Short Communication

Non-invasive Method for Evaluation of Gastrocecal Transit Time by Using a Breath Test in Conscious Rats

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Abstract. The aim of this study was to establish a method for evaluating gastrocecal transit time using a simple and noninvasive ¹³C breath-test system in conscious rats. After fasting, rats were orally administered lactose-[¹³C]ureide. ¹³CO₂ level in the expired air was measured at appropriate intervals. It was confirmed that priming dose of lactose-ureide was necessary for determining gastrocecal transit time. The level of ¹³CO₂ in the expired air increased in a lactose-[¹³C]ureide dose-dependent manner. The gastrocecal transit time determined with 30 mg/kg of lactose-[¹³C]ureide was 160 ± 24.5 min. This method is expected to contribute to the further understanding of intestinal pathophysiology.

Keywords: lactose-[¹³C]ureide, breath test in rats, gastrocecal transit time

In the clinical setting, gamma radiosciintigraphy is generally accepted as the gold standard for measuring small intestinal transit time (1–3). However, this method is stressful and involves radiation exposure. Therefore, the lactulose breath hydrogen test described by Bond and Levitt (4, 5) is now widely used to measure orocecal transit time. Recently, ¹³C-breath tests have been developed as a nonradioactive alternative. This test, in particular, is widely employed to monitor *Helicobacter pylori* infections. Lactose-[¹³C]ureide has also been used for investigating orocecal transit time. Geypens et al. (6) investigated the validity of the lactose-[¹³C]ureide breath test by comparing it with a well-established method, namely scintigraphy using ^{99m}Tc-sulfur colloid, and concluded that the breath test using lactose-[¹³C]ureide is a valid alternative to scintigraphy techniques for measuring orocecal transit time.

Despite the wide use of the breath-test system in the clinical setting, there has been no widely accepted and non-invasive method for measuring orocecal transit time in experimental animals. Recently, we developed a simple and non-invasive breath-test system for monitoring gastric emptying in conscious rats (7). The aim of the present study was to establish a method for evaluating

gastrocecal transit time by using lactose-[¹³C]ureide. Moreover, we also tested the necessity of a priming dose of lactose-ureide because it is necessary for determining oroorectal transit time in humans.

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.

Male Sprague-Dawley rats weighing about 200 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. The breath test was performed according to our previously reported method (7). In brief, this system is composed of an animal chamber (desiccator, 2000 ml), pump (Masterflex L/S; Cole-Palmer Inst. Co., Vernon Hills, IL, USA) and breath-sampling bag (Otsuka Pharmaceutical Co., Ltd., Tokyo). Collected ¹³CO₂ air was measured with infrared analyzers (UBiT IR-300 and UBiT-AS10; Otsuka Electronics Ltd., Osaka). The rats were placed in the chamber immediately after the oral administration of lactose-[¹³C]ureide. The air in the chamber was continuously aspirated during the experimental period. The expired air was collected at 60-min intervals during a 0–180-min period and at 20-min intervals during a 180–420-min period. Aspiration volume was set for 150 ml/min. At each sample point, the expired air

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was collected into a breath-sampling bag for 1.5 min. Aspirated air was discharged outside this breath-test system except for the period of collecting expired air in the breath-sampling bag. Mixed gas being composed of 5% CO₂ and 95% O₂ was used for the control. In this system, the concentration of CO₂ in the aspirated air in the breath-sampling bag was at least more than 1%. The ¹³CO₂ levels were measured by placing the breath-sampling bags into the sample joint of the infrared analyzer. The measured values were presented as the $\Delta^{13}\text{CO}_2$ (‰).

Gastrocecal transit time defined as the time when the value of ¹³CO₂ in the tested group was over the control range at each measured point (> mean of 4 control measurements + 2 S.D.) after the administration of lactose-[¹³C]ureide. The maximum concentration (C_{max} , ‰), the time taken to reach the maximum concentration (T_{max} , min) and the area under the curve ($\text{AUC}_{420\text{min}}$, ‰ · min) were calculated using the $\Delta^{13}\text{CO}_2$ values. C_{max} and $\text{AUC}_{420\text{min}}$ reflect the absorption of labeled materials.

In the first series of experiments, the priming effect of lactose-ureide was investigated. 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 h. In the control rats, distilled water was administered instead of lactose-ureide. After fasting, lactose-[¹³C]ureide at a dose of 60 mg/kg was administered orally in both lactose-ureide-treated rats and control rats. In the second series of experiments, the optimal dosage of lactose-[¹³C]ureide was determined. Three doses of lactose-[¹³C]ureide (30, 60, and 120 mg/kg) were administered orally and the breath test was performed. All these rats were treated with lactose-ureide at a dose of 60 mg/kg twice a day before the experiment. In the control rats, distilled water was administered orally instead of lactose-[¹³C]ureide.

Lactose-[¹³C]ureide and lactose-ureide were purchased from Cambridge Isotope Laboratories Inc. (Andover, MA, USA) and Otsuka Pharmaceutical Co., Ltd., respectively.

All results are presented as the mean $\pm$ S.E.M. Statistical analyses were performed by using Stat View, Version 5.0.0.0 (SAS Institute Inc., Cary, NC, USA), and P values <0.05 [two-way repeated measures analysis of variance (ANOVA), Bonferroni post test, and one-way ANOVA] were considered as statistically significant.

In the control rats, expired ¹³CO₂ was detected slightly as shown in Fig. 1. On the contrary, expired ¹³CO₂ increased with time and peaked at 360 min and decreased thereafter in lactose-ureide-pretreated rats. A significant difference was observed between the control rats (lactose-ureide non-treated rats) and lactose-ureide-

Fig. 1. Effect of pre-dosing of lactose-ureide on the time course of $\Delta^{13}\text{CO}_2$ in expired air from rats. Values represent the mean $\pm$ S.E.M. ($n=4$). Significant difference was observed between control rats and lactose-ureide pretreated rats [$*P<0.05$, $**P<0.01$ vs. control: analyzed by Bonferroni post test after two-way repeated measures analysis of variance (ANOVA)].

Fig. 2. Effect of the dose of lactose-[¹³C]ureide on the time course of $\Delta^{13}\text{CO}_2$ in expired air from rats. Values represent the mean $\pm$ S.E.M. ($n=4$).

treated rats [$P<0.01$, Bonferroni post test after two-way repeated measures analysis of variance (ANOVA)].

In the control rats, expired ¹³CO₂ was slightly detected as shown in Fig. 2. Expired ¹³CO₂ increased with time in a dose-dependent manner, peaked, and decreased thereafter. The values of gastrocecal transit time at doses of 30, 60, and 120 mg/kg were 160 ± 24.5 , 150 ± 17.3 , and 180 ± 0.0 min, respectively, showing no significant difference among the three doses (one way ANOVA). C_{max} values of 30, 60, and 120 mg/kg were $19.2 \pm 2.7\%$, $43 \pm 6.0\%$, and $73.9 \pm 13.0\%$, respectively. A positive correlation was observed between C_{max} values and the doses of lactose-[¹³C]ureide (Fig. 3A). $\text{AUC}_{420\text{min}}$ values of 30, 60, and 120 mg/kg were 2875 ± 376 , 6899 ± 905 , and 10917 ± 2386 ‰ · min, respectively. A positive correlation was observed between $\text{AUC}_{420\text{min}}$ values and the doses of lactose-[¹³C]ureide (Fig. 3B). T_{max} values of 30, 60, and 120 mg/kg were 330 ± 35.1 , 370 ± 17.3 , and 380 ± 28.3 min, respectively.

In the present study, we used lactose-[¹³C]ureide as a tool to investigate the gastrocecal transit in rats based

Fig. 3. Correlation between C_{max} and dose of lactose-[¹³C]ureide (A) and between AUC_{420min} and dose of lactose-[¹³C]ureide (B). Positive correlations were observed between the two parameters (A: $y = 39.4 \ln(x) - 116.1$, $r = 0.997$; B: $y = 5801 \ln(x) - 16854$, $r = 1$). Values represent the mean $\pm$ S.E.M. ($n = 4$).

on its proven utility in clinical studies. After passing the small bowel, the sugar-urea bond of lactose-[¹³C]ureide is split by allantoicase of bacteria colonizing in the cecum. Split urea is absorbed from the colon and metabolized in the liver to ¹³CO₂. Ruemmele et al. (8) reported that the human gut tissue possesses no allantoicase-like activity. Therefore, lactose-[¹³C]ureide seems to be an appropriate substance to test the orocecal transit time.

Wutzuke and Schutt (9) investigated the relationship between the timing of lactose-ureide pre-dosing and the orocecal transit time in humans and found that pre-dosing on the day before the measurement is the most appropriate measurement condition for the orocecal transit time. Then, in this study we investigated the effect of the priming dose of lactose-ureide. In the rats without pre-dosing of lactose-ureide, ¹³CO₂ was only slightly detected in the expired air. On the contrary, ¹³CO₂ was detected in the expired air of lactose-ureide pretreated rats. These results show that the priming dose is necessary for the evaluation of gastrocecal transit time in the rat breath test as suggested in humans. However, there was a difference between humans and rats. In the human study, even with no pre-dosing of lactose-ureide, ¹³CO₂ was detected even though the level of expired ¹³CO₂ was significantly lower than those of animals

given pre-dosing of this compound. Van Den Driessche et al. (10) reported that in all children who performed the breath test after induction of unlabeled lactose-ureide three times the prior day, the mean orocecal transit time in the group of 20 children aged 3 to 17 years was 255 min (rang of 165 – 390 min), but in all infants aged less than 6 months and in preterm infants, the ¹³CO₂ signal was absent, indicating that those individuals were unable to hydrolyze lactose-ureide. Therefore, these differences may be caused by the different intestinal flora between children and infants, and humans and rats, because the lactose-[¹³C]ureide bond is split by bacterial allantoicase. Further studies on the intestinal flora in humans and rats would clarify the cause of age- or species-related differences in orocecal transit time determined by the ¹³C-breath test.

Gastrocecal transit time determined by using 30 mg/kg of lactose-[¹³C]ureide was 160 ± 24.5 min and was not affected significantly by increasing the dose of lactose-[¹³C]ureide in the dose range of 30 – 120 mg/kg. The limitation of the present study was insufficient frequency of measurement during the 0 – 180-min period. More precise data would be obtained by increasing the reading points.

C_{max} values increased in a dose-dependent manner, and a positive correlation was observed between C_{max} values and the doses of lactose-[¹³C]ureide. Moreover, AUC_{420min} values and doses of lactose-[¹³C]ureide showed a positive correlation. These results suggest that a dosage between 30 and 120 mg/kg is suitable for evaluating gastrocecal transit time in rats.

The present study established a non-invasive and feasible method for the evaluation of rat gastrocecal transit by using the ¹³C breath-test system. This method is expected to contribute to further understanding of intestinal pathophysiology. Moreover, it was confirmed that the priming dose of lactose-ureide is necessary in the breath test also in rats as indicated in previous clinical studies.

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