Full Paper

Simple and Noninvasive Breath Test Using ^{13}C -Acetic Acid to Evaluate Gastric Emptying in Conscious Rats and Its Validation by Metoclopramide

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Abstract. The ^{13}C -breath test has been used to clinically evaluate gastric emptying. However, this method has not been sufficiently validated in experimental animals. The present study aimed to establish a simple and noninvasive ^{13}C -breath-test system in Sprague-Dawley male rats. After fasting, rats were orally administered Racol containing ^{13}C -acetic acid and housed in a desiccator. The expired air in the chamber was collected in a breath-sampling bag using a tube and aspiration pump. The level of $^{13}\text{CO}_2$ in the expired air was measured using an infrared spectrometer at appropriate intervals for 120 min. During this period, the rate of $^{13}\text{CO}_2$ excretion increased, peaked, and decreased thereafter. The maximum concentration ($C_{\max}$) and area under the curve ($\text{AUC}_{120\text{ min}}$) of $^{13}\text{CO}_2$ excretion increased in volume- and dose-dependent manners. The time taken to reach the maximum concentration ($T_{\max}$) of $^{13}\text{CO}_2$ excretion increased as the volume increased, but was not affected by the dose of ^{13}C -labeled acetic acid. Metoclopramide dose-dependently increased the $C_{\max}$ and shortened $T_{\max}$ of $^{13}\text{CO}_2$ excretion compared with those of the control rats, whereas the $\text{AUC}_{120\text{ min}}$ was not affected. These results confirm that this simple method can successfully evaluate gastric emptying. Moreover, this system is suitable for investigating additional physiological functions using other labeled compounds.

Keywords: ^{13}C -acetic acid, breath test in rats, gastric emptying, metoclopramide

Introduction

Existing techniques for testing gastric motility include gastrotonometry via the infused-catheter or micro-transducer methods, radioactive-isotope method, and acetaminophen method (1). Radioscintigraphy is generally accepted as the gold standard for measuring gastric emptying in humans (2). However, all of these methods are invasive and stressful and involve radiation exposure or have drug-related adverse effects.

Recently, ^{13}C -breath tests have been developed as a nonradioactive alternative. The ^{13}C -labeled urea breath test, in particular, is widely employed clinically to monitor *Helicobacter pylori* infections. In addition, the ^{13}C -octanoic acid breath test has been frequently applied in the clinical diagnosis of gastric-emptying disorder since it was first reported by Ghooos and colleagues in 1993 (3). The gastric-emptying time can be indirectly

measured by monitoring the $^{13}\text{CO}_2$ concentration in expired air after a ^{13}C -labeled substance, such as acetic or octanoic acid, has been ingested and absorbed from the small intestine (1, 3, 4).

Symonds and colleagues (5) and Schoonjans and co-workers (6) reported noninvasive methods for evaluating gastric emptying using ^{13}C -labeled substances in mice and rats, respectively. However, in order to measure the amount of expired $^{13}\text{CO}_2$, the authors used gas chromatography and mass spectrometry; these analytical techniques are expensive and expert knowledge is required to operate the apparatus. In addition, animal chambers are expensive and air exchange must be prevented during the sampling of expired $^{13}\text{CO}_2$.

Therefore, in the present study, we aimed to establish a simple and noninvasive system for monitoring expired $^{13}\text{CO}_2$ using a desiccator and a pump, both of which are readily available experimental instruments, along with an infrared spectrometer (UBiT-IR₃₀₀; Otsuka Electronics, Co., Ltd., Osaka) and an automatic sampler

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(UBiT-AS10, Otsuka Electronics, Co., Ltd.), which are marketed for the diagnosis of *H. pylori* infections and can be operated without any technical expertise.

Metoclopramide has been reported to enhance gastrointestinal motility in both animals (7, 8) and humans (9, 10). Therefore, the effects of this drug were studied in order to validate our breath-test system for the assessment of gastric emptying.

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 The Japanese Pharmacological Society.

Animals

Male Sprague-Dawley rats (200–250 g) were purchased from SLC (Shizuoka). The animals were fasted in mesh cages for 18 h before each experiment in order to prevent coprophagy, but were allowed free access to drinking water during this period.

Breath-test system

A schematic illustration of the system used for monitoring the $^{13}\text{CO}_2$ levels in expired air from Sprague-Dawley rats is shown in Fig. 1. The desiccator and aspiration pump were selected because they were easy to set up and relatively inexpensive. The UBiT-IR₃₀₀ and UBiT-AS₁₀ apparatus were chosen as they allowed $^{13}\text{CO}_2$ to be measured simply and effectively. A desiccator with

a volume of 2,000 ml was employed so that the rats could move freely within the chamber and the expired air could be collected effectively in the breath-sampling bag. Aspirating the expired air through the aspiration tube caused fresh air to be automatically drawn through a hole, through which the aspiration tube also passes, on the side of the desiccator (Fig. 1). The air in the chamber was continuously aspirated during the experimental period. Aspirated air was discharged outside this breath-test system except for the period collecting expired air in the breath-sampling bag. Mixed gas composed of 5% CO_2 and 95% O_2 gas was used for the control. In this system, the concentration of CO_2 in the aspirated air in the breath sampling bag was at least more than 1%.

In clinical studies, breath tests are usually performed after the subjects have eaten a solid or liquid test meal containing a ^{13}C -labeled compound. Racol, which is an enteral nutritional formulation, is one such liquid test meal. Thus, in the present study, Racol containing ^{13}C -labeled acetic acid was used as the test meal to ensure consistency with previous clinical studies.

The rats were placed in the chamber immediately after the oral administration of the test meal. The expired air was collected at 5-min intervals until 70 min after the test meal administration, with additional measurements at 90 and 120 min. At each sample point, the expired air was collected into a breath-sampling bag for 1.5 min in the case of the aspiration volume being 150 ml/min. This system allowed four rats to be tested simultaneously under the same conditions (Fig. 1).

The $^{13}\text{CO}_2$ levels in the expired air were measured by

Fig. 1. Schematic illustration of the system used for monitoring $^{13}\text{CO}_2$ levels in the expired air from rats. Briefly, this system comprised a desiccator that was used as an animal chamber, a pump, and a breath-sampling bag. The $^{13}\text{CO}_2$ was measured with an infrared spectrometer (UBiT-IR₃₀₀) and an automatic sampler (UBiT-AS₁₀). Aspirating the expired air through the aspiration tube caused fresh air to be automatically drawn through a hole, through which the aspiration tube also passes, on the side of the desiccator.

placing the breath-sampling bags into the sample joint of the UBit-IR₃₀₀ infrared analyzer. The measured values were presented as the $\Delta^{13}\text{CO}_2$ (‰). The maximum concentration (C_{max} ; ‰), the time taken to reach the maximum concentration (T_{max} ; min) and the area under the curve ($\text{AUC}_{120 \text{ min}}$; ‰·min) were calculated using the $\Delta^{13}\text{CO}_2$ values. C_{max} and $\text{AUC}_{120 \text{ min}}$ reflect the absorption of labeled materials.

Effect of aspiration volume on respiratory pattern

The aspiration volume was determined based on the fact that a volume of 75 ml/min has been used for the artificial respiration of anesthetized rats weighing 200–250 g. We therefore tested three aspiration volumes (75, 150, and 300 ml/min) to determine the suitable volume for use under our study conditions. As a result, the dose of acetic acid and the sample volume were fixed at 16 mg/kg and 2.5 ml/kg, respectively. Breath sampling time was changed to 3 min and 45 s in volumes of 75 and 300 ml/min, respectively.

Effect of test-meal volume on gastric emptying

The volume of the rat stomach varies depending upon the developmental stage. However, rats weighing 200–250 g are generally used in experimental studies and 10 ml/kg is commonly regarded as a comparatively large volume for oral administration in these rats. In order to determine a suitable sample volume under our study conditions, various volumes were tested ranging from 1.25 to 10 ml/kg. Based on our findings, the dose of ^{13}C -labeled acetic acid was fixed at 16 mg/kg.

Effect of ^{13}C -labeled acetic acid dosage on gastric emptying

The sample volume data showed that doses of 2.5 or 5 ml/kg were suitable for use in evaluating gastric emptying; in this case, the volume of Racol containing ^{13}C -acetic acid was fixed at 2.5 ml/kg based on the assumption that the smaller volume was applicable in the case of using a heavier rat. To determine the optimal dosage of ^{13}C -labeled acetic acid, three doses were evaluated: 8, 16, and 32 mg/kg.

Effect of metoclopramide on gastric emptying

Metoclopramide suspended in 1% gum arabic solution was administered orally at doses of 0.3–3 mg/kg in a total volume of 5 ml/kg. After 30 min, Racol containing ^{13}C -acetic acid was administered orally at a dose of 16 mg/kg (2.5 ml/kg). In the control rats, vehicle (1% gum arabic solution) was administered orally instead of metoclopramide, using the same protocol.

Samples

[1- ^{13}C]Acetic acid and Racol were purchased from Wako Pure Chemical (Osaka) and Otsuka Pharmaceutical Co., Ltd., respectively. Metoclopramide was purchased from Sigma (St. Louis, MO, USA). The Gum arabic was Japanese Pharmacopoeia grade.

Data analyses

All results are presented as the mean $\pm$ S.E.M. Statistical analyses were performed using a Dunnett's multiple-comparison test and P values <0.05 were considered statistically significant.

Results

Effect of aspiration volume on respiratory pattern

Similar respiratory patterns were seen at all of the

Fig. 2. Effect of aspiration volume on the time course of $\Delta^{13}\text{CO}_2$ in expired air from rats. Values represent the mean $\pm$ S.E.M. ($n = 3$ or 4).

Table 1. Effect of aspiration volume of expired air on pharmacokinetic parameters in rats

	Aspiration volume (ml/min)		
	75	150	300
C_{max} (‰)	236.3 $\pm$ 25.9	261.1 $\pm$ 12.5	290.3 $\pm$ 12.5
T_{max} (min)	26.3 $\pm$ 1.3	28.8 $\pm$ 2.4	26.7 $\pm$ 1.7
$\text{AUC}_{120 \text{ min}}$ (‰·min)	15,839 $\pm$ 1,210	16,733 $\pm$ 711	18,309 $\pm$ 341

Values represent the mean $\pm$ S.E.M. ($n = 3$ or 4).

aspiration volumes tested (Fig. 2). However, the rats demonstrated slight anoxia at a volume of 75 ml/min. Anoxia was not observed at volumes of 150 ml/min or greater.

The $C_{\max}$ and $T_{\max}$ values showed no statistically significant differences among the three aspiration volumes (Table 1).

The $AUC_{120\text{ min}}$ values showed a tendency to increase as the aspiration volume increased, although there were no statistically significant differences between the three volumes tested (Table 1).

Effect of test-meal volume on gastric emptying

As the test-meal volume increased, the amount of expired $^{13}\text{CO}_2$ increased in a dose-dependent manner (Fig. 3). However, no clear peak was observed at a volume of 10 ml/kg.

The $C_{\max}$ values at the different test-meal volumes are shown in Table 2. A positive correlation was observed between the $C_{\max}$ and the volume of the test meal ($R^2 = 0.994$, Fig. 4). When only the first three volumes (1.25, 2.5, and 5 ml/kg) were used to calculate the regression curve, the R^2 value was 0.996.

The $T_{\max}$ values at different test-meal volumes are shown in Table 2. The $T_{\max}$ values increased as the

Fig. 3. Effect of test-meal volume on the time course of $\Delta^{13}\text{CO}_2$ in expired air from rats. Values represent the mean $\pm$ S.E.M. ($n = 3$ or 4).

sample volume increased.

The $AUC_{120\text{ min}}$ values at different test-meal volumes are shown in Table 2. The $AUC_{120\text{ min}}$ values increased in a volume-dependent manner and a significant positive correlation was observed between the $AUC_{120\text{ min}}$ value and the test-meal volume ($R^2 = 0.9994$, Fig. 4). When

Fig. 4. Correlation between test-meal volume and $C_{\max}$ (A) and $AUC_{120\text{ min}}$ (B). A: A significant positive correlation was observed between the test-meal volume and the $C_{\max}$ ($y = 86.7x + 65.1$, $R^2 = 0.994$). When only the first three volumes (1.25, 2.5, and 5 ml/kg) were used to calculate the regression curve, the equation and R^2 value were $y = 100.9x + 28.6$ and 0.996, respectively. B: A significant positive correlation was observed between the test-meal volume and the $AUC_{120\text{ min}}$ values ($y = 8194x + 1422$, $R^2 = 0.9994$). When only the first three volumes (1.25, 2.5, and 5 ml/kg) were used to calculate the regression curve, the equation and R^2 value were $y = 8187x + 1441$ and 0.9963, respectively. The values represent the mean ($n = 3$ or 4).

Table 2. Effect of the test-meal volume of Racol containing ^{13}C -labeled acetic acid on pharmacokinetic parameters in rats

	Test-meal volume (ml/kg)			
	1.25	2.5	5	10
$C_{\max}$ (‰)	145.4 ± 4.6	294.5 ± 15.0	582.2 ± 4.4	917.2 ± 4.6
$T_{\max}$ (min)	31.3 ± 6.6	33.8 ± 2.4	43.8 ± 4.3	56.7 ± 3.3
$AUC_{120\text{ min}}$ (‰·min)	$10,954 \pm 234$	$22,988 \pm 1,041$	$42,015 \pm 1,865$	$83,369 \pm 3,349$

Values represent the mean $\pm$ S.E.M. ($n = 3$ or 4).

only the first three volumes (1.25, 2.5, and 5 ml/kg) were used to calculate the regression curve, the R^2 value was 0.9963.

Effect of ^{13}C -labeled acetic acid dosage on gastric emptying

As the dose of ^{13}C -labeled acetic acid increased, the amount of $^{13}\text{CO}_2$ in the expired air increased in a dose-dependent manner and clear peaks were observed with all of the doses tested (Fig. 5).

The $C_{\max}$ values at different doses are shown in Table 3. A positive correlation was observed between the $C_{\max}$ and the dose of ^{13}C -labeled acetic acid ($R^2 = 0.9981$, Fig. 6).

The $T_{\max}$ values at different doses are shown in Table 3. The $T_{\max}$ increased as the dose of ^{13}C -labeled acetic acid increased, but there was no difference between 16 and 32 mg/kg.

The $\text{AUC}_{120\text{ min}}$ values at different doses are shown in Table 3. The $\text{AUC}_{120\text{ min}}$ values increased in a dose-dependent manner and a significant positive correlation was observed between these variables ($R^2 = 0.9991$, Fig. 6).

Effect of metoclopramide on gastric emptying

Metoclopramide dose-dependently and significantly enhanced gastric emptying (Fig. 7). In the control rats,

Fig. 5. Effect of the dose of ^{13}C -labeled acetic acid on the time course of $\Delta^{13}\text{CO}_2$ in expired air from rats. The values represent the mean $\pm$ S.E.M. ($n = 4$).

the $C_{\max}$ value was $310.7 \pm 21.5\%$ (Fig. 7 and Table 4). Metoclopramide significantly increased the $C_{\max}$ value at a dose of 3 mg/kg ($C_{\max} = 380.0 \pm 14.7\%$, $P < 0.05$), although no significant differences were observed at other doses (Fig. 7 and Table 4).

In the control rats, the $T_{\max}$ value was 31.3 ± 1.3 min (Table 4). Metoclopramide significantly decreased the $T_{\max}$ values at doses of 1 and 3 mg/kg ($T_{\max} = 21.7 \pm 1.7$ and 22.5 ± 1.4 min, respectively; $P < 0.01$; Table 4).

The $\text{AUC}_{120\text{ min}}$ value in the control rats was

Fig. 6. Correlation between the dose of ^{13}C -labeled acetic acid and $C_{\max}$ (A) and $\text{AUC}_{120\text{ min}}$ (B). A: A significant positive correlation was observed between the dose of ^{13}C -labeled acetic acid and the $C_{\max}$ ($y = 18.6x + 8.1$, $R^2 = 0.9981$). B: A significant positive correlation was observed between the dose of ^{13}C -labeled acetic acid and the $\text{AUC}_{120\text{ min}}$ ($y = 1339x + 1005$, $R^2 = 0.9991$). The values represent the mean ($n = 4$).

Table 3. Effect of the dose of ^{13}C -acetic acid on pharmacokinetic parameters in rats

	^{13}C -Acetic acid (mg/kg)		
	8	16	32
$C_{\max}$ (‰)	164.5 ± 10.5	294.5 ± 15.0	607.4 ± 7.2
$T_{\max}$ (min)	22.5 ± 1.4	33.8 ± 2.4	35.0 ± 2.0
$\text{AUC}_{120\text{ min}}$ (‰·min)	$11,335 \pm 931$	$22,988 \pm 1,041$	$43,648 \pm 701$

Values represent the mean $\pm$ S.E.M. ($n = 4$).

Table 4. Effect of metoclopramide on the pharmacokinetic parameters of gastric emptying in rats

	Control	Metoclopramide (mg/kg)		
		0.3	1	3
$C_{\max}$ (‰)	310.7 ± 21.5	297.2 ± 29.5	276.1 ± 19.2	380.0 ± 14.7*
$T_{\max}$ (min)	31.3 ± 1.3	27.5 ± 1.4	21.7 ± 1.7**	22.5 ± 1.4**
$AUC_{120\text{ min}}$ (‰ · min)	22,229 ± 1,313	21,398 ± 1,442	20,195 ± 760	22,750 ± 969

Values represent the mean ± S.E.M. (n = 3 or 4). *, **: Significant difference from the control ($P < 0.05$, $P < 0.01$).

Fig. 7. Effect of metoclopramide on the time course of $\Delta^{13}\text{CO}_2$ in expired air from rats. The values represent the mean ± S.E.M. (n = 3 or 4).

22,229 ± 1,313‰ · min (Table 4). Metoclopramide did not significantly affect the $AUC_{120\text{ min}}$ values at any of the doses tested.

Discussion

Several methods for the measurement of gastric emptying have been reported previously. $^{99\text{m}}\text{Tc}$ -Colloid-based scintigraphy is considered to be the reference method (11–14); and the other techniques include real-time ultrasonography (15, 16), the radiopaque method (17), the metal-sphere method (18), and the sulfamethiazole capsule method (14). In 1993, Ghoos and colleagues (3) reported a noninvasive ^{13}C -octanoic acid gastric-emptying breath test based on the analysis of the time course of $^{13}\text{CO}_2$ enrichment in exhaled air from humans. Subsequently, many studies have used ^{13}C -octanoic acid or ^{13}C -acetic acid to monitor gastric emptying clinically.

Breath tests have also been applied in some animal studies. Ishii and co-workers (19) used ^{13}C -aminopyrine to monitor the liver function in anesthetized rats. Wyse and colleagues used ^{13}C -octanoic acid breath tests in ponies (20) and dogs (21) to evaluate gastric emptying. For smaller experimental animals, Schoonjans and co-workers (6) reported the use of a breath test with ^{13}C -octanoic acid in rats placed in metabolic air-tight cages.

In their method, the breath tests were performed by a fully automated system of computer-guided switching valves within the metabolic cages. The breath samples were then analyzed by gas chromatography and isotope mass spectrometry; the latter is the established standard method for the sensitive and accurate measurement of the $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratio in breath samples (22). Symonds and co-workers (5) reported noninvasive methods for evaluating gastric emptying using ^{13}C -labeled substances in mice housed in gas-tight rubber-sealed glass containers. The exhaled air containing $^{13}\text{CO}_2$ was sampled with a plastic syringe and the breath samples were analyzed using isotope mass spectrometry.

In the reports discussed above, specialized animal chambers were needed to collect the expired air and gaseous exchange in the animal chamber was stopped during the $^{13}\text{CO}_2$ sampling. As a result, we identified the need to establish a simple and cheap breath-test system that did not alter the air conditions in the animal chamber, even while collecting the expired air. For this purpose, we used a desiccator as the animal chamber and a pump to aspirate the expired air into a breath-sampling bag; these instruments are widely available at a reasonable price. Moreover, air exchange is not stopped in this system, even during $^{13}\text{CO}_2$ sampling; fresh air automatically flows into the desiccator through a hole, through which the aspiration tube also passes, on the side of the chamber in order to replace the aspirated air.

Considering the analytical instruments, isotope mass spectrometry has been used previously to measure $^{13}\text{CO}_2$ concentrations. Ohara and colleagues (23) and Leodolter and co-workers (24) have developed a new compact instrument for nondispersive isotope-selective infrared spectroscopy (UBiT-IR₃₀₀). Using this apparatus, the $^{13}\text{CO}_2$ concentration in the respired air can be measured simply by placing the breath-sampling bags in the sample joint without the need for technical procedures. In the present study, we used the UBiT-IR₃₀₀ instrument to measure $^{13}\text{CO}_2$ due to its convenience, simplicity, and reliability of measurement. Braden and colleagues (4) reported that the half-emptying times for the ^{13}C -acetate breath test were closely correlated with those measured by radiosciintigraphy using both semisolids and liquids

and that the $T_{\max}$ of $^{13}\text{CO}_2$ exhalation was itself a reliable parameter compared with the half-emptying times obtained by scintigraphy.

In addition, Kato and colleagues (25) reported that the data obtained from mass spectrometry were significantly correlated with the data from infrared spectroscopy ($r = 0.99989$, $P < 0.0001$). As a result, in the present study, we used the $T_{\max}$ rather than the half-emptying time. Moreover, in order to analyze pharmacokinetic parameters, the $C_{\max}$ and $\text{AUC}_{120 \text{ min}}$ values were used.

In our experimental system, the rate of air exchange was important because it might affect both the respiratory pattern and the condition of the animals. A rate of 75 ml/min, which has been used previously for the artificial respiration of anesthetized rats, was taken as the standard rate of air exchange. Three different rates of aspiration (75, 150, and 300 ml/min) were evaluated. At 75 ml/min, the rats showed slight anoxia. Although there were slight differences in the $C_{\max}$, $T_{\max}$, and $\text{AUC}_{120 \text{ min}}$ values among the three aspiration volumes, these were not statistically significant. Thus, the aspiration volume in the present study was fixed at 150 ml/min.

A diagnosis of impaired gastric emptying is generally based on an assessment of the gastric emptying of solids, because disturbances of solid emptying usually precede impairments of liquid emptying (26). ^{13}C -Acetic acid and ^{13}C -octanoic acid have been used to evaluate gastric emptying clinically. Braden and co-workers (4) reported that the ^{13}C -acetic acid breath test accurately reflects gastric emptying after both liquid and semisolid test meals. Enteral nutritional formulations, such as Racol, have been used as liquid test meals for previous gastric-emptying tests. In the present study, we used Racol to measure gastric emptying, as the administration of solid meals to rats presents technical difficulties.

After the administration of Racol containing ^{13}C -acetic acid, the concentration of $^{13}\text{CO}_2$ in the expired air increased over time, peaked, and decreased thereafter. A volume-dependent increase in the amount of expired $^{13}\text{CO}_2$ was observed, although no clear peak was detected at a test-meal volume of 10 ml/kg. This finding might indicate saturation in the metabolism of acetic acid. Indeed, a more reliable linear relationship was observed between the $C_{\max}$ and the test-meal volume at 1.25–5 ml/kg, even though significant dose-dependency was also observed at 1.25–10 ml/kg. These findings confirmed that a sample volume of 5 ml/kg or less was suitable in this study.

The test-meal volume was fixed as 2.5 ml/kg and the dosage of ^{13}C -acetic acid was changed from 8 to 32 mg/kg. A dose-dependent increase of $^{13}\text{CO}_2$ in the expired air was observed. Clear and significant dose-

dependent curves were obtained for the $C_{\max}$ and $\text{AUC}_{120 \text{ min}}$ values, even including the high dose of 32 mg/kg. On the other hand, 100 mg of ^{13}C -acetic acid has been used in clinical studies. The dose of ^{13}C -acetic acid is calculated as 2 mg/kg, if the weight of human were supposed to be 50 kg, although a species difference exists. Therefore, it may be possible to use a dose lower than 8 mg/kg for evaluating gastric motility. Based on these results and findings, the experimental conditions were fixed as follows: the test-meal volume and dose of ^{13}C -acetic acid were 2.5–5 ml/kg and 8–16 mg/kg, respectively, and the aspiration volume was 150 ml/min.

The effect of metoclopramide, which is a prokinetic drug, was investigated in order to validate the present method. Metoclopramide has been reported to enhance gastric emptying both in clinical studies of humans (7, 8) and in animal studies (9, 10). In the present system, metoclopramide dose-dependently enhanced gastric emptying. However, the $\text{AUC}_{120 \text{ min}}$ values were not affected by metoclopramide even at a dose of 3 mg/kg, which significantly increased the $C_{\max}$. On the contrary, in the present breath test system, atropine sulfate increased $T_{\max}$ in a dose-dependent manner (data not shown). These findings show that this system is suitable for evaluating the gastric emptying.

In conclusion, we have reported a simple and non-invasive method for investigating gastric emptying without causing stress to rats. This method could be adapted for use with mice instead of rats by changing the chamber and aspiration volumes. Moreover, by using alternative stable isotopes (such as lactose and aminopyrine) it will be possible to investigate the functions of other organs (in this example, the colon and liver, respectively).

References

- 1 Kim DY, Myung SJ, Camilleri M. Novel testing of human gastric motor and sensory functions: rationale, methods, and potential application in clinical practice. *Am J Gastroenterol*. 2000;95:3365–3373.
- 2 Heading R, Tothill P, McLoughlin G, Shearman D. Gastric emptying rate measurement in man. A double isotope scanning technique for simultaneous study of liquid and solid components of a meal. *Gastroenterology*. 1976;71:45–50.
- 3 Ghos YF, Maes BD, Geypens BJ, Mys G, Hiele MI, Rutgeerts PJ, et al. Measurement of gastric emptying rate of solids by means of a carbon-labeled octanoic acid breath test. *Gastroenterology*. 1993;104:1640–1647.
- 4 Braden B, Adams S, Duan LP, Orth KH, Maul FD, Lambecke B, et al. The [^{13}C]acetate breath test accurately reflects gastric emptying of liquids in both liquid and semisolid test meals. *Gastroenterology*. 1995;108:1048–1055.
- 5 Symonds EL, Butler RN, Omari TI. Assessment of gastric

- emptying in the mouse using the [^{13}C]-octanoic acid breath test. *Clin Exp Pharmacol Physiol*. 2000;27:671–675.
- 6 Schoonjans R, Van Vlem B, Van Heddeghem N, Vandamme W, Vanholder R, Lameire N, et al. The ^{13}C -octanoic acid breath test: validation of a new noninvasive method of measuring gastric emptying in rats. *Neurogastroenterol Motil*. 2002;14:287–293.
- 7 Dkhar SA, Shewade DG, Shashindran CH, Suchitra AD. Relative efficacy of some prokinetic drugs in morphine-induced gastrointestinal transit delay in mice. *World J Gastroenterol*. 2003;9:779–783.
- 8 Osinski MA, Seifert TR, Cox BF, Gintant GA. An improved method of evaluation of drug-evoked changes in gastric emptying in mice. *J Pharmacol Toxicol Methods*. 2002;47:115–120.
- 9 Vanderplas Y, Hauser B, Salvatore S. Current pharmacological treatment of gastroparesis. *Expert Opin Pharmacother*. 2004;5:2251–2254.
- 10 Ferris CD, Smith DS. Current concepts in diabetic gastroparesis. *Drugs*. 2003;63:1339–1358.
- 11 Ziegler D, Schadevaldt P, Pour Mirza A, Piolot R, Schommartz B, Reinhardt M, et al. [^{13}C]Octanoic acid breath test for non-invasive assessment of gastric emptying in diabetic patients: variation and relationship to gastric symptoms and cardiovascular autonomic function. *Diabetologia*. 1996;39:823–830.
- 12 Tothil P, McLaughlin GP, Heading RC. Techniques and errors in scintigraphic measurement of gastric emptying. *J Nucl Med*. 1978;19:256–261.
- 13 Siegel JA, Urbain JL, Adler LP, Charkes ND, Maurer AH, Krevsky B, et al. Biphasic nature of gastric emptying. *Gut*. 1988;29:85–89.
- 14 Asada T, Murakami M, Sako Y, Fukushima Y, Yonekura Y, Konishi J, et al. Sulfamethizole capsule method. A new method for assessing gastric emptying of solids. *Dig Dis Sci*. 1994;39:2056–2061.
- 15 Arienti V, Magri F, Boriani L, Maconi G, Bassein L, Baraldini M, et al. Effect of single dose of oral erythromycin on gastric and gallbladder emptying. Simultaneous assessment by ultrasound. *Dig Dis Sci*. 1994;39:1309–1312.
- 16 Cucchiara S, Raia V, Minella R, Frezza T, De Vizia B, De Ritis G. Ultrasound measurement of gastric emptying time in patients with cystic fibrosis and effect of ranitidine on delayed gastric emptying. *J Pediatr*. 1996;128:485–488.
- 17 Meyer BM, Werth BA, Beglinger C, Hildebrand P, Jansen JB, Zach D, et al. Role of cholecystokinin in regulation of gastrointestinal motor functions. *Lancet*. 1989;ii:12–15.
- 18 Ueberschaer B, Ewe K, Alles U, Schmidtman I. Effect of 4 x 250 mg erythromycin on human gastrointestinal transit. *Z Gastroenterol*. 1995;33:340–344.
- 19 Ishii Y, Asai S, Kohno T, Suzuki S, Ishii M, Hosoi I, et al. $^{13}\text{CO}_2$ peak value of L-(1- ^{13}C)phenylalanine breath test reflects hepatopathy. *J Surg Res*. 1999;86:130–135.
- 20 Wyse CA, Murphy DM, Preston T, Sutton DG, Morrison DJ, Christley RM, et al. The (^{13}C)-octanoic acid breath test for detection of effects of meal composition on the rate of solid-phase gastric emptying in ponies. *Res Vet Sci*. 2001;71:81–83.
- 21 Wyse CA, Preston T, Love S, Morrison DJ, Cooper JM, Yam PS. Use of the ^{13}C -octanoic acid breath test for assessment of solid-phase gastric emptying in dogs. *Am J Vet Res*. 2001;62:1939–1944.
- 22 Craig H. Isotope standards for carbon and oxygen and correlation factors for mass spectrometric analysis of carbon dioxide. *Geochim Cosmochim Acta*. 1957;12:133–149.
- 23 Ohara S, Kato M, Asaka M, Toyama T. The U-BiT-100 $^{13}\text{CO}_2$ infrared analyzer: comparison between infrared spectrometric analysis and mass spectrometric analysis. *Helicobacter*. 1998;3:49–53.
- 24 Leodolter A, Arnim UV, Gerards C, Kahl S, Malfertheiner P. Pre- and post-treatment diagnosis of *H. pylori* infection by the ^{13}C -urea breath test (^{13}C -UBT): variation of a new isotope-selective infrared spectrometer (ISIS) (abstract). *Gastroenterology*. 2000;118 Suppl 2:A506.
- 25 Kato M, Saito M, Fukuda S, Kato C, Ohara S, Hamada S, et al. ^{13}C -Urea breath test, using a new compact nondispersive isotope-selective infrared spectrophotometer: comparison with mass spectrometry. *J Gastroenterol*. 2004;39:629–634.
- 26 Chaudhuri TK, Fink S. Gastric emptying in human disease states. *Am J Gastroenterol*. 1991;86:533–538.