (1, 2). It was considered that the levels of penetration of [14C]CLR and [14C]AMX into gastric tissue increased depending on the gastric pH elevation caused by LPZ. In addition, the level of radioactivity recovered in gastric contents after oral administration of [14C]CLR was increased with the coadministration of LPZ (1).

To clarify the mechanisms involved in the recovery of increased amounts of [14C]CLR in gastric contents, we investigated the effects of CLR, AMX, and LPZ on gastric emptying in rats. We also evaluated the gastrointestinal absorption of CLR and AMX to determine if penetration of the drugs into gastric tissue participates in absorption.

[6-O-methyl-[14C]CLR and [carbonyl-[14C]AMX were obtained from Nemoto & Co., Ltd. (Tokyo, Japan), and Daiichi Pure Chemicals Co., Ltd. (Tokyo, Japan), respectively. The specific activities were 2.72 and 5.59 MBq/mg, respectively. The radiochemical purities of both compounds were 97% or higher, based on high-pressure liquid chromatography analysis. Unlabeled CLR, LPZ, AMX, and commercially available reagents were used as described previously (1).

Seven-week-old male Wistar rats purchased from Nihon SLC Co., Ltd. (Shizuoka, Japan), were acclimatized and used in the study (1). Rats were given 5 mg of CLR, 10 mg of AMX, and 10 mg of LPZ per kg of body weight.

In the study on gastric emptying, CLR, AMX, and LPZ were suspended in 5% gum arabic adjusted to pH 7.0 with 1 M KOH. Five hundred milligrams of glass powder (particle diameter, 0.200 to 0.295 mm; Nihon Rikagaku Kikai, Tokyo, Japan) was suspended in 1 ml of 0.4% sodium carboxymethyl cellulose. Phenol red was dissolved in purified water at a concentration of 0.05%. The rats were randomly distributed into five groups, with each group consisting of four to nine rats: the vehicle-only group, the CLR group, the AMX group, the LPZ group, and the coadministration (CLR, AMX, and LPZ) group. From days 1 to 3 the rats received LPZ or vehicle (5% gum arabic adjusted to pH 7.0 with 1 M KOH) orally once daily under conditions of no fasting. On day 4, after the rats had fasted for 16 h, CLR, AMX, or vehicle was administered to rats that had been given the vehicle from days 1 to 3 and LPZ alone or CLR, AMX, and LPZ together were administered to rats that had been given LPZ for 3 days. One hour after administration 1 ml of glass powder suspension or phenol red solution was administered orally. In the case of administration of the glass powder suspension, gastric emptying was determined as described by Ito et al. (6) After oral administration of phenol red solution, gastric emptying was measured by a modification of the method of Shoji et al. (11) Thirty minutes after phenol red administration, each rat was killed and the stomach was removed. Rats killed immediately after the administration of phenol red served as controls. The stomach was cut into small pieces and rinsed in 40 ml of 0.1 M Na2HPO4. The rinsed solution was centrifuged (1,600 × g) for 10 min, and the supernatant was diluted several times with 0.1 M Na2HPO4. Each diluted supernatant was measured at a wavelength of 560 nm with a spectrophotometer (DU 650; Beckman Instruments Ltd., Fullerton, Calif.). Gastric emptying (in percent) was calculated as follows: 100 − ([A/B] × 10), where A is the amount of phenol red recovered from the test stomach and B is the average amount of phenol red recovered from the control stomach.

When gastrointestinal absorption was studied, [14C]CLR was dissolved in diluted hydrochloric acid. [14C]AMX was dis-
solved in 2.33% KH₂PO₄–1.44% NaHCO₃ isotonic buffer (pH 7.4). The rats were distributed randomly into four groups. Each group included two to four rats. Fasting rats were anesthetized with diethyl ether, and isolation ligatures were applied to the stomach (whole) and duodenum (approximately 1.5 cm). [¹⁴C]CLR or [¹⁴C]AMX was injected into each isolated loop. At 0.5, 1, 2, 3, and 4 h after injection, a blood sample (20 μl) was taken from the tail vein and radioassayed. At 4 h after injection, each loop was excised and the contents were washed out with saline. The levels of radioactivity in part of the loop contents (500 mg) and the washed tissue (100 mg) were measured. The radioactivity of each biological sample was measured in a liquid scintillation counter, as described previously (1).

Results are expressed as means and standard deviations (SDs). The detection limit for radioactivity was twice that of the background. The significance of differences was evaluated by Tukey’s analysis with the SAS/STAT package. A significance level of 0.01 was used for all tests.

FIG. 1. Effects of CLR (5 mg/kg), AMX (10 mg/kg), and LPZ (10 mg/kg) on gastric emptying in rats. (A) Glass powder method; (B) phenol red method. Each value represents the mean ± SD for nine (a), eight (b), five (c), four (d), and six (e) animals.

The effects of CLR, AMX, and LPZ on gastric emptying in rats are shown in Fig. 1. By both the glass powder method and the phenol red method, the effects of the drugs on gastric emptying were sparse when CLR, AMX, or LPZ was administered alone or when they were coadministered. Proton pump inhibitors such as omeprazole (OPZ) (10) and LPZ (7) had no apparent effect on gastric emptying of the liquid meals. In the present study, the results obtained with LPZ were similar to those obtained by previous investigators (7). The gastric emptying of radioactivity after oral administration of [¹⁴C]CLR to rats decreased when LPZ was coadministered (1). We found no significant change in the gastric emptying when CLR was given alone and when it was coadministered with AMX and LPZ. It has been reported that when OPZ is administered to healthy volunteers the viscosity of the gastric mucus decreases because of an increased intragastric pH (4). In addition, a lesser degree of viscosity of the gastric mucus facilitates the penetration of antibiotic (5). Therefore, it was suggested that the amount of [¹⁴C]CLR excreted from the rat stomach de-

FIG. 2. Level of radioactivity in blood after injection of [¹⁴C]CLR (5 mg/kg) (A) or [¹⁴C]AMX (10 mg/kg) (B) into loops of the stomach (△) and duodenum (●) in rats. Each point represents the mean ± SD for four animals (A) or the mean ± range for two animals (B).
was high after injection of [14C]AMX into the duodenum loop, up to 4 h. On the other hand, the level of radioactivity in blood was lower than those in the stomach and duodenum loops was investigated (3). It has been reported that orally administered [14C]CLR or [14C]AMX in the stomach and duodenum loops was investigated (3). It has been reported that orally administered [14C]CLR and [14C]AMX were distributed in gastric tissue at high levels when they were coadministered with LPZ (1, 2), and this synergism was observed only in the target site and not in plasma or the main organs. The absorption of [14C]CLR and [14C]AMX in the stomach and duodenum loops was investigated in the present study. The levels of radioactivity in blood after injection of [14C]CLR or [14C]AMX into the stomach and duodenum loops are shown in Fig. 2. After injection of [14C]CLR into the duodenum loop, the level of radioactivity in blood rapidly increased and was maintained at a high level for 4 h. In the case of injection of [14C] CLR into the stomach loop, the levels of radioactivity in blood were lower than those in the duodenum loop, although they were continuously detected for up to 4 h. On the other hand, the level of radioactivity in blood was high after injection of [14C]AMX into the duodenum loop, but radioactivity was not detected at any time point when [14C]AMX was injected into the stomach loop.

The remaining radioactivity noted 4 h after injection of [14C]CLR or [14C]AMX into gastrointestinal loops is given in Table 1. After injection of [14C]CLR into the stomach loop, much of the radioactivity administered was recovered in the stomach contents. In the case of the duodenum loop, only 1.36% of the radioactivity administered was recovered in tissue and duodenum contents. Similarly, a large amount of the radioactivity remained in the stomach contents after injection of [14C]AMX into the stomach loop. The amount of radioactivity recovered in the contents of the duodenum loop was less than the amount recovered in the contents of the stomach loop, and approximately half of the injected [14C]AMX was recovered. These results suggest that both [14C]CLR and [14C]AMX are absorbed in the duodenum and are passed into the blood circulation, yet the level of gastric absorption which contributed to the levels of radioactivity in whole blood was low. We propose that both [14C]CLR and [14C]AMX penetrate gastric tissues and, hence, that their use would enhance the eradication of H. pylori.

**REFERENCES**


**TABLE 1. Gastrointestinal radioactivity after injection of [14C]CLR (5 mg/kg) or [14C]AMX (10 mg/kg) into loops of the stomach and duodenum in rats**

<table>
<thead>
<tr>
<th>Site</th>
<th>Contents</th>
<th>Tissue</th>
<th>Contents</th>
<th>Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>86.63 ± 21.36</td>
<td>0.85 ± 0.36</td>
<td>80.01 ± 5.73</td>
<td>1.36 ± 0.83</td>
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<tr>
<td>Duodenum</td>
<td>1.27 ± 0.42</td>
<td>0.09 ± 0.02</td>
<td>55.88 ± 12.93</td>
<td>0.62 ± 0.22</td>
</tr>
</tbody>
</table>

- Mean ± SD for four animals.
- Mean ± SD for three animals.
- Mean with range for two animals.