Simultaneous measurement of gastric emptying and gastrocecal transit times in conscious rats using a breath test after ingestion of [1-\textsuperscript{13}C] acetic acid and lactose-[13C] ureide

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Abstract

This study reports a method for the evaluation of both gastric emptying and gastrocecal transit times in rats simultaneously by using the same breath testing system measuring equipment. Male rats were used after fasting. Gastric emptying and gastrocecal transit time were evaluated by using [1-\textsuperscript{13}C] acetic acid (8 mg/kg) and lactose-[\textsuperscript{13}C] ureide (60 mg/kg), respectively. A mixture of both \textsuperscript{13}C-labelled compounds dissolved in Racol (liquid nutrient formula) was administered orally. The level of \textsuperscript{13}CO\textsubscript{2} in the expired air was measured using an infrared spectrometer at appropriate intervals for a period of 420 min. The level of \textsuperscript{13}CO\textsubscript{2} in the expired air from [1-\textsuperscript{13}C] acetic acid increased with time and peaked at about 30 min before decreasing, while that from lactose-[\textsuperscript{13}C] ureide increased after about 180 min. The time taken to reach the maximum value of gastric emptying ($T_{\text{max}}$) was 27.5 ± 0.9 min. Gastrocecal transit time was 180 ± 11.5 min, which was calculated as the time before the \textsuperscript{13}CO\textsubscript{2} value increased again. These results accorded with the results of gastric emptying and gastrocecal transit time evaluated by using each \textsuperscript{13}C-labelled compound separately. These results demonstrate that this method is useful for the simultaneous evaluation of gastric emptying and gastrocecal transit times in rats.

Key words: gastric emptying, gastrocecal transit time, breath test in rats, [\textsuperscript{13}C] acetic acid, lactose-[\textsuperscript{13}C] ureide

Introduction

Radioscintigraphy is generally accepted as the gold standard for measuring gastric emptying in humans (Heading \textit{et al.}, 1976). Existing techniques for testing gastric motility include gastrotonometry via infused-catheter or microtransducer, the radioactive-isotope method or the acetaminophen method.
(Kim et al., 2000). However, all of these methods are invasive and stressful, and either involve radiation exposure or have drug-related adverse effects. Ghoos et al. reported in 1993 that the $^{13}$C-octanoic acid breath test was applicable for the clinical diagnosis of gastric-emptying disorder. Since then, this breath test has been used widely in clinical research studies. Sakamoto et al. (2011), Nonaka et al. (2011) and Akimoto et al. (2009) have evaluated the effects of domperidone, itopride and postprandial coffee intake, respectively, using [1-$^{13}$C] acetic acid.

Recently, methods involving double balloon endoscopy and capsule endoscopy have been developed to diagnose diseases of the small intestine (Yen et al., 2012; Nishimura et al., 2011). Therefore, it is of great value to have a technique that can be used in clinical studies to simply and reliably monitor global intestinal motor activity. In 1999, Geypens et al. studied the validity of the lactose-$[^{13}$C] ureide breath test by comparing it with a well-established method, namely scintigraphy, using $^{99m}$Te-sulfur colloid, and concluded that the breath test using lactose-$[^{13}$C] ureide is a valid alternative to existing scintigraphic techniques for measuring orocecal transit time.

In basic research, we have already reported a simple and non-invasive breath test system for evaluating gastric emptying and pancreatic exocrine secretion in conscious rats (Uchida et al., 2005; Uchida et al., 2007; Uchida et al., 2008; Matsumoto et al., 2008; Uchida et al., 2011). Moreover, we have also established a non-invasive and feasible method for the evaluation of rat gastrocecal transit time by using lactose-$[^{13}$C] ureide (Uchida et al., 2009a) and the effect of atropine sulfate which significantly delayed the gastrocecal transit time (Uchida et al., 2009b).

On the other hand, Urita et al. (2002) and Gonzalez et al. (1998) reported the efficacy of a lactulose plus $^{13}$C-acetate breath test in the diagnosis of gastrointestinal motility disorders, detecting hydrogen levels and $^{13}$CO$_2$ levels separately with different equipment in a clinical study. In 1997, Galati et al. reported gastric emptying and orocecal transit times in portal hypertension and end-stage chronic liver disease using a scintigraphic technique involving radiation exposure. Imamura et al. (2007) also reported that gastric emptying and orocecal transit times could be measured at the same time by using $^{13}$C-labelled octanoic acid and lactose-$[^{13}$C] ureide in a clinical research study. However, a precise method for simultaneously determining gastric emptying and gastrocecal transit times using the same measuring equipment and different $^{13}$C-labelled substances has not been reported. Moreover, the simultaneous measurement of gastric emptying and gastrocecal transit times by using breath testing in experimental animals has not been reported. Therefore in this study, we aimed to establish a method for the simultaneous evaluation of gastric emptying and gastrocecal transit times in conscious rats using [1-$^{13}$C] acetic acid and lactose-$[^{13}$C] ureide.

**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 weighing about 200 g were purchased from SLC (Shizuoka, Japan) and used in the following experiments after 1 week acclimation in a room maintained at $21 \pm 2^\circ$C with
Simultaneous measurement of GE and GCTT

a humidity of 55 ± 15%. The animals were exposed to an alternating 12 h period of light/dark with the light cycle occurring between 07:00–19:00 hrs. 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

The breath test was performed according to the method reported by us (Uchida et al., 2005) as shown in Fig. 1. Briefly, a desiccator with a volume of 2,000 mL was employed so that the rats could move freely within the chamber. The rats were placed in the chamber immediately after the oral administration of the test meal. The air in the chamber was continuously aspirated during experimental period with a pump and the expired air was able to be collected effectively in the breath-sampling bag. The $^{13}$CO$_2$ levels in the expired air were measured by placing the breath-sampling bags into the sample joint of the UBiT-IR$_{300}$ infrared analyzer (Otsuka Electronics Ltd., Osaka). Mixed gas composed of 5% CO$_2$ and 95% O$_2$ gas was used as the control. The measured values were represented as $\Delta^{13}$CO$_2$ (‰).
Effects of primed lactose-ureide and lactose-ureide contained in test meal on gastric emptying

It has been shown clinically that priming with lactose-ureide is necessary to investigate the orocecal transit time (Wutzke et al., 2007). We also confirmed that a priming dose of lactose-ureide is necessary in the breath testing of rats (Uchida et al., 2009a). Therefore, the first phase of each experiment involved evaluating the effect of this priming dose of lactose-ureide on gastric emptying. Next, the effect of a two-component mixture of both primed lactose-ureide and non-labeled lactose-ureide contained in a test meal was investigated, as it is necessary to use a two-component mix of labeled acetic acid and labeled lactose-ureide when performing the simultaneous measurement of gastric emptying and gastrocecal transit time in the same rat.

Then, we set up the following three groups; Group 1: distilled water instead of priming with lactose-ureide and [1-13C] acetic acid (control), Group 2: primed with lactose-ureide and [1-13C] acetic acid, and Group 3: primed with lactose-ureide and [1-13C] acetic acid containing lactose-ureide. Four rats were use in each group.

The priming dose of lactose-ureide dissolved in distilled water was administered orally at a dose of 60 mg/kg twice a day (10:00 and 17:00) before fasting for 18 hrs. In the control rats, distilled water was administered instead of lactose-ureide.

Gastric emptying was evaluated by using test meals of Racol (liquid nutrient formula) containing [1-13C] acetic acid or [1-13C] acetic acid plus lactose-ureide (60 mg/kg). [1-13C] Acetic acid was administered orally at a dose of 4 mg/kg (2.5 mL/kg). This dose was selected because expired 13CO2 from [1-13C] acetic acid would not have an affect on the level of expired 13CO2 derived from labeled lactose-ureide.

The expired air was collected at 5-min intervals over a 70 min period after the administration of the test meal, with additional measurements at 90 and 120 min. The aspiration volume was set at 150 mL/min. The time taken to reach the maximum concentration ($T_{max}$; min) was used for the evaluation.

Effect of acetic acid on gastrocecal transit time

In this experiment, the effect of acetic acid instead of [1-13C] acetic acid was evaluated on the gastrocecal transit time. Lactose-ureide was pre-loaded before the experiments as described above. After fasting, rats were orally administered with lactose-[13C] ureide dissolved in Racol containing non-labeled acetic acid in a volume of 2.5 mL/kg (4 mg/kg of acetic acid and 60 mg/kg of lactose-[13C] ureide). After this, expired air was collected at 5-min intervals for a period of 70 min after administration of the test meal, with additional measurements at 90 and 120 min, and after 120 min at 20 min intervals until 420 min had elapsed following the test meal. The aspiration volume was again set at 150 mL/min. The gastrocecal transit time was essentially defined as in our earlier experiments (Uchida et al., 2009a) but with a slight modification. We defined gastrocecal transit time as the time when the measured value of 13CO2 was greater than the mean + 2 S.D. of the values measured in the period between 10 and 120 min following the administration of lactose-[13C] ureide. Eight rats were used in this study.

Simultaneous measurement of gastric emptying and gastrocecal transit time

Simultaneous measurement of gastric emptying and gastrocecal transit time was performed as follows. Lactose-ureide was pre-loaded before the experiments in rats as described above. The test meal was composed of [1-13C] acetic acid and lactose-[13C] ureide dissolved in Racol (4 and 60 mg/kg,
respectively), and was administered orally at a volume of 2.5 mL/kg. Expired air was collected at 5-min intervals for the period to 70 min after the test meal administration, with additional measurements at both 90 and 120 min, and then after 120 min at 20 min intervals for the period to 420 min. The aspiration volume was again set at 150 mL/min.

A regression curve, plotted from the values obtained during gastric emptying from 5 to 120 min after the test meal, was calculated by using non-compartmental analysis with WinNonlin computer software (Pharsight Corporation, Mountain View CA, USA).

The gastrocecal transit time was defined by the following two criteria: firstly the time when the measured value of $^{13}$CO$_2$ was minimal, and secondly as the time when the measured value crossed the calculated value from the regression curve of gastric emptying. Eight rats were used in this study.

**Drugs and agents**

$[1-^{13}C]$ Acetic acid, lactose-$[^{13}C]$ureide, and Racol and lactose-ureide were purchased from Wako Pure Chemical (Tokyo, Japan), Cambridge Isotope Laboratories Inc. (Andover, MA, USA) and Otsuka Pharmaceutical Co., Ltd. (Tokyo), respectively.

**Statistical analysis**

All results are presented as the mean ± S.E.M. Statistical analyses were performed by using either the Student’s $t$-test or Dunnet’s multiple comparison test (Stat View, Version 5.0.0.0), and $P$ values <0.05 were considered to be statistically significant.

**Results**

**Effects on gastric emptying of priming with lactose-ureide and lactose-ureide contained in a test meal**

In the control rats, expired $^{13}$CO$_2$ air increased with time after the oral administration of the test meal, peaked at about 30 min and decreased thereafter as shown in Fig. 2. The time taken to reach the maximum concentration ($T_{\text{max}}$) value was calculated to be 28.8 ± 3.8 min.

After priming with lactose-ureide, the pattern of expired $^{13}$CO$_2$ air was not significantly affected as compared with the control (Fig. 2). Moreover, the $T_{\text{max}}$ value was also not affected (27.5 ± 1.4 min, Cohen’s d value: 0.227 as compared with $[1-^{13}C]$ acetic acid alone; 28.8 ± 3.8 min), suggesting that priming with lactose-ureide had no effect on the gastric emptying of $[1-^{13}C]$ acetic acid.

In the group primed with lactose-ureide plus co-administration of lactose-ureide with $[1-^{13}C]$ acetic acid, the pattern of expired $^{13}$CO$_2$ air was not affected as compared with the control (Fig. 2). Moreover, the $T_{\text{max}}$ value was also not affected (28.8 ± 2.4 min, Cohen’s d value: 0 as compared with $[1-^{13}C]$ acetic acid alone; 28.8 ± 3.8 min), suggesting that the co-administration of lactose-ureide with $[1-^{13}C]$ acetic acid had no effect on gastric emptying.

**Effects of acetic acid on gastrocecal transit time**

Expired $^{13}$CO$_2$ air was not detected before 160 min (Fig. 3). On and after about 180 min following lactose-$[^{13}C]$ ureide administration, the content of $^{13}$CO$_2$ in the expired air increased gradually, peaked and then decreased thereafter. Gastrocecal transit time was found to be 178 ± 12.8 min (n=8). In this
study, there were no non-respondent rats that did not show an increase in the levels of $^{13}$CO$_2$ derived from lactose-$[^{13}$C] ureide in their expired air.

**Simultaneous measurement of gastric emptying and gastrocecal transit time**

With the simultaneous administration of $[1-^{13}$C] acetic acid and lactose-$[^{13}$C] ureide, expired $^{13}$CO$_2$ air increased with time, peaked at about 30 min and then decreased as shown in Fig. 5. Thereafter, the levels of $^{13}$CO$_2$ in the expired air increased again at about 180 min after the administration of the test meal (Fig. 4).

The $T_{max}$ value for gastric emptying was $27.1 \pm 1.0$ min, being almost equal to the values when $[1-^{13}$C] acetic acid was administered alone ($28.8 \pm 3.8$, Cohen’s d: 0.235 as compared with $[1-^{13}$C] acetic acid alone; $28.8 \pm 3.8$ min).

The gastrocecal transit time was found to be $180 \pm 11.5$ min (n=7) when the measured value of expired $^{13}$CO$_2$ was at a minimum. On the other hand, the gastrocecal transit time was found to be 190
Simultaneous measurement of GE and GCTT ± 11.5 min (n=7) when the measured value crossed the value calculated from the regression curve for gastric emptying. These values were not significantly different as compared with that using acetic acid plus lactose-[13C] ureide (178 ± 12.8 min, n=7). In this study, only one non-respondent rat was observed out of 8 rats.

Discussion

In the present study, we found that simultaneous measurement of gastric emptying and gastrocecal transit time became possible by way of breath testing using [1-13C] acetic acid and lactose-[13C] ureide simultaneously in conscious rats.

Breath tests have been used clinically to evaluate gastric emptying using [1-13C] acetic acid or [1-13C] octanoic acid (Katsube et al., 2007, Cardoso-Júnior et al., 2007). We have already reported that [1-13C] acetic acid is more sensitive for the evaluation of gastric emptying in rats as compared with [1-13C] octanoic acid if used at the same molarity. In clinical research studies, 13C-labeled acetic acid has been administered in the form of a liquid test meal, while 13C-labeled octanoic acid has been administered in the form of solid test meal. This is because octanoic acid is only slightly soluble in water and acetic acid only slightly soluble in lipid. In this study, we used a liquid test meal containing [1-13C] acetic acid as the labeled compound, because a solid test meal is hard for the rats to ingest within a short limited time, although Hoshino et al. (2008) reported breath testing using a solid test meal in mice. Until now, we have usually used 8 mg/kg of [1-13C] acetic acid as the dose for the evaluation of gastric emptying (Uchida et al., 2005; Uchida et al., 2007; Uchida et al., 2011; Uchida et al., 2009b). However, in the present study we used 4 mg/kg of [1-13C] acetic acid, because a larger dose of [1-13C] acetic acid would affect the level of the expired 13CO2 derived from the simultaneously administered lactose-[13C] ureide. In other words, it would be difficult to separate the expiration of 13CO2 levels derived from [1-13C] acetic acid from that derived from the lactose-[13C] ureide. As expected, by using 4 mg/kg of [1-13C] acetic acid, the level of expired 13CO2 became almost 0 % after a period of 120 min following the [1-13C] acetic acid administration. We have already reported that the gastrocecal transit time was about 180 min by using lactose-[13C] ureide in conscious rats (8). Therefore the reduced dose of [1-13C] acetic acid (4 mg/kg) separated the peaks of expired 13CO2 and enabled us to distinguish the [1-13C] acetic acid-derived

Fig. 4. Changes in the expired 13CO2 levels after simultaneous administration of [1-13C] acetic acid and lactose-[13C] ureide in conscious rats. Values represent mean ± S.E.M. of 7 rats.
expired $^{13}$CO$_2$ from the lactose-$^{13}$C ureide-derived expired $^{13}$CO$_2$.

Lactose-$^{13}$C ureide has a proven utility in clinical studies as a tool to investigate the gastrocecal transit time. After passing the small bowel, the sugar-urea bond of lactose-$^{13}$C ureide is split by al-lantoicase, which is produced by bacteria colonizing the cecum. Ruemmele et al. (1997) reported that human gut tissues possess no allantoicase-like activity. In basic research, we reported that a priming dose of lactose-ureide is also needed for rats to enable the investigation of gastrocecal transit time using lactose-$^{13}$C ureide (Uchida et al., 2009a). Therefore, we evaluated at first the effects of a priming dose of lactose-ureide and the simultaneous administration of lactose-ureide instead of lactose-$^{13}$C ureide on gastric emptying. We found that gastric emptying using $^{[1-13]}$C acetic acid was not significantly affected by the priming dose of lactose-ureide or co-administration of lactose-ureide with $^{[1-13]}$C acetic acid. These results show that lactose-ureide itself does not influence the results of gastric emptying using $^{[1-13]}$C acetic acid.

We evaluated next the effect of acetic acid on the gastrocecal transit time. We found that with the simultaneous administration of acetic acid and lactose-$^{13}$C ureide, gastrocecal transit time was 178 ± 12.8 min, which was not so different from the gastrocecal transit time reported in our previous report (Uchida et al. 2009 a).

Both $^{[1-13]}$C acetic acid and lactose-$^{13}$C ureide were then administered for simultaneous evaluation of gastric emptying and gastrocecal transit time. Gastric emptying time was clearly evaluated, with $T_{max}$ values being almost equal to those obtained when $^{[1-13]}$C acetic acid was administered alone, showing that lactose-$^{13}$C ureide administration did not influence the expired level of $^{13}$CO$_2$ from $^{[1-13]}$C acetic acid. Two methods were used to evaluate gastrocecal transit time; firstly we defined it as the time when the measured value of $^{13}$CO$_2$ was minimal and secondly it was defined as the time when the measured value coincided with the calculated value from the regression curve. According to the first method, gastrocecal transit time was 180 ± 11.5 min and according to the second method, gastrocecal transit time was 190 ± 11.5 min. These values were very close to the value obtained from lactose-$^{13}$C ureide plus acetic acid (178 ± 12.8 min). These findings show that both methods can be used in the calculation of the gastrocecal transit time. However, measurement of the lowest value of expired $^{13}$CO$_2$ may be more convenient as it obviates the need to use a curve fit program. Imamura et al. (2007) in a clinical research study defined the end of the orocecal transit time to be just before the second increase in the $^{13}$CO$_2$ curve, supporting our decision to define the end of the gastrocecal transit time as the time when the measured value of $^{13}$CO$_2$ was minimal.

In conclusion, it was found from the present study that breath testing after administration of both $^{[1-13]}$C acetic acid and lactose-$^{13}$C ureide was a successful method for the simultaneous evaluation of gastric emptying and gastrocecal transit time in conscious rats. Moreover, the gastrocecal transit time was simply defined as the time when the measured value of $^{13}$CO$_2$ was at its lowest value.

References

Akimoto, K., Inamori, M., Iida, H., Endo, H., Akiyama, T., Ikeda, T., Fujita, K., Takahashi, H., Yoneda, M., Goto, A., Abe, Y., Kobayashi, N., Kirikoshi, H., Kubota, K., Saito, S. and Nakajima, A. (2009). Does postprandial coffee intake enhance gastric emptying?: a crossover study using continuous real time $^{13}$C breath test (Breath ID system). *Hepatogastroenterol.* **56**: 918–920.
Cardoso-Júnior, A., Coelho, L.G., Savassi-Rocha, P.R., Vignolo, M.C., Abrantes, M.M., de Almeida, A.M., Dias, E.E., Vieira Júnior, G., de Castro, M.M. and Lemos, Y.V. (2007). Gastric emptying of solids and semi-solids in morbidly obese and non-obese subjects: an assessment using the $^{13}$C-octanoic acid and $^{13}$C-acetic acid breath tests. Obes. Surg. 17: 236–241.

Galati, J.S., Holdeman, K.P., Bottjen, P.L. and Quigley, E.M. (1997). Gastric emptying and orocecal transit in portal hypertension and end-stage chronic liver disease. Liver Transpl. Surg. 3: 34–38.

Geypens, B., Bennink, R., Peeters, M., Evenepoel, L., Maes, B., Ghoos, Y. and Rutgeerts, P. (1999). Validation of the lactose-[$^{13}$C]ureide breath test for determination of orocecal transit time by scintigraphy. J. Nucl. Med. 40: 1451–1455.

Ghoos, Y.F., Maes, B.D., Geypens, B.J., Mys, G., Hiele, M.I., Rutgeerts, P.J. and Vantrappen, G. (1993). Measurement of gastric emptying rate of solids by means of a carbon-labeled octanoic acid breath test. Gastroenterol. 104: 1640–1647.

Gonzalez, R., Dunkel, R., Koletzko, B., Schusdziarra, V. and Allescher, H.D. (1998). Effect of capsaicin-containing red pepper sauce suspension on upper gastrointestinal motility in healthy volunteers. Dig. Dis. Sci. 43: 1165–1171.

Heading, R., Tothill, P., McLoughlin, G. and Shearman, D. (1976). Gastric emptying rate measurement in man. A double isotope scanning technique for simultaneous study of liquid and solid components of a meal. Gastroenterol. 71: 45–50.

Hoshino, T., Oikawa, T., Endo, M. and Hanawa, T. (2008). The utility of noninvasive $^{13}$C-acetate breath test using a new solid test meal to measure gastric emptying in mice. J. Smooth Muscle Res. 44: 159–165.

Imamura, H., Konagaya, T., Kaneko, H., Ohno, K. and Kamakura, S. (2007). Reliability of simultaneous measurement of gastric emptying and orocecal transit time by breath test. J. Aichi Med. Univ. Assoc. 35: 79–86 (in Japanese with English abstract).

Katsube, T., Konnno, S., Murayama, M., Yoshimitsu, K., Shiozawa, S., Shimakawa, T., Naritaka, Y. and Ogawa, K. (2007). Gastric emptying after pylorus-preserving gastrectomy: assessment using the $^{13}$C-acetic acid breath test. Hepatogastroenterol. 54: 639–642.

Kim, D.Y., Myung, S.J. and Camilleri, M. (2000). Novel testing of human gastric motor and sensory functions: rationale, methods, and potential application in clinical practice. Am. J. Gastroenterol. 295: 3365–3373.

Matsumoto, K., Kimura, H., Tashima, K., Uchida, M. and Horie, S. (2008). Validation of $^{13}$C-acetic acid breath test by measuring effects of loperamide, morphine, mosapride, and itopride on gastric emptying in mice. Biol. Pharm. Bull. 31: 1917–1922.

Nishimura, N., Yamamoto, H., Yano, T., Hayashi, Y., Sato, H., Miura, Y., Shinhata, H., Sunada, K. and Sugano, K. (2011). Balloon dilation when using double-balloon enteroscopy for small-bowel strictures associated with ischemic enteritis. Gastrointest. Endosc. 74: 1157–1161.

Nonaka, T., Kessoku, T., Ogawa, Y., Yanagisawa, S., Shiba, T., Sahaguchi, T., Atsukawa, K., Takahashi, H., Sekino, Y., Iida, H., Hosono, K., Endo, H., Sakamoto, Y., Koide, T., Takahashi, H., Tokoro, C., Abe, Y., Maeda, S., Nakajima, A. and Inamori, M. (2011). Does postprandial itopride intake affect the rate of gastric emptying? A crossover study using the continuous real time $^{13}$C breath test (Breath ID system). Hepatogastroenterol. 58: 224–228.

Ruemmele, F.M., Heine, W.E., Keller, K.M. and Lentze, M.J. (1997). Metabolism of glycosyl ureides by human intestinal brush border enzymes. Biochim. Biophys. Acta. 1336: 275–280.

Sakamoto, Y., Kato, S., Sekino, Y., Sakai, E., Uchiyama, T., Iida, H., Hosono, K., Endo, H., Fujita, K., Koide, T., Takahashi, H., Yoneda, M., Tokoro, C., Goto, A., Abe, Y., Kobayashi, N., Kubota, K., Maeda, S., Nakajima, A. and Inamori, M. (2011). Effects of domperidone on gastric emptying: a crossover study using a continuous real-time $^{13}$C breath test (Breath ID system). Hepatogastroenterol. 58: 637–641.

Uchida M. (2011) $^{13}$C-Breath Test for Studying Physiology and Pathophysiology by Using Experimental Animals. Gas Biology Research in Clinical Practice. Ed by Yoshikawa T, Naito Y: Basel, Karger, pp100–111.
Uchida, M., Endo, N. and Shimizu, K. (2005). Simple and noninvasive breath test using $^{13}$C-acetic acid to evaluate gastric emptying in conscious rats and its validation by metoclopramide. *J. Pharmacol. Sci.* 98: 388–395.

Uchida, M. and Mogami, O. (2008). Usefulness of breath test for evaluating pancreatic exocrine function using N-benzoyl-L-tyrosyl-$^{13}$C-L-alanine sodium in non-invasive and conscious rats. *Biol. Pharm. Bull.* 31: 785–788.

Uchida, M. and Shimizu, K. (2007). $^{13}$C-acetic acid is more sensitive than $^{13}$C-octanoic acid for evaluating gastric emptying of liquid enteral nutrient formula by breath test in conscious rats. *Biol. Pharm. Bull.* 30: 487–489.

Uchida, M. and Yoshida, K. (2009a). Non-invasive method for evaluation of gastrocecal transit time by using breath test in conscious rats. *J. Pharmacol. Sci.* 110: 227–230.

Uchida, M., Yoshida, K. and Shimizu, K. (2009b). Effect of atropine sulfate on gastric emptying and gastrocecal transit time evaluated by using the [1-$^{13}$C] acetic acid and lactose-$^{[13}$C] ureide breath test in conscious rats. *J. Breath Res.* 3: 047003.

Urita, Y., Hike, K., Torii, N., Kikuchi, Y., Sasajima, M. and Miki, K. (2002). Efficacy of lactulose plus $^{13}$C-acetate breath test in the diagnosis of gastrointestinal motility disorders. *J. Gastroenterol.* 37: 442–448.

Wutzke, K.D. and Schütt, M. (2007). The duration of enzyme induction in orocecal transit time measurements. *Eur. J. Clin. Nutr.* 61: 1162–1166.

Yen, H.H., Chen, Y.Y., Yang, C.W., Liu, C.K. and Soon, M.S. (2012). Clinical impact of multidetector computed tomography before double-balloon enteroscopy for obscure gastrointestinal bleeding. *World J. Gastroenterol.* 18: 692–697.