^{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

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Breath test using ^{13}C -labeled compound has been used as a convenient method to evaluate gastric emptying. ^{13}C -Labeled acetic acid or octanoic acid has been used in clinic. However, there is few report comparing two compounds. This study aimed to compare ^{13}C -acetic acid and ^{13}C -octanoic acid in newly-constituted breath test for monitoring gastric emptying in conscious rats. After fasting, rats were orally administered Racol (liquid enteral nutrient formula) containing ^{13}C -labeled compound (same molar of ^{13}C -acetic acid or ^{13}C -octanoic acid) and housed in a chamber. 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. Expired $^{13}\text{CO}_2$ air from ^{13}C -acetic acid changed at significantly higher levels than that from ^{13}C -octanoic acid. $C_{\max}$ and $AUC_{120\text{ min}}$ values of expired $^{13}\text{CO}_2$ from ^{13}C -acetic acid were significantly higher than those from ^{13}C -octanoic acid, but $T_{\max}$ was not different between them. These results show that ^{13}C -acetic acid is more sensitive for monitoring gastric emptying than ^{13}C -octanoic acid in liquid test meal although both acids clearly monitored gastric emptying.

Key words breath test in rat; octanoic acid; acetic acid; gastric emptying

For evaluating gastric motility many methods have been reported, such as the gastrotonometry by the infused catheter method or microtransducer method, the radioactive isotope method and the acetaminophen method.¹⁾

In 1993, Ghooos and colleagues²⁾ developed ^{13}C -breath test for monitoring gastric emptying as a nonradioactive alternative method. By using this method, gastric emptying time can be indirectly measured by monitoring the $^{13}\text{CO}_2$ concentration in the expired air after a ^{13}C -labeled substance, such as acetic acid or octanoic acid, was ingested and absorbed from the duodenum.^{1–3)} Thereafter breath tests using ^{13}C -labeled compounds have been used in laboratory animals for evaluating physiological function, such as gastric emptying^{4,5)} or liver function.⁶⁾ Recently, the present authors reported simple and noninvasive breath test system for monitoring gastric emptying by using ^{13}C -labeled acetic acid in conscious rats.⁷⁾

^{13}C -Labeled acetic acid or ^{13}C -labeled octanoic acid has been used generally in clinic for evaluating gastric emptying. ^{13}C -Labeled acetic acid has been used in the form of liquid test meal, such as coffee or juice. On the contrary, ^{13}C -labeled octanoic acid has been used in the form of solid test meal, such as egg yolk or steak. This reason is that octanoic acid is slightly soluble in water and acetic acid in lipid. Therefore, there are few reports comparing ^{13}C -labeled acetic acid and ^{13}C -labeled octanoic acid in the same test meal. Racol is liquid enteral nutrient and used in Japan as a liquid test meal for evaluating gastric emptying in clinic. Although Racol is liquid form, it contains lipid at a concentration of 4.46 g/200 ml. Therefore, octanoic acid can be solved in lipid if added to Racol.

Then, in the present study the expired air pattern from ^{13}C -labeled acetic acid was compared with that from ^{13}C -labeled octanoic acid in the same test meal of Racol by using the breath test system newly developed by the present authors.⁷⁾

MATERIALS AND METHODS

Animals The following animal studies were performed according to “Guiding Principles for the Care and Use of Laboratory Animals” approved by The Japanese Pharmacological Society.

Male Sprague-Dawley rats (200–250 g) were purchased from SLC (Shizuoka, Japan), and fasted in meshed cages for 18 hr to prevent coprophagy before the each experiment, but free access to drinking water.

Evaluation System of Gastric Emptying by Breath Test Schematic illustration of apparatus for monitoring $^{13}\text{CO}_2$ expired air from rats reported by the present authors⁷⁾ was shown in Fig. 1. In brief, this system is composed with animal chamber (desiccator; 2000 ml), pump (Masterflex L/S, Cole-Palmer Inst. Co., U.S.A.) and breath sampling bag (Ohtsuka Pharmaceutical Co. Ltd., Japan). Collected $^{13}\text{CO}_2$ air was measured with UBiT IR-300 and UBiT-AS10 (Ohtsuka Electronics Co., Ltd., Japan).

Rats were placed in the chamber just after the oral admin-

Fig. 1. Schematic Illustration of the System Used for Monitoring $^{13}\text{CO}_2$ Levels in the Expired Air from Rats

Briefly, this system composed a desiccator that was used as animal chambers, a pump and breath-sampling bags. Aspirating the expired air caused fresh air to automatically flow into the desiccator to replace it through a hole in the side of the chamber.

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istration of sample contained ^{13}C -labeled acetic acid or ^{13}C -labeled octanoic acid. Expired $^{13}\text{CO}_2$ air was collected and measured at 5 min intervals until 70 min, followed by at 90 and 120 min after sample administration. Expired $^{13}\text{CO}_2$ air was collected for 1.5 min at each time. Ventilation volume was 150 ml/min.

To compare the sensitivity of ^{13}C -labeled compounds for monitoring gastric emptying, same molar of ^{13}C -labeled compound (0.5 mol/kg), acetic acid or octanoic acid, was used for the sake of one molar of $^{13}\text{CO}_2$ being metabolized from each one molar of ^{13}C -labeled compound. Labeled compounds were solved in Racol (enteral nutritional formula) and administered in a volume of 2.5 ml/kg.

Evaluation Parameters 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 ($AUC_{120\text{min}}$; ‰·min) were calculated using the $\Delta^{13}\text{CO}_2$ values. $C_{\max}$ and $AUC_{120\text{min}}$ reflect the absorption of labeled materials.

Samples ^{13}C -Labeled compounds, [$1\text{-}^{13}\text{C}$] acetic acid and [$1\text{-}^{13}\text{C}$] octanoic acid, and Racol were purchased from Wako Pure Chemical (Tokyo, Japan) and Ohtsuka Pharmaceutical Co., Ltd. (Tokyo, Japan), respectively.

Data Analysis Results were represented as the mean $\pm$ S.E.M. for used animals. Statistical analyses were performed by using a Student's *t*-test, and values of $p < 0.05$ were regarded as significant.

RESULTS

Comparison of ^{13}C -Labeled Acetic Acid and ^{13}C -Labeled Octanoic Acid In the rats treated with ^{13}C -acetic acid, expired $^{13}\text{CO}_2$ air increased in course of time, peaked at about 30 min after ingestion and decreased thereafter (Fig. 2). On the contrary, in the rats treated with ^{13}C -octanoic acid, the levels of expired $^{13}\text{CO}_2$ were significantly lower than those in ^{13}C -acetic acid-treated rats (Fig. 2).

Evaluation Parameters $T_{\max}$, $C_{\max}$ and $AUC_{120\text{min}}$ values were shown in Table 1.

$C_{\max}$ value was significantly higher in acetic acid-treated rats than that in octanoic acid-treated rats ($p < 0.01$).

$T_{\max}$ values in acetic acid-treated rats and octanoic acid treated-rats were almost the same values.

The value of $AUC_{120\text{min}}$ in acetic acid-treated rats was significantly higher than that in octanoic acid-treated rats ($p < 0.01$).

DISCUSSION

It was found that Racol containing ^{13}C -labeled acetic acid was more sensitive than that containing ^{13}C -labeled octanoic acid for monitoring gastric emptying of liquid test meal by breath test in conscious rats.

Diagnosis of impaired gastric emptying and control of therapy is generally based on the assessment of gastric emptying of solids because disturbances of solid emptying preceded impairment of liquid emptying.⁸⁾ Duan and colleagues⁹⁾ reported that in patient with dyspepsia, gastric half emptying times of solids were significantly delayed as compared to the emptying of solid in the controls and that the gastric emptying of liquids did not differ in patients and con-

Fig. 2. Time Course of $\Delta^{13}\text{CO}_2$ in Expired Air from Rats Administered ^{13}C -Labeled Acetic Acid or ^{13}C -Labeled Octanoic Acid in Rats

The values represent the mean $\pm$ S.E.M. ($n=4$). *,** Significantly different from the rats treated ^{13}C -labeled acetic acid ($p < 0.05$, 0.01).

Table 1. Pharmacokinetic Parameters after Oral Administration of ^{13}C -Acetic Acid or ^{13}C -Octanoic Acid in Rats

	^{13}C -Acetic acid	^{13}C -Octanoic acid
$C_{\max}$ (‰)	246.0 ± 15.5	$127.3 \pm 18.6^{**}$
$T_{\max}$ (min)	31.3 ± 2.4	35.0 ± 2.9
$AUC_{120\text{min}}$ (‰·min)	17353 ± 1387	$10897 \pm 893^{**}$

Values represent the mean $\pm$ S.E.M. ($n=4$). ** Significantly different from ^{13}C -acetic acid group ($p < 0.01$).

trols. On the other hand, Braden and colleagues³⁾ reported that the ^{13}C -labeled acetate breath test accurately reflects gastric emptying of liquids in both liquid and semisolid test meals.

Then, in this study, we compared ^{13}C -labeled acetic acid with ^{13}C -labeled octanoic acid in the form of liquid test meal for monitoring gastric emptying in conscious rats and used liquid nutrient, Racol, because it is difficult to administer solid test meal containing ^{13}C -labeled compound to rats, although ^{13}C -labeled octanoic acid and ^{13}C -labeled acetic acid were used in clinic to measure gastric emptying of solids and liquids, respectively.¹⁰⁾ For strictly comparing the specificity of two ^{13}C -labeled compounds in breath test, we used the same molar of labeled compound, because molar difference affects the amount of expired $^{13}\text{CO}_2$ air.

After the administration of Racol containing ^{13}C -labeled compound, expired $^{13}\text{CO}_2$ air increased in course of time, reached peak and decreased thereafter. $T_{\max}$ values were almost the same between two labeled compounds. This finding shows that gastric emptying was almost the same in both compounds. However, expired $^{13}\text{CO}_2$ from ^{13}C -labeled acetic acid changed at significantly higher levels as compared with that from ^{13}C -labeled octanoic acid. Moreover, $AUC_{120\text{min}}$ of acetic acid was also significantly higher than that of octanoic acid. In this study, we used same molar of compounds. If one molar of labeled compound metabolized, same molar of $^{13}\text{CO}_2$ would be expired in the air. Therefore, we speculated that the difference in the change of expired $^{13}\text{CO}_2$ air might be caused by the different absorption rate and metabolic rate of labeled compound in the intestine and the liver, respectively.

On the other hand, Beauvieux and colleagues¹¹⁾ reported that the addition of acetate and octanoate stabilized ATP level at 58% and 45% of the initial level in isolated liver from fed rats, respectively, suggesting that acetate is metabo-

lized at lower energy and keep ATP at higher level as compared with octanoate. In other words, acetic acid may be more effectively metabolized to CO₂ as compared with octanoic acid. On this point further studies would be needed.

Duan and colleagues⁹⁾ reported that gastric emptying of solids was significantly accelerated by cisapride both in the patients with functional dyspepsia and in the controls and that the gastric emptying of liquids did not differ in patients and controls, and cisapride had no effect on the emptying of liquids within the normal range. However, in our preliminary experiments by using this breath test system and liquid test meal (Racol) containing ¹³C-labeled acetic acid, mosapride, serotonin 4 receptor agonist, significantly enhanced the gastric emptying (data not shown). This finding supports usefulness of our breath test system for evaluating gastric emptying even using liquid test meal.

In conclusion, it was found that Racol containing ¹³C-labeled acetic acid was more sensitive than that containing ¹³C-labeled octanoic acid from the findings that the values of C_{max} and AUC_{120 min} from ¹³C-labeled acetic acid were significantly higher than those from ¹³C-labeled octanoic acid, although T_{max} values were almost the same between two labeled compounds.

REFERENCES

- 1) Kim D. Y., Myung S. J., Camilleri M., *Am. J. Gastroenterol.*, **95**, 3365—3373 (2000).
- 2) Ghos Y. F., Maes B. D., Geypens B. J., Mys G., Hiele M. I., Rutgeerts P. J., Vantrappen G., *Gastroenterology*, **104**, 1640—1647 (1993).
- 3) Braden B., Adams S., Duan L. P., Orth K. H., Maul F. D., Lembcke B., Hor G., Caspary W. F., *Gastroenterology*, **108**, 1048—1055 (1995).
- 4) Symonds E. L., Butler R. N., Omari T. I., *Clin. Exp. Pharmacol. Physiol.*, **27**, 671—675 (2000).
- 5) Schoonjans R., Van Vlem B., Van Heddeghem N., Vandamme W., Vanholder R., Lameire N., Lefebvre R., De Vos M., *Neurogastroenterol. Motil.*, **14**, 287—293 (2002).
- 6) Ishii Y., Asai S., Kohno T., Suzuki S., Ishii M., Hosoi I., Fuji M., Iwai S., Ishikawa K., *J. Surg. Res.*, **86**, 130—135 (1999).
- 7) Uchida M., Endo N., Shimizu K., *Pharmacol. Sci.*, **98**, 388—395 (2005).
- 8) Chaudhuri T. K., Fink S., *Am. J. Gastroenterol.*, **86**, 533—538 (1991).
- 9) Duan L. P., Braden B., Caspary W. F., Lembcke B., *Dig. Dis. Sci.*, **40**, 2200—2206 (1995).
- 10) Gonzalez A., Monreal I., Mugueta C., Gil M. J., Betes M., Varo N., *Rev. Med. Univ. Navarra*, **42**, 83—90 (1998).
- 11) Beauvieux M. C., Tissier P., Gin H., Canioni P., Gallis J. L., *J. Nutr.*, **131**, 1986—1992 (2001).