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Effect of Concomitant ^{18}O in ^{13}C-Urea on the Urea Breath Test

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Summary

Helicobacter pylori infection in stomach has been extensively assessed by the ^{13}C-urea breath test(UBT) with infra-red or mass spectrometer. However, analyses of commercially available ^{13}C-urea for UBT showed about 10 atom% of ^{18}O abundance. It is therefore very important to investigate the ^{18}O-isotope effect on UBT. Using a mass spectrometer, breath samples of UBT were analyzed to investigate the change of mass spectra by the isotopic exchange reaction between ^{18}O of carbon dioxide and ^{16}O of water. As the result, the ^{18}O content of ^{13}C, ^{18}O-carbon dioxide exhaled in UBT reached its natural abundance level immediately after the administration of ^{13}C, ^{18}O-urea. It was concluded that the ^{18}O content of ^{13}C, ^{18}O-urea has no effect on UBT using either infra-red or mass spectroscopy.

Key words: ^{18}O-Isotopic Exchange Reaction, Urea Breath Test, ^{13}C, ^{18}O -Urea, Mass Spectrometry

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Introduction

The ^{13}C-urea breath test (UBT) has been widely used to diagnose *Helicobacter pylori* (HP) infection, closely related to gastritis, gastric ulcers and gastric cancer (1). This unique diagnosis exploits two facts, that HP is a potent urease producer and that ^{13}C-carbon dioxide derived from ^{13}C-urea can be selectively detected in breath.

In our previous report, we established the analytical method of ^{18}O abundance in ^{13}C, ^{18}O-carbon dioxide or ^{13}C, ^{18}O-urea (2). The ^{18}O abundance in commercially available ^{13}C-urea has been found in the range from 0.2 to 12 atom%. Of late most of ^{13}C-urea contain approximately 10 atom% of ^{18}O. It is quite possible that the high ^{18}O content causes not only the isotopic shift in infrared spectra but also affects the mass spectra of ^{18}O-isotope species of carbon dioxide exhaled in UBT (2, 3). These spectral changes would result in the wrong diagnosis for gastric HP infection. It is therefore very important for UBT to investigate the ^{18}O-isotope effect on infra-red or mass spectra. From a clinical standpoint, we had to prove that the concomitant ^{18}O in ^{13}C, ^{18}O-urea did not entirely affect the routinely available UBT. Here it should be noted that the isotopic exchange reaction occurs between ^{18}O of carbon dioxide and ^{16}O of water. Mass spectrometry was used in this study, since it could easily monitor the spectral change by the isotope exchange reaction. Preliminary the isotopic exchange reaction between ^{18}O of carbon dioxide and ^{16}O of excess water was first investigate. The reaction required more than one hour to attain to the equilibrium state. Subsequently ^{13}C, ^{18}O-carbon dioxide formed in UBT was analyzed to investigate whether the ^{18}O abundance in ^{13}C, ^{18}O-urea affects mass spectra of UBT or not.

In this report, the isotopic ^{18}O-exchange reaction of ^{13}C, ^{18}O-carbon dioxide exhaled in UBT is described in detail.

Materials & Methods

Urea (Lot No. MTI-10390-S1) containing 9.85 atom% ^{18}O and 99.14 atom% ^{13}C was obtained from MassTrace (Woburn, USA) for UBT. 2L plastic bags for breath samples of UBT were purchased

from GL Sciences Inc. (Tokyo, Japan). Breath samples of UBT were analyzed using a Finnigan mass spectrometer Model delta E (Bremen, Germany).

The isotopic exchange reaction between ^{18}O and ^{16}O in carbon dioxide exhaled in UBT was investigated for five healthy volunteers. Three subjects of them were diagnosed as HP-positive and the other two as HP-negative by routine UBT. They were fasted overnight, brushed their teeth and mouth-rinsed thoroughly with tap water to avoid the effect of urease-producing microbial flora in oral cavity (4, 5). Prior to the ingestion of ^{3}C, ^{18}O-urea, the breath sample for the baseline was collected in an aluminized bag. 100 mg of ^{13}C, ^{18}O-urea, which was dissolved in 100 ml of tap water, was orally administered in sitting position. Breath samples were collected in bags at 5, 10, 15, 20, 30, 45 and 60 min after the administration. About 100 mL of breath gas immediately after each sampling was transferred into a 100 mL vacuum bottle using 50 mL syringe. Carbon dioxide was separated from the sample in vacuum using liquid nitrogen and dry-ice methanol to trap in a glass tube within 5 min (3). ^{18}O and ^{13}C abundance in purified carbon dioxide was analyzed using the mass spectrometer and expressed as $\Delta\delta$. The enrichment of stable isotope is expressed as the excess (δ) of a paticular isotope species being measured to a known reference material. For UBT, the change in δ value over baseline can be described as $\Delta\delta$.

Results & Discussion

Figure 1 shows the time course of $\Delta\delta$ (^{13}C) for HP-positive (P1～P3)and -negative subjects (N1, N2). $\Delta\delta$ (^{13}C) values for HP-positive subjects were usually higher than those for HP-negative ones during whole UBT. Using 2.5‰ as a cut-off value at 10 min, three HP-positive subjects were reassessed to be HP-positive and the other two HP-negative. One HP-negative subject (N1) gave a small peak at 5 min as shown in Figure 1. This shows that even careful mouth rinsing cannot completely exclude the hydrolysis of ^{13}C-urea by microbial flora in oral cavity (4, 5).

Time course of $\Delta\delta$ (^{18}O) in UBT for HP-positive (P1～P3) and -negative subjects (N1～N2) is presented in Table 1. In the table, there is no significant difference between $\Delta\delta$ (^{18}O) of all

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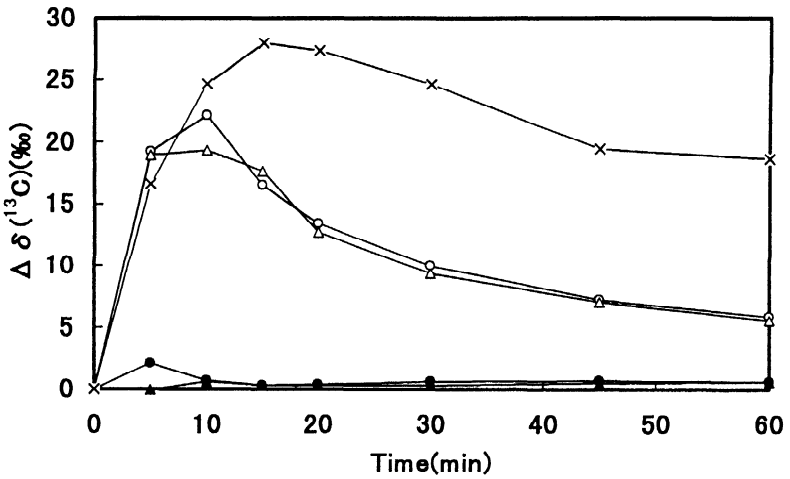

Figure 1; Changes of Δ δ (^{13}C) in ^{13}C-urea breath test for H. pylori -positive (P) and -negative (N) subjects. ●;N1, ▲;N2, ○;P1, △;P2, ×;P3.

Table 1; Changes of Δ δ (^{18}O) in ^{13}C-urea breath test for *H. pylori*-positive (P) and -negative (N) subjects.

Time(min)	HP-negative(‰)		HP-positive(‰)		
	N1	N2	P1	P2	P3
0	0	0	0	0	0
5	-0.35	0.01	0.44	-0.02	-0.03
10	0.04	-0.04	0.33	-0.28	-0.12
15	-0.27	-0.07	0.50	-0.21	0.10
20	-0.21	-0.06	0.40	-0.14	-0.06
30	-0.2	-0.01	0.18	-0.02	-0.10
45	-0.37	-0.13	0.37	-0.02	-0.07
60	-0.11	0.18	0.61	-0.13	-0.06
Average±SD*	-0.20±017	-0.02±0.09	0.36±0.18	-0.05±0.14	-0.04±0.07

*: Standard deviation.

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subjects within 60 min after the administration of ^{13}C-urea. This proved that ^{18}O of carbon dioxide exchanged completely ^{16}O even in the earlier UBT. In other words the ^{18}O content of ^{13}C, ^{18}O -urea cannot affect the routine UBT using either infra-red or mass spectrometer.

The effect of ^{18}O in carbon dioxide on UBT can be estimated using mass spectrometry and an equation of Craig correction, which is used to compensate for the contribution of ^{17}O to m/z 45 (5). This correction can be calculated using $\delta(^{18}O)$ instead of $\delta(^{17}O)$. Accordingly the ^{18}O abundance of ^{13}C, ^{18}O-urea affects the Craig correction to a large extent. The atomic delta($\delta\ ^{13}C$) for ^{13}C can be calculated from the molecular δ_{45} and $\delta(^{18}O)$ as follows:

$$\delta^{13}C = 1.0676\,\delta_{45} - 0.0338\,\delta(^{18}O)$$

Effect of ^{18}O on $\delta^{13}C$ reflects the second term of the above equation. As listed in Table 1, only a small alteration in $\Delta\,\delta(^{18}O)$, i.e., $\delta(^{18}O)$ was observed for both HP-positive and -negative subjects. Even the maximum change of $\Delta\,\delta(^{18}O)$ or $\delta(^{18}O)$ was only 0.61‰, which corresponded to the contribution of 0.021‰ to $\delta^{13}C$. The result obtained here shows clearly that the ^{18}O abundance of commercially available ^{13}C-urea has no effect on the clinical assessment of HP infection by standard UBT. More precisely the isotopic exchange reaction between ^{18}O and ^{16}O *in vivo* is very fast compared to that *in vitro* (in water). This suggests that carbonic anhydrase takes part in the isotopic exchange reaction in UBT.

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