Validity of the ^{13}C-Urea Breath Test for the Diagnosis of *Helicobacter pylori* Infection

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Infection with *Helicobacter pylori* (*H. pylori*) plays an important role in the pathogenesis of gastritis, peptic ulcer and gastric cancer, and the ^{13}C-urea breath test (^{13}C-UBT) is a convenient and non-invasive method for the detection of *H. pylori* in the stomach. We have examined the sensitivity, specificity and accuracy of ^{13}C-UBT.

The $^{13}CO_2/^{12}CO_2$ ratio was measured using infrared spectroscopy (IR) and gas chromatography/mass spectrometry (GC-MS).

Key words *Helicobacter pylori*; ^{13}C-urea breath test; diagnosis; infrared spectroscopy; gas chromatography/mass spectrometry

We have previously developed a breath test involving the administration of ^{13}C-phenacetin and the detection of $^{13}CO_2$ in exhaled air for the diagnosis of liver disease without blood sampling.[1] We have also employed a breath test using ^{13}C-methacetin to study the relationship between liver function and atopic dermatitis.[2] The ^{13}C-urea breath test (^{13}C-UBT) is used for non-invasive diagnosis of *Helicobacter pylori* (*H. pylori*) infection, which is involved in the pathogenesis of gastritis, peptic ulcer and gastric cancer. In 1982, Marshall and Warren[3] reported the isolation of *Campylobacter pylori* (renamed *H. pylori* in 1987) from the stomach of gastric ulcer patients, and in 1987, a ^{13}C-UBT for the detection of *H. pylori* infection was reported for the first time by Graham *et al.* using gas chromatography/mass spectrometry (GC-MS).[4] Graham also showed that gastric disease returns in 80—100% of patients within one year if *H. pylori* is not eradicated.[5] The National Institutes of Health (NIH) in the U.S.A. issued a consensus statement that the eradication of *H. pylori* is recommended for the treatment of initial or relapsed stomach ulcer in February 1994, and a working group of the International Agency for Research on Cancer (IARC), World Health Organization (WHO), classified *H. pylori* as a definite carcinogen (group 1) in June 1994.

Figure 1 shows the principle of the ^{13}C-UBT. ^{13}C-Urea is used as a substrate for the measurement of the urease activity of *H. pylori* in the stomach (higher animals have no endogenous urease activity in the stomach). Orally administered ^{13}C-urea (100 mg) is hydrolyzed to $^{13}CO_2$ and ammonia by *H. pylori* in the stomach, and the ratio of $^{13}CO_2/^{12}CO_2$ in the expired air is evaluated.

We have examined the sensitivity, specificity, and accuracy of ^{13}C-UBT, comparing it with histology using an infrared spectroscopy (IR) and GC-MS for isotope detection. IR is cheap and easy to operate for ^{13}C-UBT, and a suitable IR instrument has been developed for this purpose in Japan.[6] In contrast, GC-MS is a complex technique and relatively expensive. We also examined a new triple therapy for the eradication of *H. pylori* and the stability of the $^{13}CO_2/^{12}CO_2$ ratio in breath sampled in 250 ml aluminized bags.

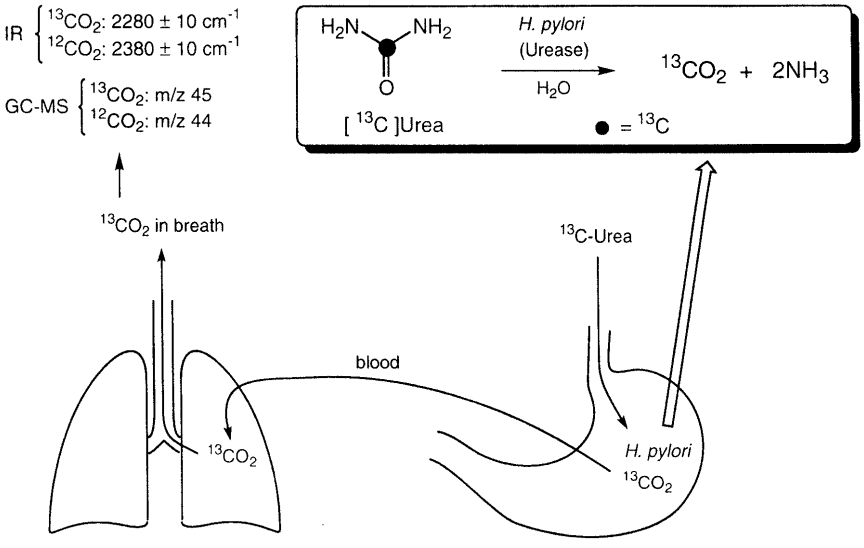

Fig. 1. Principle of the ^{13}C-UBT

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Experimental

Materials ^{13}C-Urea (99% atom ^{13}C) was prepared as reported previously[7] and supplied by Masstrace, Inc. (Somerville, MA, U.S.A.).

Instruments IR spectra were measured with a $^{13}CO_2$ analyzer (EX-130S, JASCO, Tokyo, Japan) for the ^{13}C-UBT. The $^{13}CO_2$ excess permil (‰) was calculated from the IR absorption intensities of $^{13}CO_2$ ($2280 \pm 10\,cm^{-1}$) and $^{12}CO_2$ ($2380 \pm 10\,cm^{-1}$). GC-MS was done with a Breath MAT (Finnigan MAT, Bremen, Germany) to detect $^{12}CO_2$ (m/z 44) and $^{13}CO_2$ (m/z 45) for the ^{13}C-UBT.

Method Written informed consent was obtained from all of the patients, who had biopsy-comfirmed ulcers. Each person was orally given 100 mg of ^{13}C-urea in 100 ml of water. In some cases, the mouth was subsequently washed with water. Breath samples were collected in 250 ml aluminized bags (supplied by Shiseido Co., Ltd., Tokyo, Japan) at 5, 10, 15, 20, 25, 30, 45 and 60 min after the ingestion of ^{13}C-urea.

The effect of the new triple therapy with lansoprazole (30 mg once daily), clarithromycin (200 mg twice daily), and metronidazole (500 mg twice daily), which is sufficient to eradicate *H. pylori* in one week, was also examined.

Detection of *H. pylori* was made in all of the patients by smear, culture and histology, as previously described.[8] Briefly, two paired biopsies were obtained from the antrum and the body. One of each paired biopsy was used for the microbiological examinations and the other for histological studies. When at least one of the three methods yielded a positive result, the patient was judged *H. pylori*-positive. The patient was considered as *H. pylori*-negative when all of three were negative. The data from this detection method (biopsy method) were used as a standard in this study.

Results and Discussion

Figure 2 shows the results of ^{13}C-UBT at various times after the administration of ^{13}C-urea. Closed circles are *H. pylori*-positive patients and open circles are *H. pylori*-negative by histology. Figure 3 shows the average values of 30 measurements. In *H. pylori*-positive cases, the $^{13}CO_2/^{12}CO_2$ ratio in expired air after administering 100 mg of ^{13}C-urea peaked at 15 to 30 min, being quite distinct from that in *H. pylori*-negative patients. The

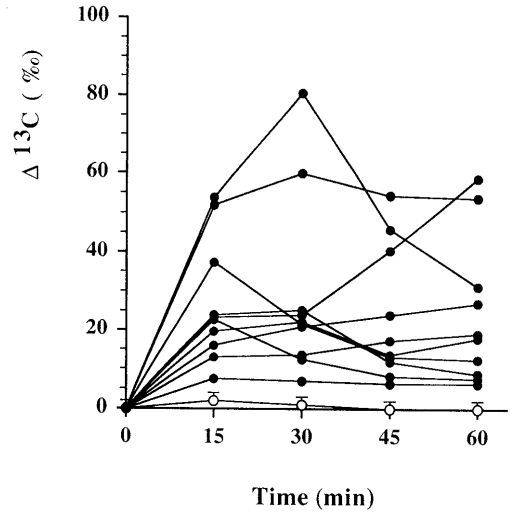

Fig. 2. Time Course of ^{13}C-UBT

H. pylori-positive patients (—●—) and 10 *H. pylori*-negative patients (—○—) from histology.

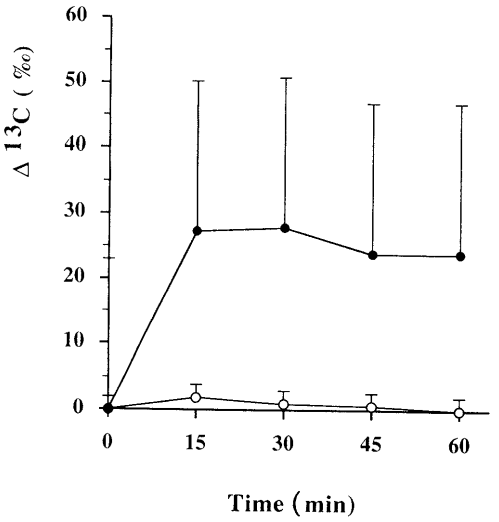

Fig. 3. Average Time Course of ^{13}C-UBT

20 *H. pylori*-positive patients (—●—) and 10 *H. pylori*-negative patients (—○—) from histology.

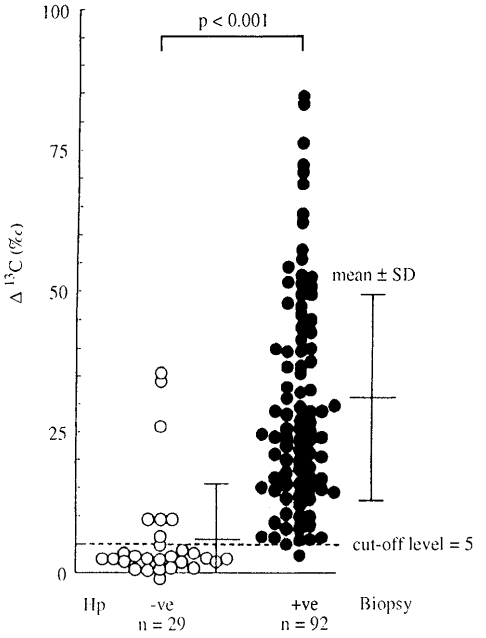

Fig. 4. Results of ^{13}C-UBT at 15 min

H. pylori-positive patients (—●—) and *H. pylori*-negative patients (—○—) from histology.

Table 1. Results of ^{13}C-UBT Compared with Histology Results

	Total biopsy		After administration of ^{13}C-urea (mouth washed with water) biopsy		After administration of ^{13}C-urea (mouth not washed with water) biopsy	
	Positive	Negative	Positive	Negative	Positive	Negative
^{13}C-UBT Positive	241	39	43	9	198	30
Negative	12	107	3	37	9	70
Sensitivity (%)	95.3		93.5		95.7	
Specificity (%)	73.3		80.4		70.0	
Accuracy (%)	87.2		87.0		87.3	

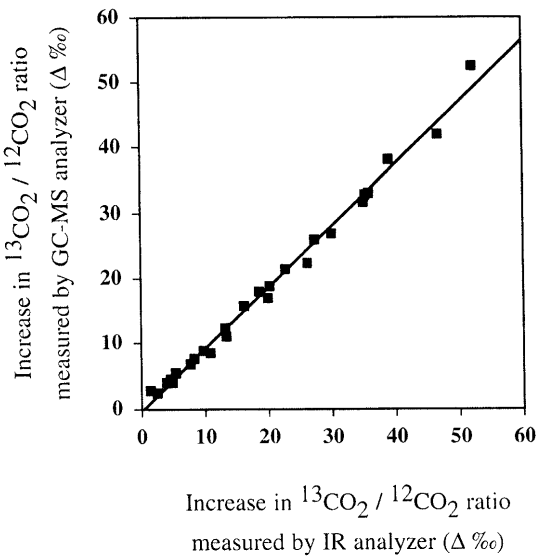

Fig. 5. Comparison of ^{13}C-UBT Results with GC-MS and with IR Analyzers Using Standard Gas Samples

$y=0.945x-0.347$, $r^2=0.992$.

results obtained when the assay was conducted at 15 min are shown in Fig. 4. The cut-off level was set at 5 (‰) as Δ ^{13}C permil. The results of the ^{13}C-UBT are compared with the histology findings in Table 1. The sensitivity of positive cases using IR and MS was 241/253 (95.3%). The specificity was 107/146 (73.3%). The accuracy of the ^{13}C-UBT was 348/399 (87.2%). Thus, ^{13}C-UBT is a non-invasive and accurate method for the detection of *H. pylori* infection. As shown in Fig. 5, there was a good correlation between the results obtained with the GC-MS and IR analyzers for 26 standard gas samples. The IR analyzer should be particularly useful for the diagnostic testing of *H. pylori* infection using ^{13}C-UBT.

We also determined ^{13}C-UBT before and 4 weeks after triple drug therapy to check the eradication of *H. pylori* for 26 *H. pylori*-positive patients. ^{13}C-UBT gave excellent sensitivity (100%), specificity (92.9%), and accuracy (100%) at 4 weeks post-eradication (data not shown).

Conclusion

^{13}C-UBT is a highly sensitive, specific, and accurate method for the diagnosis of *H. pylori* infection. Since it is also cheap, easy and reproducible, it is expected to be more useful for evaluating infection and the effectiveness of treatments of *H. pylori* infection than other currently available diagnostic methods.

The standard procedure is as follows. 1) Before the administration of 100 mg of ^{13}C-urea in 100 ml of water, the control ratio of $^{13}CO_2/^{12}CO_2$ in the breath is measured. 2) After the administration, residual ^{13}C-urea is washed out of the mouth with water. 3) After 15 min, a breath sample is taken in a 250 ml aluminized bag. The $^{13}CO_2/^{12}CO_2$ ratio in the breath is measured with IR or GC-MS. 4) The cut-off level was set at 5 (‰) Δ ^{13}C permil for the detection of *H. pylori* infection. 5) The breath samples in 250 ml aluminized bags can be stored for one year.

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