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 $^{13}$CO$_2$ levels in the expired air were measured and the maximum concentration ($C_{\text{max}}$; ‰) and the time to reach the maximum concentration ($T_{\text{max}}$; min) were determined. Metoclopramide significantly and dose-dependently increased $C_{\text{max}}$ and decreased $T_{\text{max}}$. On the other hand, atropine sulfate significantly and dose-dependently decreased $C_{\text{max}}$ and increased $T_{\text{max}}$. 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 ± 2°C) with a relative humidity of 55 ± 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 [1-13C] 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 CO2 in the aspirated air was kept below 3%. The 13CO2 levels in the trapped air were measured by PO Cone (Ohtsuka Electronics Co., Ltd., Japan) and presented as the Δ13CO2 (‰). The CO2 level of control gas was adjusted to 5 ± 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 ± SEM. The maximum concentration (C\(_{\text{max}}\); ‰) and the time to reach the maximum concentration (T\(_{\text{max}}\); 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\(_{\text{max}}\) was 15.0 ± 0.7‰ (Table 1). Metoclopramide significantly and dose-dependently increased the C\(_{\text{max}}\) at doses of 0.3, 1 and 3 mg/kg (17.3 ± 0.8, 18.2 ± 0.8 and 20.1 ± 0.7‰, respectively).

In control mice, the T\(_{\text{max}}\) was 18.2 ± 1.1 min. Metoclopramide significantly decreased the T\(_{\text{max}}\) 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\(_{\text{max}}\) was 15.7 ± 0.9‰ (Table 2). Atropine sulfate significantly and dose-dependently decreased the C\(_{\text{max}}\) at doses of 0.3, 1 and 3 mg/kg (12.0 ± 1.1, 10.2 ± 0.9 and 9.3 ± 0.7‰, respectively).

In control mice, the T\(_{\text{max}}\) was 18.3 ± 2.5 min. Atropine sulfate significantly and dose-dependently increased the T\(_{\text{max}}\) 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).
We applied the 13C-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 CO₂ 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 13C

![Figure 1](image)

**Figure 1.** Effect of metoclopramide on the time course of Δ13CO₂ in expired air from mice. The values represent the mean ± SE (n=14).

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

| Metoclopramide (mg/kg) | Control | 0.3 | 1 | 3 |
|------------------------|---------|-----|---|---|
| C<sub>max</sub> (%)    | 15.0 ± 0.7 | 17.3 ± 0.8* | 18.2 ± 0.8** | 20.1 ± 0.7** |
| T<sub>max</sub> (min)  | 18.2 ± 1.1 | 16.6 ± 1.4 | 15.4 ± 1.0 | 13.6 ± 0.8* |

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

### Discussion

We applied the 13C-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 CO₂ concentration and sufficient volume sampling in every two minutes.

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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 $^{13}$C-acetate as a substitute for $^{13}$C-octanoate, which is widely applied in measuring solid gastric emptying since the first report by Ghoos et al. (Ghoos 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 $^{13}$C 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 $C_{\text{max}}$ and $T_{\text{max}}$ as simple parameters of expiratory curves. Although half-$^{13}$CO$_2$ recovery ($T_{1/2}$) is routinely used as another parameter in breath tests, $T_{1/2}$ has limited capability to reflect gastric emptying (Sanaka

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

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

Values represent the mean ± SEM (n=7, 8) (*, $P<0.05$; **, $P<0.01$).
et al., 2007; Sanaka et al., 2008). Because the animal chambers become additional compartment to delay the recovery of $^{13}$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.

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