

Gastric emptying after artificial ulceration in rats: differences due to the site of the ulcer and the effects of prokinetic drugs

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Abstract

Background This study aimed to evaluate the effects of the position of an acetic acid-induced gastric ulcer and the effects of prokinetic drugs on gastric emptying. **Materials and Methods** Male Sprague-Dawley rats were used in this study. Acetic acid ulcers were induced either in the region between the fundus and pylorus on the anterior wall of the stomach or in the glandular region on the greater curvature of the stomach to determine whether there were regional differences in the effect of the ulcers. Gastric emptying was evaluated with a breath test using [1-¹³C] acetic acid. In addition, the effects of the prokinetic drugs, metoclopramide and mosapride, on gastric emptying were also evaluated. **Results** Acetic acid induced ulcers in the region between the fundus and pylorus on the anterior wall of the stomach significantly delayed gastric emptying as compared with control rats, but not the acetic acid induced ulcers in the glandular region on the greater curvature of the stomach. Metoclopramide and mosapride did not improve the delayed gastric emptying even at doses that enhanced gastric emptying in normal rats. **Conclusion** These findings show that gastric emptying is influenced by the position of the ulcer and the region between the fundus and pylorus on the anterior wall plays an important role in gastric emptying. Moreover, it was found that metoclopramide and mosapride do not improve the delayed gastric emptying caused by acetic acid ulcers induced on the anterior wall in the region between the fundus and pylorus.

Key words: Gastric emptying, ¹³C-Breath test in rats, gastric ulcer, metoclopramide, mosapride

Introduction

Many diseases are stressful for patients and induce sleep disturbance. Stress is known to release corticotropin-releasing factor (CRF) from the nucleus paraventricularis. We have previously reported that urocortin,

a CRF agonist, induced sleep disturbance by acting on CRF receptors, because astressin, a CRF antagonist, significantly attenuated the urocortin-induced sleep disturbance (1). Million et al. (2) found that human recombinant CRF and rat urocortin delayed gastric emptying in conscious rats. In preliminary experiments, we have also found that urocortin significantly delayed gastric emptying and that this delay was significantly attenuated by pre-treatment with astressin (data not shown). These findings show that stress by disease induces the delay of gastric emptying. In other words, diseases may have an effect on gastric emptying as a result of CRF released by stress.

On the other hand, Takahashi et al. (3) found that oral administration of the H₂ receptor antagonists, ranitidine and famotidine, had no significant effect on gastric emptying, while rabeprazole delayed gastric emptying. Anjiki et al. (4) also reported that rabeprazole has dual effects on the emptying of solids: an initial acceleration with a subsequent deceleration, resulting in a delay in gastric emptying. These findings show that drug therapy can influence gastric emptying.

A correlation between ulcer and gastric emptying has been reported by Maconi et al. (5), who showed a relationship among acid secretion, antral distension, and liquid gastric emptying in active and healed duodenal ulcers. However, Higuchi et al. (6) found that endoscopic submucosal dissection preserved gastric emptying in patients with early gastric cancer. Ulcers have been defined histologically as a lesion that penetrates the muscularis mucosae and the muscle layer at the base of the mucosa. Therefore, it is easy to speculate that gastric ulcers may have an influence on gastric emptying.

The present study will evaluate the effects of acetic acid-induced gastric ulcers on gastric emptying evaluated by the ¹³C-breath test. In addition, we investigated the effects on gastric emptying of gastric ulcers located in different regions and the variation in gastric emptying that occurred with administration of prokinetic drugs.

Materials and Methods

The following animal studies were performed in accordance with the *Guiding Principles for the Care and Use of Laboratory Animals* approved by Meiji Co., Ltd.

Animals

Male Sprague-Dawley rats (200–250 g) were purchased from SLC (Shizuoka, Japan). The light and dark cycle was 12 h, and light period was from 7:00 to 19:00. The animals were fasted in mesh cages for 18 h before each experiment in order to prevent coprophagy in the breath test, but were allowed free access to drinking water during this period.

Gastric ulcers induced by acetic acid

Gastric ulcers were induced with acetic acid according to the method reported by Okabe et al. (7). In brief, the abdomen was incised and the stomach exposed under anesthesia with an intraperitoneal administration of sodium pentobarbital at a dose of 35 mg/kg. Acetic acid solution (glacial acetic acid; 100 µl) was applied into a cylindrical tube (ID: 6mm) attached on the serosa for 30 sec. The acetic acid was aspirated and the lesion washed with saline, after which the lesion was wiped softly and repeatedly with saline-soaked cotton-wool. Acetic acid ulcers were induced in the region between the fundus and pylorus on the anterior wall of the stomach (antral ulcer) or in the glandular region on the greater curvature of the stomach (corpus ulcer) as shown in Fig. 1. Sham operated rats were used as control.

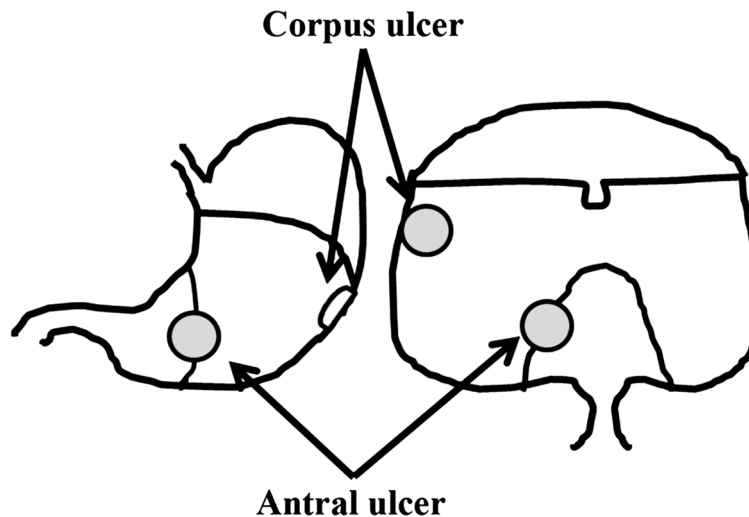


Fig. 1. Schematic representation of the ulcerated regions between the fundus and pylorus on the anterior wall (antral ulcer) or in the glandular region on the greater curvature of the rat stomach (corpus ulcer).

Breath-test system

Breath testing was performed using the method reported by the present author (8). In brief, rats were orally administered a test meal composed of Racol (liquid enteral nutrition formula), containing [$1\text{-}^{13}\text{C}$] acetic acid at a dosage of 16 mg/kg (2.5 ml/kg). The rats were placed in the chamber immediately after the oral administration of the test meal. Aspiration volume was set at 150 ml/min. The expired air was collected at 5-min intervals for 70 min after the test meal administration, with additional measurements at 90 and 120 min. At each sampling point, the expired air was collected into a breath-sampling bag for 1.5 min.

The $^{13}\text{CO}_2$ levels in the expired air were measured by placing the breath-sampling bags into the sample joint of the POCone infrared analyzer (Otsuka Pharmaceutical Co., Ltd., Tokyo). The measured values were presented as $\Delta^{13}\text{CO}_2$ (%).

After breath testing, the presence of a gastric ulcer was confirmed by sacrificing rats under anesthesia.

Pharmacokinetic parameters of the breath test

$\Delta^{13}\text{CO}_2$ values were plotted against the time after the test meal administration as shown in Fig. 2. The maximum concentration (C_{max} ; %), the time taken to reach the maximum concentration (T_{max} ; min) and the area under the curve ($\text{AUC}_{120\text{ min}}$; % \cdot min) were calculated using measured $\Delta^{13}\text{CO}_2$ values.

Effects of metoclopramide and mosapride on gastric emptying in antral ulcer-induced rats

Metoclopramide and mosapride were suspended in distilled water for injection and administered orally at a dosage of 3 mg/kg in a volume of 5 ml/kg, which is the dosage significantly enhancing gastric emptying in normal rats as reported previously (8, 9). Breath testing was performed 30 min after metoclopramide or mosapride administration. In control rats, distilled water was administered instead of metoclopramide and mosapride.

Data analysis

All results are presented as the mean \pm standard error of the mean (S.E.M.). Statistical analysis was performed using the Student's *t*-test or Dunnett's multi comparison test, with $P < 0.05$ being considered to be statistically significant.

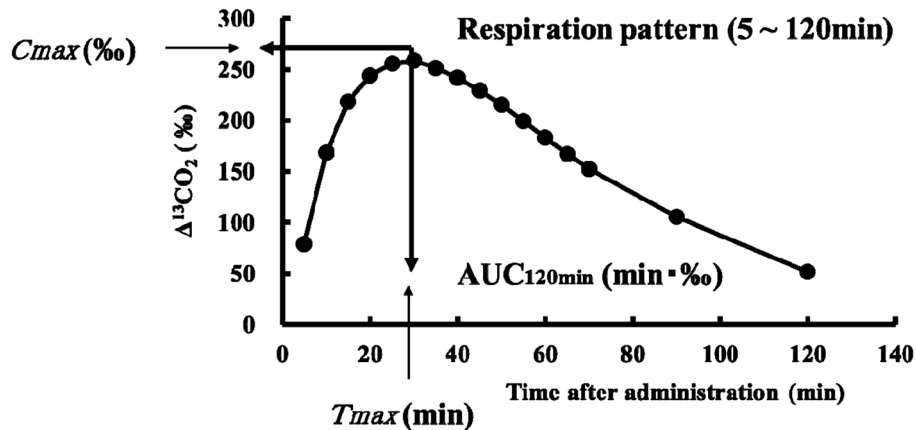


Fig. 2. Schematic representation of the criteria of the breathe test. $\Delta^{13}\text{CO}_2$ values were plotted against the time after test meal administration. The maximum concentration (C_{max} ; ‰), the time taken to reach the maximum concentration (T_{max} ; min) and the area under the curve ($\text{AUC}_{120\text{min}}$; ‰·min) were calculated using measured $\Delta^{13}\text{CO}_2$ values.

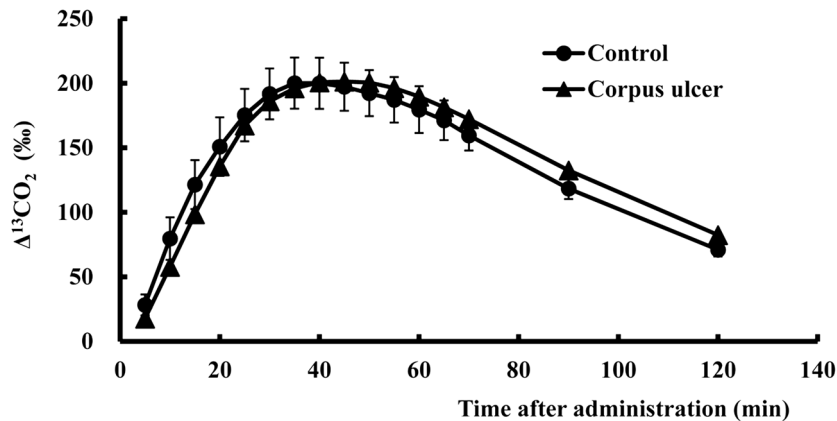


Fig. 3. The change of expired $^{13}\text{CO}_2$ in rats with (▲) and without (●) the corpus ulcers. Values represent the mean \pm standard error of the mean ($n = 4$ or 5). No significant differences were observed between two groups.

Agents

Both [1- ^{13}C]Acetic acid and glacial acetic acid, were purchased from Wako Pure Chemical (Tokyo, Japan) and Racol from Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan). Sodium pentobarbital (Somnopentil) was obtained from Kyoritsuseiyaku Corporation (Tokyo). Distilled water for injection was purchased from Otsuka Pharmaceutical Factory, Inc. (Tokushima, Japan).

Results

Effect of acetic acid-induced corpus ulcer

The changes in the levels of expired $^{13}\text{CO}_2$ are shown in Fig. 3. In control rats, expired $^{13}\text{CO}_2$ increased with time and peaked at about 40 min and decreased thereafter. In the ulcerated rats, no significant differences were observed as compared with the controls. There were no significant differences between the pharmacokinetic parameters (Table 1).

Table 1. Pharmacokinetic parameters of the changes in the levels of expired $^{13}\text{CO}_2$ in rats with and without the corpus ulcers

	C_{max} (‰)	T_{max} (min)	$AUC_{120\text{ min}}$ (‰ · min)
Control	202.4 ± 24.1	38.8 ± 3.2	16,396 ± 1,613
Corpus ulcer	206.8 ± 12.2	45.0 ± 3.7	16,848 ± 785

Values represent the mean ± standard error of the mean ($n = 4$ or 5). No significant differences were observed as compared with control group.

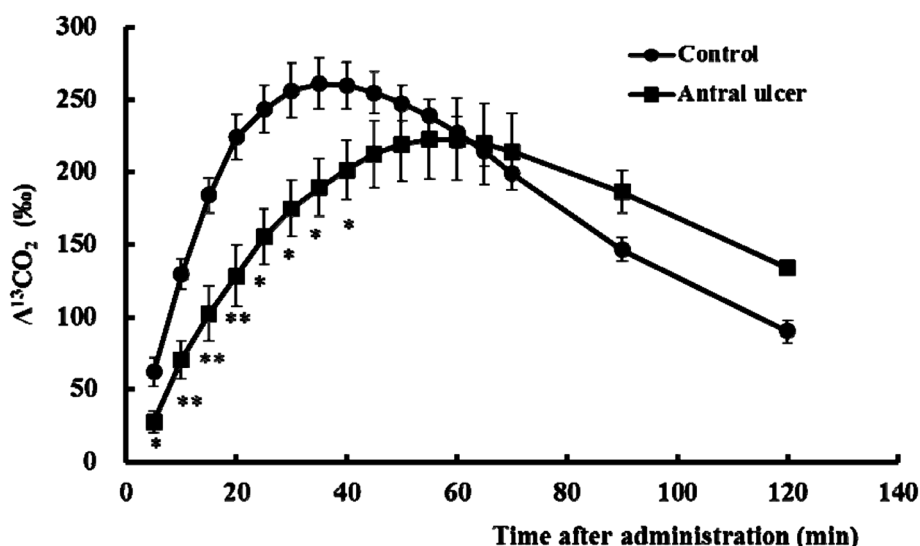


Fig. 4. The change of expired $^{13}\text{CO}_2$ in rats with (■) and without (●) the antral ulcers. Values represent the mean ± standard error of the mean ($n = 4$). *, **: Significant difference from the control ($P < 0.05$, 0.01).

Table 2. Pharmacokinetic parameters of the changes in the levels of expired $^{13}\text{CO}_2$ in rats with and without the antral ulcers

	C_{max} (‰)	T_{max} (min)	$AUC_{120\text{ min}}$ (‰ · min)
Control	264.9 ± 15.5	33.8 ± 3.1	21,188 ± 759
Antral ulcer	201.2 ± 31.6	57.5 ± 4.3**	19,751 ± 1,474

Values represent the mean ± standard error of the mean ($n = 4$). **: Significant difference from the control group ($P < 0.01$)

Effect of acetic acid-induced antral ulcer

The changes in the levels of expired $^{13}\text{CO}_2$ are shown in Fig. 4. In control rats, expired $^{13}\text{CO}_2$ increased with time, peaked at about 30 min and decreased thereafter. In the ulcerated rats, the change of expired $^{13}\text{CO}_2$ was shifted to the right, with significant differences observed as compared with the controls.

A comparison of the pharmacokinetic parameters showed a significant difference for the T_{max} value as compared with that of controls, but not for either of C_{max} or $AUC_{120\text{ min}}$ (Table 2).

Effects of metoclopramide and mosapride on the gastric emptying in rats with antral ulcer

The changes in the levels of expired $^{13}\text{CO}_2$ are shown in Fig. 5. In the ulcerated rats (Control), the levels of expired $^{13}\text{CO}_2$ increased with time, peaked at about 40 min and decreased thereafter. In the rats administered

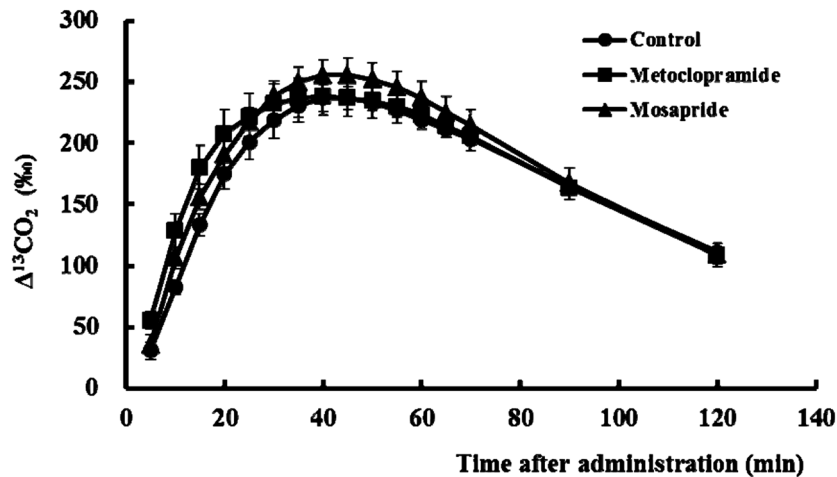


Fig. 5. The effects of metoclopramide (■) or mosapride (▲) on the change of expired $^{13}\text{CO}_2$ in rats having antral ulcers. Values represent the mean \pm standard error of the mean ($n = 4$). No significant differences were observed as compared with the control (●).

Table 3. Effects of metoclopramide or mosapride on the pharmacokinetic parameters of the changes in the levels of expired $^{13}\text{CO}_2$ in rats having antral ulcers

	C_{max} (‰)	T_{max} (min)	$AUC_{120 \text{ min}}$ (‰ · min)
Control	237.8 ± 25.8	42.5 ± 2.5	$19,394 \pm 877$
Metoclopramide	239.4 ± 12.5	41.2 ± 2.4	$21,475 \pm 1,213$
Mosapride	256.2 ± 25.8	42.5 ± 1.4	$21,818 \pm 1,629$

Values represent the mean \pm standard error of the mean ($n = 4$). No significant differences were observed as compared with the control.

with metoclopramide and mosapride, the levels of expired $^{13}\text{CO}_2$ increased with time, peaked at about 40 min and decreased thereafter, with no significant difference observed when compared with the controls.

No significant differences were observed between the pharmacokinetic parameters when compared with the gastric ulcer group (Table 3).

Discussion

In the present study, we evaluated the influences of acetic acid-induced gastric ulcers on gastric emptying as evaluated by $^{13}\text{CO}_2$ breath testing and found that the presence of a gastric ulcer delays gastric emptying in rats. Moreover, it became obvious that the position of the induced gastric ulcer influences gastric emptying.

With regard to the relationship between gastrointestinal disease and gastric emptying, Kristinsson et al. (10) stated that clinicians should consider impaired gastric emptying when evaluating patients with Crohn's disease and severe symptoms of upper gut dysmotility, which cannot be attributed to active inflammation or organic obstruction of the digestive tract. Higher disease activity in inflammatory bowel disease was associated with prolonged gastric emptying and increased release of a glucagon-like peptide-1 (11). The gastric emptying of water by active duodenal ulcer patients showed a delay (12). Bar-Natan et al. (13) reported that a delay in gastric emptying was observed in a considerable number of patients after gastric surgery, but gastric motility returned in 3 to 6 weeks in most patients. In the present study, a delay in gastric emptying was observed as

a result of acetic acid-induced antral ulcer. However, no delay of gastric emptying was observed as a result of acetic acid-induced corpus ulcer. These findings would suggest that laparotomy, at least, should not have an influence on gastric emptying and that there are differences in the regions that influence gastric emptying. Kanaizumi et al. (14) reported that proximal gastric ulcers enhanced, while distal gastric ulcers delayed, gastric emptying in patients with gastric ulcers, suggesting that the location of ulcer sites affects gastric emptying differently. This finding supports our present data. On the contrary, there was no change in the rate of gastric emptying reported after endoscopic submucosal dissection in early gastric cancer patients (6). This observation may be explained by differing effects on the muscle layers, which plays an important role in moving the digested food to the duodenum. While both the external muscle layers and the muscularis mucosa are kept intact in the endoscopic submucosal dissection, they would have been destroyed in the present experiments because the glacial acetic acid affected the mucosa through both the external muscle layers and the muscularis mucosa from the point of application on the serosa.

Bar-Natan et al. (13) reported that the delay in gastric emptying resulting from gastric surgery reversed in 3 to 6 weeks. Kanaizumi et al. (14) also found that the rate of gastric emptying as ulcers healed was closer to that in healthy subjects. The present study was performed on Day 3 following ulcer induction, in the active stage of the ulcer. Therefore, it would be of interest to perform the ^{13}C -breath test for the evaluation of gastric emptying during the healing stage.

The antral region has been known to play an important role in the migration of the digested food to the duodenum. As mentioned above, the smooth muscle layers would have been destroyed in the present experiments. Therefore, the damage to the smooth muscle of the antral region might induce the delay in the gastric emptying. However, the corpus ulcer did not affect the rate of gastric emptying, although the damage to the smooth muscle would be the same as for antral ulcers, suggesting that the corpus region does not play an important role in gastric emptying. In addition, it was found that the operation for inducing gastric ulcers does not correlate to the delay in gastric emptying. We have already reported that the healing process was different according to the regional location of acetic acid induced-ulcers from our sequential endoscopic observations (15). Corpus ulcers healed within 133 days and did not recur or relapse. On the contrary, the antral ulcers diminished in size with the progress of time (10–50 days), and 50% of the ulcerated rats healed with convergences of mucosal folds (35–154 days). On and after the 50th day, some diminished or healed ulcers showed signs of relapse (37% of used rats) or recurrence (40% of healed rats, 21% of used rats), with the cumulative relapse and recurrence percentage (CR%) reaching 59% on the 365th day. This finding shows that antral ulcers are intractable. Therefore, delayed gastric emptying may have an effect on the healing process of ulcers, because the delay of gastric emptying induces stasis of digested chow and enhances acid secretion and a decrease in the pH of the digested chow. Decreased pH may delay the healing of ulcers, because lower pH is unfavorable for ulcer healing. To clarify this point, further study would be needed.

Both metoclopramide and mosapride have been known to enhance the rate of gastric emptying through the activation of dopamine and serotonin receptors, respectively. We have already reported that metoclopramide and mosapride significantly enhance the rate of gastric emptying using ^{13}C -breath testing in rats and mice (8, 9, 16). However, in this study metoclopramide and mosapride did not improve the delayed gastric emptying in rats with antral ulcers induced by acetic acid, although the dosage was effective in enhancing the rate of gastric emptying in normal rats (8, 9). With mosapride, we have previously observed dual effects on gastric emptying with different dosages (9). Higher dosage inhibits gastric emptying probably caused by M1, a metabolite of mosapride, which has the ability to inhibit 5-HT₃ receptors. Therefore, we did not investigate higher dosages in this study. These findings may show that the position of the gastric ulcer is important in

determining the strategy of the therapy.

In conclusion, it was found that gastric emptying was delayed by gastric ulcers induced in the region between the fundus and pylorus on the anterior wall of the stomach (antral ulcer), but not on the glandular region of the greater curvature (corpus ulcer). Metoclopramide and mosapride did not improve the delayed gastric emptying. These findings may be useful in gastric surgery or in the therapy of gastric ulcers.

Conflict of interest

The authors declare that they have no conflict of interest.

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