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A rapid-release 50-mg tablet-based ^{13}C-urea breath test for the diagnosis of Helicobacter pylori *infection*

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SUMMARY

Background: Recently, a rapid-release 100-mg ^{13}C-urea tablet with citrate supplement (Diabact UBT) showed excellent performance in a European population.

Aim: To investigate the accuracy of a 50-mg tablet-based ^{13}C-urea breath test protocol.

Methods: Consecutive dyspeptic patients referred for upper endoscopy were recruited. ^{13}C-Urea breath test was performed using a 50-mg ^{13}C-urea tablet (Diabact UBT) and compared with the gold standard (rapid urease test and histology). Baseline, 10-min, 20-min and 30-min breath samples were collected in all cases. The cut-off values at each measurement interval were determined by three standard deviations above the mean excess $\delta^{13}CO_2$ excretion of *Helicobacter pylori*-negative patients.

Results: Two hundred patients (150 before therapy and 50 after therapy) were available for analysis, with a mean age of 48.4 years, and 99 patients (50%) were *H. pylori* positive. The sensitivity and specificity of the 50-mg tablet-based ^{13}C-urea breath test at 10 min, 20 min and 30 min were 100% and 98%, 100% and 100%, and 100% and 98%, respectively.

Conclusion: A 20-min, 50-mg tablet-based ^{13}C-urea breath test (Diabact UBT) protocol is highly accurate for the diagnosis of *H. pylori* infection.

INTRODUCTION

The ^{13}C-urea breath test (^{13}C-UBT) is a technically simple and non-invasive test for the diagnosis of *Helicobacter pylori* infection. The accuracy of ^{13}C-UBT in the diagnosis of *H. pylori* infection is high, with a sensitivity of 90–98% and a specificity of 92–100%.[1–6] The sensitivity and specificity of post-treatment ^{13}C-UBT are as high as those of pre-treatment ^{13}C-UBT, making it a reliable test for the determination of post-treatment *H. pylori* status.[7] Since the first description of ^{13}C-UBT by Graham *et al.*,[8] several modifications have been published to simplify and optimize the test, including the amount of ^{13}C-urea used, type of test meal, number of samples and sampling time. Citrate has been described to be the best test meal.[9, 10] Previously, we have shown that a 50-mg ^{13}C-UBT protocol with or without citrate, using a sampling point at 20 min, is an accurate and cost-saving approach for the diagnosis of *H. pylori* infection in the Chinese population.[11] Recently, a newly developed rapid-release tablet containing ^{13}C-urea and citric acid has been described.[12] By enclosing the ^{13}C-urea in a capsule, contamination by urease-producing oropharyngeal bacteria is avoided. This protocol has been investigated in a European population, with a sensitivity of 95% and a specificity of 100%.[12] However, the dose of ^{13}C-urea used was 100 mg (two tablets),[12] which increased the cost of the test. On the basis of our previous experience with the 50-mg ^{13}C-UBT protocol (powder), we postulate that this novel ^{13}C-urea tablet at a dose of 50 mg may be equally reliable for the diagnosis of *H. pylori* infection.

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Thus, the aims of this study were: (i) to investigate the 50-mg ^{13}C-urea tablet protocol and determine the optimal cut-off value; (ii) to determine the optimal measurement interval of this protocol; and (iii) to investigate whether the accuracy of this protocol can be maintained in patients post-eradication.

METHODS

Patient population

Patients referred to the endoscopy unit of the Department of Medicine, Queen Mary Hospital, Hong Kong for the investigation of dyspepsia or for follow-up after *H. pylori* eradication and/or ulcer healing were recruited. Dyspepsia was defined as persistent or recurrent upper abdominal pain or discomfort over the preceding 3-month period. Informed written consent was obtained from all patients participating in the trial. Exclusion criteria included patients with previous gastric surgery and patients taking antibiotics, H_2-receptor antagonists, bismuth compounds or proton pump inhibitors in the 4 weeks before endoscopy. Patients with a past history of *H. pylori* eradication were allowed to enter the study provided that the medication for eradication had been stopped for at least 4 weeks and the other inclusion and exclusion criteria were met.

Gastric biopsies

During upper endoscopy, two antral biopsies and one corpus biopsy were taken. One antral biopsy was used for rapid urease test and the rest were sent for histological examination of *H. pylori* status by haematoxylin and eosin staining and modified Giemsa staining if necessary. For patients who had received eradication therapy within the past 3 months, an additional body biopsy was obtained for rapid urease test. The definition of *H. pylori* infection required both rapid urease test and histology to be positive and was used as the 'gold standard' in this study. Equivocal cases were excluded from the analysis. This approach has been validated previously in our centre, with a sensitivity and specificity of 100%, and less than 1% of cases cannot be diagnosed by this method.[13]

Tablet-based ^{13}C-UBT

Each ^{13}C-urea tablet (Diabact UBT, Diabact AB, Uppsala, Sweden) contained 50 mg of ^{13}C-urea with 456 mg of anhydrous citric acid. The 50-mg tablet-based ^{13}C-UBT was performed after an overnight fast. After the collection of a baseline exhaled breath sample in a vacutainer, the 50-mg ^{13}C-urea tablet (Diabact UBT) was swallowed with 200 mL of water. Further breath samples were taken at 10, 20 and 30 min. No test meal was used before the breath test. All patients were recruited between December 2001 and May 2002. None of the recruited patients had participated in any previous ^{13}C-UBT trials. Collected samples were analysed by a purpose-built isotope ratio mass spectrometer at the Simon K. Y. Lee Digestive Disease Laboratory, Queen Mary Hospital, Hong Kong. Results were expressed as the excess $\delta^{13}CO_2$ excretion. The cut-off values at each measurement interval were determined by three standard deviations above the mean excess $\delta^{13}CO_2$ excretion of *H. pylori*-negative patients. The sensitivities, specificities and accuracies of ^{13}C-UBT were calculated at 10, 20 and 30 min.

Statistical analysis

The statistics used included Student's *t*-test, the chi-squared test and Fisher's exact test when appropriate. A *P* value of 0.05 or less was considered to be statistically significant.

RESULTS

Two hundred and nine patients were recruited. Nine cases were excluded from the analysis. Four cases had incomplete breath test data and five cases could not be classified according to our gold standard for the diagnosis of *H. pylori* infection, i.e. only one positive test out of the two tests (histology and rapid urease test). Thus, a total of 200 patients were available for analysis. Of these 200 patients, 50 had received eradication therapy within 3 months before the breath test, i.e. post-eradication patients. The mean age of the 200 patients was 48.4 years (range, 18–86 years). There were 87 males and 113 females. Ninety-nine per cent of the study patients were ethnic Chinese. Ninety-nine patients (50%) were diagnosed as *H. pylori* positive by the gold standard, 20 of which were post-eradication patients. Only one of the 20 *H. pylori*-positive post-eradication patients had a discordant rapid urease test between the antrum and the corpus (antral negative but corpus positive). Of these 99 *H. pylori*-positive patients, three had gastric ulcer (3%), 17 had duodenal ulcer

Table 1. Mean excess $\delta^{13}CO_2$ of *Helicobacter pylori*-negative patients ($n = 101$) at the 10-min, 20-min and 30-min sampling points

Sampling point	Mean excess $\delta^{13}CO_2$	Standard deviation	Cut-off (mean + 3 s.d.)
10 min	0.16	0.36	1.2‰
20 min	0.16	0.64	2.1‰
30 min	0.24	0.31	1.2‰

(17%), one had gastric and duodenal ulcers (1%), 17 had gastro-duodenal erosions (17%) and one had oesophagitis (1%). One hundred and one patients (50%) were *H. pylori* negative by our gold standard. Of these 101 patients, two had duodenal ulcer (2%), one had gastric ulcer (1%), six had gastro-duodenal erosions (6%), one had gastric polyp (1%) and five had oesophagitis (5%). *H. pylori* status correlated strongly with the presence of duodenal ulcers ($P < 0.001$) and gastro-duodenal erosions ($P = 0.006$).

The calculated cut-off values were 1.2‰, 2.1‰ and 1.2‰ for the 10-min, 20-min and 30-min measurement points, respectively (Table 1). The sensitivity, specificity and accuracy of the 50-mg tablet-based ^{13}C-UBT at 10, 20 and 30 min were 100%, 98% and 99%, 100%, 100% and 100%, and 100%, 98% and 99%, respectively. The ^{13}C-urea tablet was easy to swallow and well tolerated by all patients. There was no difference in accuracy between patients before eradication therapy and those after eradication therapy (data not shown).

The mean excess $\delta^{13}CO_2$ excretion at the 10-min (30.2 vs. 24.0, $P < 0.001$) and 20-min (29.8 vs. 24.0, $P < 0.001$) sampling points was significantly higher than that at the 30-min sampling point in *H. pylori*-positive patients before eradication therapy (Figure 1). Such a difference was also observed for the 20-min sampling point when compared with the 30-min sampling point in *H. pylori*-positive patients after eradication therapy (24.7 vs. 20.6, $P = 0.004$). The mean excess $\delta^{13}CO_2$ excretion at the 20-min sampling point was similar to the reading at 10 min (29.8 vs. 30.2, $P = 0.73$) before *H. pylori* eradication. Similarly, the mean excess $\delta^{13}CO_2$ excretion at the 10-min sampling point was similar to the reading at 20 min (25.4 vs. 24.7, $P = 0.79$) after *H. pylori* eradication. The reading at the 10-min sampling point was greater than that at 30 min (25.4 vs. 20.6, $P = 0.14$), after *H. pylori* eradication, but the P value was insignificant.

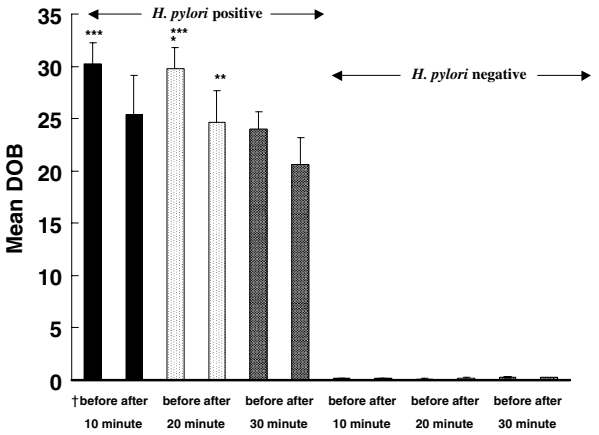

Figure 1. Mean delta over baseline (DOB) ± S.E.M. in *Helicobacter pylori*-positive and *H. pylori*-negative patients at 10-, 20- and 30-min sampling points (before, before eradication; after, after eradication). ***$P < 0.001$ when compared with the mean DOB at 30 min in patients before eradication. **$P < 0.01$ when compared with the mean DOB at 30 min in patients after eradication. *$P = 0.73$ when compared with the mean DOB at 10 min before eradication.

However, the small number of patients ($n = 20$) in this particular subgroup may limit the power to detect a difference between the 10-min and 30-min measurements.

For *H. pylori*-negative patients, the mean excess $\delta^{13}CO_2$ excretion was less than 0.3 at all three sampling points irrespective of eradication status (Table 1, Figure 1).

Five patients could not be classified according to our gold standard of *H. pylori* infection due to discordant results between rapid urease test and histology. Two patients had received eradication therapy previously. All five patients were invited to repeat a standard ^{13}C-UBT using a validated 75-mg ^{13}C-urea protocol.[6] There was total agreement between the 50-mg tablet-based ^{13}C-UBT and the conventional 75-mg ^{13}C-UBT, suggesting that the discrepant results were due to the inaccuracy of the biopsy-based tests (Table 2).

DISCUSSION

^{13}C-UBT is one of the most important non-invasive methods for the detection of *H. pylori* infection. The low-dose 50-mg ^{13}C-UBT protocol with citrate as test meal has been validated in the Chinese population previously, with a sensitivity and specificity of 100% and 98%, respectively, using a cut-off of 3‰ and a

Table 2. ^{13}C-Urea breath test (^{13}C-UBT) results of the five patients with discordant results between rapid urease test (RUT) and histology

Eradication status	RUT	Histology	50-mg tablet-based ^{13}C-UBT	75-mg conventional ^{13}C-UBT
Before	–	+	+	+
Before	–	+	+	+
Before	–	+	–	–
After	+	–	+	+
After	–	+	–	–

measurement interval of 20 min.[11] The calculated cut-off values of the novel 50-mg Diabact UBT were 1.2‰, 2.1‰ and 1.2‰ for the 10-min, 20-min and 30-min measurement points, respectively. The sensitivity and specificity of the 50-mg Diabact UBT were 100% and 98%, 100% and 100%, and 100% and 98% at 10 min, 20 min and 30 min, respectively. The accuracy of this protocol is preserved in post-eradication patients.

At present, there is universal agreement that two samples should be taken: a baseline sample collected at time zero and a 30-min sample collected after the ingestion of ^{13}C-urea. ^{13}C is measured as the $^{13}CO_2$: $^{12}CO_2$ isotope ratio and is expressed as the excess $\delta^{13}CO_2$ excretion. The cut-offs in the three measurement intervals were determined by three standard deviations above the mean excess $\delta^{13}CO_2$ excretion of *H. pylori*-negative patients. The cut-off determined by this method correlated well with that of Hamlet *et al.*[12]

The major advance of this novel 50-mg Diabact UBT is that it has an almost instantaneous disintegration and dissolution after entering the stomach.[12] This rapid-release formulation is supported by the fact that the mean excess $\delta^{13}CO_2$ excretion peaked at the 10-min and 20-min sampling points. For *H. pylori*-negative patients, the mean excess $\delta^{13}CO_2$ excretion at the 10-min, 20-min and 30-min sampling points was close to zero, indicating that ^{13}C-urea hydrolysis was minimal. Although this is not a head-to-head comparison, the mean excess $\delta^{13}CO_2$ excretion values of this 50-mg tablet-based ^{13}C-UBT at 10, 20 and 30 min were 0.16, 0.16 and 0.24, respectively, which are 5–10 times smaller than the corresponding mean excess $\delta^{13}CO_2$ excretion values of 1.6 and 1.0 at 20 and 30 min (10-min measurement not available), respectively, using 50 mg of ^{13}C-urea powder.[11] Our data suggest that the ^{13}C-urea tablet confers protection against oropharyngeal urease activity and discriminates well between *H. pylori*-positive and *H. pylori*-negative cases. Two *H. pylori*-negative patients showed false positive breath test results at the 10-min and 30-min sampling points according to our cut-off value. The corresponding excess $\delta^{13}CO_2$ excretion values at 10 min, 20 min and 30 min in the first patient were 1.3, 1.3 and 1.8, respectively, and in the second patient were 2.1, 1.6 and 1.4, respectively. Although there was no statistical difference between the three time points, our data suggest that the 20-min sampling point has the highest accuracy. However, to power the study to detect a difference between the three time points would require an enormous investigation, which is probably inappropriate within the context of this study.

Five patients had discordant results between rapid urease test and histology; they were all invited for a conventional 75-mg ^{13}C-UBT used in our laboratory.[6] There was total agreement between the 50-mg tablet-based ^{13}C-UBT and the conventional protocol, suggesting that the 50-mg tablet-based ^{13}C-UBT may be more accurate than the biopsy-based tests.

The use of a test meal in ^{13}C-UBT is employed to increase the residence time of ^{13}C-urea in the stomach and to improve the contact between urease produced by *H. pylori* and the substrate. Citric acid has been suggested to be the best liquid test meal.[9, 10] The tablet formulation used in this study contains 456 mg of anhydrous citric acid. Although the dose of citrate is low when compared with that used in previous studies, the high accuracy obtained with the 50-mg ^{13}C-urea tablet suggests that low-dose citrate is equally efficacious. This tablet is well tolerated, and the ^{13}C-UBT can easily be performed outside the hospital/clinic, or even at home, after which a test-tube of expired air can be sent by regular mail for analysis. This may allow a potentially wider application of this non-invasive test for the diagnosis of *H. pylori* infection in regions at high risk of gastric cancer, such as China.

One of the limitations of this study is that we did not perform a head-to-head comparison of the 50-mg ^{13}C-urea tablet with 50 mg of ^{13}C-urea powder. As mentioned previously, in order to detect a small difference in accuracy between the tablet and powder, an enormous investigation would be required, which may be impractical.

In conclusion, we have shown that the 50-mg tablet-based ^{13}C-UBT (Diabact UBT), using a measurement interval of 20 min, is highly accurate for the diagnosis of *H. pylori* infection.

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REFERENCES

1 Goddard AF, Logan RPH. Review article: urea breath tests for detecting *Helicobacter pylori*. Aliment Pharmacol Ther 1997; 11: 641–9.
2 Savarino V, Mela GS, Zentilin P, *et al.* Comparison of isotope ratio mass spectrometry and nondispersive isotope-selective infrared spectroscopy for 13C-urea breath test. Am J Gastroenterol 1999; 94: 1203–8.
3 Klein PD, Graham DY. Minimum analysis requirements for the detection of *Helicobacter pylori* infection by the ^{13}C-urea breath test. Am J Gastroenterol 1993; 88: 1865–9.
4 Cutler AF, Havstad S, Ma CK, Blaser MJ, Perez-Perez GI, Schubert TT. Accuracy of invasive and noninvasive tests to diagnose *Helicobacter pylori* infection. Gastroenterology 1995; 109: 136–41.
5 Savarino V, Vigneri S, Celle G. The ^{13}C urea breath test in the diagnosis of *Helicobacter pylori* infection. Gut 1999; 45(Suppl. 1): I18–22.
6 Wong WM, Wong BCY, Wong KW, *et al.* ^{13}C-urea breath test with or without citric acid is equally accurate for the detection of *Helicobacter pylori* infection in Chinese. Aliment Pharmacol Ther 2000; 14: 1353–8.
7 Lam SK, Talley NJ. Report of the 1997 Asia Pacific consensus conference on the management of *Helicobacter pylori* infection. J Gastroenterol Hepatol 1998; 13: 1–12.
8 Graham DY, Klein PD, Evans DJ Jr, *et al. Campylobacter pylori* detected noninvasively by the ^{13}C-urea breath test. Lancet 1987; 1: 1174–7.
9 Dominguez-Muñoz JE, Leodolter A, Sauerbruch T, Malfertheiner P. A citric acid solution is an optimal test drink in the ^{13}C-urea breath test for the diagnosis of *Helicobacter pylori* infection. Gut 1997; 40: 459–62.
10 Graham DY, Runke D, Anderson SY, Malaty HM, Klein PD. Citric acid as the test meal for the ^{13}C-urea breath test. Am J Gastroenterol 1999; 94: 1214–7.
11 Wong WM, Wong BCY, Li TM, *et al.* Twenty-minute 50-mg ^{13}C-urea breath test without test meal for the diagnosis of *Helicobacter pylori* infection in Chinese. Aliment Pharmacol Ther 2001; 15: 1499–504.
12 Hamlet A, Stage L, Lonroth H, Cahlin C, Nystrom C, Pettersson A. A novel tablet-based 13C urea breath test for Helicobacter pylori with enhanced performance during acid suppression therapy. Scand J Gastroenterol 1999; 34: 367–74.
13 Wong BCY, Wong WM, Wang WH, *et al.* An evaluation of invasive and non-invasive tests for the diagnosis of *Helicobacter pylori* infection in Chinese — the best tests for routine clinical use and research purposes. Aliment Pharmacol Ther 2001; 15: 505–11.