The utility of noninvasive ^{13}C -acetate breath test using a new solid test meal to measure gastric emptying in mice

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Abstract

In clinical and experimental settings, the ^{13}C breath test is performed to measure gastric emptying and has advantages of noninvasiveness and repeatability. We intended to apply the ^{13}C breath test method to mice with an easy-to-handle solid test meal that is more physiological than liquid meals. Male *ddY* mice were trained to eat ^{13}C -acetate-containing pellets as the solid test meal. Thirty minutes after administration of metoclopramide (0.3–3 mg/kg, *p.o.*) or atropine sulfate (0.3–3 mg/kg, *i.p.*), mice received the test meal and were placed in chambers. The $^{13}\text{CO}_2$ levels in the expired air were measured and the maximum concentration ($C_{\max}$; %) and the time to reach the maximum concentration ($T_{\max}$; min) were determined. Metoclopramide significantly and dose-dependently increased $C_{\max}$ and decreased $T_{\max}$. On the other hand, atropine sulfate significantly and dose-dependently decreased $C_{\max}$ and increased $T_{\max}$. The ^{13}C -acetate breath test using a solid test meal is sensitive enough to detect both enhanced and delayed gastric emptying of the reference drugs.

Key words: breath test, gastric emptying, ^{13}C -acetate, metoclopramide, atropine sulfate

Introduction

In the clinical setting, the ^{13}C -urea breath test is widely used to diagnose *Helicobacter pylori* infections (Ohara *et al.*, 1998) because of its ease of sampling and simple analytical technique. Recently, other ^{13}C breath tests have been devised to evaluate digestive organ function noninvasively. Gastric emptying is well assessed by the ^{13}C -acetate breath test with both liquid and semisolid test meals, which is considered to be a substitute for the radioactive-isotope method (Braden *et al.*, 1995; Urita *et al.*, 2002).

In the experimental setting as well, ^{13}C breath tests to measure gastric emptying in small laboratory animals have recently been reported to be both repeatable and noninvasive (Symonds

et al., 2000; Schoonjans *et al.*, 2002; Kitazawa *et al.*, 2005; Choi *et al.*, 2007). In particular, the system developed by Uchida *et al.* (2005, 2007) is superior to the other methods, because the instruments are readily available and the experimental animals are allowed to move freely in chambers allowing studies under physiological conditions. For now, however, the system is applied using only liquid test meals, which not a routine dietary form and must be administered by the stressful gavage technique.

In this study, we applied the system to mice and established a less invasive breath test method with a new, easy-to-handle solid test meal.

Methods

Animals

Male *ddY* mice (36–38 g, Japan SLC, Hamamatsu, Japan) were obtained at 10 weeks of age and housed under a 12-hour light/dark cycle at room temperature ($23 \pm 2^\circ\text{C}$) with a relative humidity of $55 \pm 10\%$. Food and water were supplied *ad libitum*. All animal experiments were performed according to the Guidelines for Care and Use of Laboratory Animals at Kitasato Institute and Kitasato University.

Test meal

The solid test meal was prepared as follows. Ninety food pellets (20 mg Bioserv dustless precision pellets, Bioserv Inc., Frenchtown NJ, USA) were placed side by side in a 35-mm Petri dish, soaked with a solution of 1.8-ml of Millipore water and 9-mg of [$1\text{-}^{13}\text{C}$] sodium acetate (Cambridge Isotope Laboratories, Woburn, MA, USA), desiccated at 60°C for eight hours, and then stored at -20°C until needed. We used the two prepared pellets (approximately 40 mg of meal) as a single test meal.

Breath test procedure

Before the experiments, fasted (more than 18 hours) mice were trained twice a week (three or four day intervals) for two weeks to eat the test meal spontaneously within two minutes. In both experiments, the order of allocation was randomly assigned to avoid the influence of habituation. The time interval between the two breath tests was set at more than three days. Before each test, mice were fasted overnight with free access to water in cages with meshed floors. Thirty minutes after administration of test drugs or the control, mice were given the solid test meal and placed in the chambers. To collect the air in the chambers, we used the noninvasive breath test system (Uchida *et al.*, 2005), comprised of four animal chambers (desiccators; 1,850 ml), a pump (Masterflex L/S, Cole-Palmer Inst. Co., USA) and breath sampling bags (Ohtsuka Pharmaceutical Co. Ltd., Japan). Expired air was collected and measured at 2 min intervals until 30 min (Experiment 1) or 40 min (Experiment 2), with additional measurements at 10 min intervals until 60 min. The aspiration volume was 70 ml/min and the concentration of CO_2 in the aspirated air was kept below 3%. The $^{13}\text{CO}_2$ levels in the trapped air were measured by POcone (Ohtsuka Electronics Co., Ltd., Japan) and presented as the $\Delta^{13}\text{CO}_2$ (‰). The CO_2 level of control gas was adjusted to $5 \pm 0.5\%$.

Effect of metoclopramide on gastric emptying (Experiment 1)

Metoclopramide (Astellas Pharma Inc.) was dissolved in distilled water at doses of 0.3–3 mg/kg in a total volume of 10 ml/kg. Thirty minutes after oral administration of metoclopramide or the same amount of control water, mice were given the test meal.

Effect of atropine sulfate on gastric emptying (Experiment 2)

Atropine sulfate (Mitsubishi Tanabe Pharma Co.) was dissolved in sterile water at doses of 0.3–3 mg/kg in a total volume of 10 ml/kg. The doses were determined according to the previous reports (Choi *et al.*, 2007; Qiu *et al.*, 2008). Thirty minutes after intraperitoneal administration of atropine or the same amount of control water, mice were given the test meal.

Data analysis

All data are presented as means $\pm$ SEM. The maximum concentration ($C_{\max}$; %) and the time to reach the maximum concentration ($T_{\max}$; min) were analyzed by a Dunnett's multiple-comparison test. Probabilities less than 5% ($P < 0.05$) were considered statistically significant.

Results

Experiment 1

Excretion curves obtained for the three different doses of metoclopramide and the control are shown in Fig. 1. In control mice, the $C_{\max}$ was $15.0 \pm 0.7\%$ (Table 1). Metoclopramide significantly and dose-dependently increased the $C_{\max}$ at doses of 0.3, 1 and 3 mg/kg (17.3 ± 0.8 , 18.2 ± 0.8 and $20.1 \pm 0.7\%$, respectively).

In control mice, the $T_{\max}$ was 18.2 ± 1.1 min. Metoclopramide significantly decreased the $T_{\max}$ at a dose of 3 mg/kg (13.6 ± 0.8 min).

In time course analysis, the concentration values at 6–10 min were obviously significant for all three doses ($P < 0.01$, detailed data are not shown).

Experiment 2

Excretion curves obtained for the three different doses of atropine sulfate and the control are shown in Fig. 2. In control mice, the $C_{\max}$ was $15.7 \pm 0.9\%$ (Table 2). Atropine sulfate significantly and dose-dependently decreased the $C_{\max}$ at doses of 0.3, 1 and 3 mg/kg (12.0 ± 1.1 , 10.2 ± 0.9 and $9.3 \pm 0.7\%$, respectively).

In control mice, the $T_{\max}$ was 18.3 ± 2.5 min. Atropine sulfate significantly and dose-dependently increased the $T_{\max}$ at doses of 0.3, 1 and 3 mg/kg (28.9 ± 3.2 , 31.3 ± 2.0 and 38.4 ± 4.8 min, respectively).

In time course analysis, the concentration values at 10–20 min were obviously significant for all three doses ($P < 0.01$, detailed data are not shown).

Fig. 1. Effect of metoclopramide on the time course of $\Delta^{13}\text{CO}_2$ in expired air from mice. The values represent the mean $\pm$ SE (n=14).

Table 1. Effect of metoclopramide on the pharmacokinetic parameters of gastric emptying in mice

	Control	Metoclopramide (mg/kg)		
		0.3	1	3
$C_{\max}$ (‰)	15.0 ± 0.7	$17.3 \pm 0.8^*$	$18.2 \pm 0.8^{**}$	$20.1 \pm 0.7^{**}$
$T_{\max}$ (min)	18.2 ± 1.1	16.6 ± 1.4	15.4 ± 1.0	$13.6 \pm 0.8^*$

Values represent the mean $\pm$ SEM (n=14) (*, $P < 0.05$; **, $P < 0.01$).

Discussion

We applied the ^{13}C -acetate breath test with a new solid test meal to mice. In this setting, metoclopramide dose-dependently enhanced gastric emptying and atropine sulfate dose-dependently delayed gastric emptying.

The system previously reported (Uchida *et al.*, 2005) has the advantage of reducing stress, because it allows rats to move freely in the chambers. We adapted this system to mice, by reason of its greater usefulness in transgenic research (Oshinski *et al.*, 2002). Several modifications were needed to adapt the system to mice. The amount of test meal was decreased to 40 mg, which is empirically determined to accomplish an optimal measurement for recording the peaks of excretion curves. In addition, the chamber volume was decreased to 1,850 ml and the rate of air exchange was decreased to 70 ml/min. The previous report showed the effect of aspiration volume in expired air from rats (Uchida *et al.*, 2005). In this study, 70 ml/min was adequate to fulfill both measurable CO_2 concentration and sufficient volume sampling in every two minutes.

The solid test meal, which we newly prepared in this study, is more physiological than a liquid meal, because a liquid meal is not the usual dietary form consumed by mice and requires restraint or gavage-feeding, both of which will affect gastrointestinal motility (Schoonjans *et al.*, 2002). Additionally, the 20 mg pellets are easy to handle and can be used in other types of ^{13}C

Fig. 2. Effect of atropine sulfate on the time course of $\Delta^{13}\text{CO}_2$ in expired air from mice. The values represent the mean $\pm$ SE (n=7 or 8).

Table 2. Effect of atropine sulfate on the pharmacokinetic parameters of gastric emptying in mice

	Control	Atropine sulfate (mg/kg)		
		0.3	1	3
$C_{\max}$ (‰)	15.7 $\pm$ 0.9	12.0 $\pm$ 1.1**	10.2 $\pm$ 0.9**	9.3 $\pm$ 0.7**
$T_{\max}$ (min)	18.3 $\pm$ 2.5	28.9 $\pm$ 3.2*	31.3 $\pm$ 2.0*	38.4 $\pm$ 4.8**

Values represent the mean $\pm$ SEM (n=7, 8) (*, $P<0.05$; **, $P<0.01$).

breath tests. The pellet-type test meal cannot be readily adjusted based on body weight, but the experiments were well conducted by providing a control group. Although a training period is needed with our method, this will contribute to reducing the stress of administration.

In this study, we selected ¹³C-acetate as a substitute for ¹³C-octanoate, which is widely applied in measuring solid gastric emptying since the first report by Ghooos *et al.* (Ghooos *et al.*, 1993), because a recent report revealed the former to be more sensitive than the latter for monitoring gastric emptying in rats (Uchida *et al.*, 2007; Sanaka *et al.*, 2008). As for conscious mice, the behavior of the marker in the aqueous and solid phase has been difficult to evaluate to date.

Moreover, the monitoring interval of 2 min was shorter than those in previous reports and allowed us to conduct a more thorough investigation of gastric emptying. Although a continuous real-time ¹³C breath test is desirable and is possible with the Oridion BreathID system (Shirin *et al.*, 2001), the cost of this system is prohibitive and multiple monitoring is nearly impossible.

Focusing on measurement of gastric emptying, we analyzed $C_{\max}$ and $T_{\max}$ as simple parameters of expiratory curves. Although half-¹³CO₂ recovery ($T_{1/2}$) is routinely used as another parameter in breath tests, $T_{1/2}$ has limited capability to reflect gastric emptying (Sanaka

et al., 2007; Sanaka *et al.*, 2008). Because the animal chambers become additional compartment to delay the recovery of $^{13}\text{CO}_2$ in this setting, $T_{1/2}$ is unsuitable for our purpose.

Our results showed that metoclopramide dose-dependently enhanced gastric emptying and atropine dose-dependently delayed gastric emptying as same as the previous report (Uchida *et al.*, 2005; Pan *et al.*, 2004).

In conclusion, this study has shown that the breath test, using the readily available setting and a new solid test meal, is sensitive enough to detect both enhanced and delayed gastric emptying of the reference drugs. It enables us to perform repeatable and noninvasive pharmacological intervention studies in mice without euthanasia. This new breath test method is anticipated to be a useful tool for whole-animal investigations with ^{13}C compounds.

References

- Braden, B., Adams, S., Duan, L.P., Orth, K.H., Maul, F.D., Lembcke, B., Hor, G. and Caspary, W.F. (1995). The [^{13}C]-acetate breath test accurately reflects gastric emptying of liquids in both liquid and semisolid test meals. *Gastroenterol.* **108**: 1048–1055.
- Choi, K.M., Zhu, J., Stoltz, G.J., Vernino, S., Camilleri, M., Szurszewski, J.H., Gibbons, S.J. and Farrugia, G. (2007). Determination of gastric emptying in nonobese diabetic mice. *Am. J. Physiol.* **293**: G1039–G1045.
- 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.
- Kitazawa, T., De Smet, B., Verbeke, K., Depoortere, I. and Peeters, T. (2005). Gastric motor effects of peptide and non-peptide ghrelin agonists in mice *in vivo* and *in vitro*. *Gut* **54**: 1078–1084.
- Ohara, S., Kato, M., Asaka, M. and Toyota, T. (1998). Studies of ^{13}C -urea breath test for diagnosis of *Helicobacter pylori* infection in Japan. *J. Gastroenterol.* **33**: 6–13.
- Osinski, M.A., Seifert, T.R., Cox, B.F. and Gintant, G.A. (2002). An improved method of evaluation of drug-evoked changes in gastric emptying in mice. *J. Pharmacol. Toxicol. Methods* **47**: 115–120.
- Pan, S.Y. and Han, Y.F. (2004). Comparison of the inhibitory efficacy of four belladonna drugs on gastrointestinal movement and cognitive function in food-deprived mice. *Pharmacol.* **72**: 177–183.
- Qiu, W.C., Wang, Z.G., Wang, W.G., Yan, J. and Zheng, Q. (2008). Gastric motor effects of ghrelin and growth hormone releasing peptide 6 in diabetic mice with gastroparesis. *World J. Gastroenterol.* **14**: 1419–1424.
- Sanaka, M., Yamamoto, T., Nakayama, S., Nagasawa, K. and Kuyama, Y. (2007). Reliability of the time to maximal [$^{13}\text{CO}_2$] excretion and the half-[$^{13}\text{CO}_2$] excretion time as a gastric emptying parameter: assessments using the Wagner-Nelson method. *J. Smooth. Muscle Res.* **43**: 201–209.
- Sanaka, M., Yamamoto, T. and Kuyama, Y. (2008). Retention, fixation, and loss of the [^{13}C] label: a review for the understanding of gastric emptying breath tests. *Dig. Dis. Sci.* **53**: 1747–1756.
- Schoonjans, R., Van Vlem, B., Van Heddeghem, N., Vandamme, W., Vanholder, R., Lameire, N., Lefebvre, R. and De Vos, M. (2002). The ^{13}C -octanoic acid breath test: validation of a new noninvasive method of measuring gastric emptying in rats. *Neurogastroenterol. Motil.* **14**: 287–293.
- Shirin, H., Kenet, G., Shevah, O., Wardi, Y., Birkenfeld, S., Shahmurov, M., Bruck, R., Niv, Y., Moss, S.F. and Avni, Y. (2001). Evaluation of a novel continuous real time ^{13}C urea breath analyser for *Helicobacter pylori*. *Aliment. Pharmacol. Ther.* **15**: 389–394.
- Symonds, E.L., Butler, R.N. and Omari, T.I. (2000). Assessment of gastric emptying in the mouse using

- the [¹³C]-octanoic acid breath test. *Clin. Exp. Pharmacol. Physiol.* **27**: 671–675.
- Symonds, E.L., Butler, R. and Omari, T.I. (2007). A mouse model for assessing the impact of ingested nutrients on gastric emptying rate. *Clin. Exp. Pharmacol. Physiol.* **34**: 132–133.
- Symonds, E.L., Tran, C.D., Butler, R.N. and Omari, T.I. (2008). Gastric emptying is altered with the presence of gastritis. *Dig. Dis. Sci.* **53**: 636–641.
- Uchida, M., Endo, N. and Shimizu, K. (2005). Simple and noninvasive breath test using ¹³C-acetic acid to evaluate gastric emptying in conscious rats and its validation by metoclopramide. *J. Pharmacol. Sci.* **98**: 388–395.
- Uchida, M. and Shimizu, K. (2007). ¹³C-Acetic acid is more sensitive than ¹³C-octanoic acid for evaluating gastric emptying of liquid enteral nutrient formula by breath test in conscious rats. *Biol. Pharm. Bull.* **30**: 487–489.
- Urita, Y., Hike, K., Torii, N., Kikuchi, Y., Sasajima, M. and Miki, K. (2002). Efficacy of lactulose plus ¹³C-acetate breath test in the diagnosis of gastrointestinal motility disorders. *J. Gastroenterol.* **37**: 442–448.