A citric acid solution is an optimal test drink in the ^{13}C -urea breath test for the diagnosis of *Helicobacter pylori* infection

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Abstract

Background—The ^{13}C -urea breath test (^{13}C -UBT) is a simple, non-invasive and reliable test for the diagnosis of *Helicobacter pylori* infection. The duration of the test, the timing of breath sampling, and the accuracy of the method vary according to the test meal used.

Aim—To identify the optimal test meal or drink for rapid and accurate performance of the ^{13}C -UBT for the detection of *H. pylori* infection.

Patients—Eighty patients with dyspeptic symptoms were included. Of these, 48 patients had a positive *H. pylori* status and 32 a negative one according to the results of the rapid urease test, histological examination, and culture.

Methods—A ^{13}C -UBT was performed after an overnight fast, on three consecutive days. On each study day a different test meal or drink was given (0.1 N citric acid solution, a standard semiliquid meal, or a semiliquid fatty meal) 10 minutes before giving 75 mg ^{13}C -urea. Breath samples were collected at 0, 15, 30, 45, and 60 minutes, and analysed by isotope ratio mass spectrometry. Results were expressed as delta (δ) and considered as positive for *H. pylori* if the highest δ (peak) was greater than 4.0.

Results—The δ peak obtained with the citric acid drink in *H. pylori* positive subjects (24.1 (SEM 1.5)) was significantly higher than that obtained with any of the semiliquid meals (13.3 (SEM 1.1) and 17.1 (SEM 1.0) respectively, $p < 0.001$). Furthermore, this δ peak was obtained earlier with the citric acid drink (30 (SEM 2) minutes) than with the other two meals tested (53 (SEM 2) min and 45 (SEM 2) min, $p < 0.001$). The sensitivity of the ^{13}C -UBT for the diagnosis of *H. pylori* infection was 96–100% with all three test meals. This high sensitivity was, however, obtained from 15 minutes by giving citric acid as the test drink, from 45 minutes by giving a semiliquid fatty meal, and at 60 minutes by giving the semiliquid standard meal. The specificity was 100% for all test meals. Citric acid is inexpensive and palatable to patients.

Conclusions—The ^{13}C -UBT procedure with citric acid as the test drink is superior to the previously proposed semiliquid test meals in terms of $^{13}\text{CO}_2$ recovery, time requirement, and cost. In routine clinical sampling, collection at times 0 and 30

minutes seems to be optimal and gives a high diagnostic accuracy.

(Gut 1997; 40: 459–462)

Keywords: *Helicobacter pylori*, diagnosis, breath test.

The ^{13}C -urea breath test (^{13}C -UBT) is a non-invasive and simple test that reflects the hydrolysis of ^{13}C -labelled urea by *Helicobacter pylori* urease. The reliability of this test in diagnosing *H. pylori* infection is very high, with a sensitivity ranging from 90 to 98% and a specificity from 92 to 100%.^{1–6} The ^{13}C -UBT may become the preferred method of assessing the effect of therapeutic regimens. Furthermore, this test is adequate for epidemiological research, with an advantage over serological methods of detecting active infection.

Since the original description by Graham *et al.*⁷ of the non-invasive and non-radioactive ^{13}C -UBT to identify the presence of *H. pylori* urease activity, several modifications have been published aiming at simplifying and optimising the test.^{1–4 8 9} Quantity of substrate given, type of test meal, number of samples, and timing of sample collection have been the key variables investigated.

A sufficiently high amount of substrate is required to avoid exhaustion by oral bacteria containing urease. However, the ^{13}C -urea dose should be reduced as far as possible for cost reasons. The amount of substrate used varies widely among different authors from 75 mg⁸ to 350 mg (5 mg/kg body weight).⁷ A dose of 100 mg has been proposed in a standardised European protocol,² but 75 mg ^{13}C -urea has been shown to be equally effective.⁸ The number of samples to be taken depends basically on the aim of the test: clinical diagnosis or research. For clinical routines, in which the ^{13}C -UBT is required as a “yes or no” test, collection of two samples (before and after ^{13}C -urea ingestion) is sufficient to provide a high diagnostic accuracy.^{4 8 9} Both dose of ^{13}C -urea and composition of the test meal influence the optimal timing for sample collection. A readily exhaustible amount of substrate will provide an early and short $^{13}\text{CO}_2$ recovery peak, limiting the time frame for sample collection. On the other hand, timing of sample collection does not seem to be critical for at least one hour if a high substrate dose is given.¹⁰ Besides the basal sample, a single sample at 30 minutes is usually recommended.^{2 3 6 8}

The need for giving a test meal in the ^{13}C -UBT has been shown in several studies,^{4 7}

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Accepted for publication
15 October 1996

because administration of the urea with no additional test meal leads to gastric emptying of the substrate before sufficient reaction with *H. pylori* urease can take place. To inhibit gastric emptying a semiliquid fatty test meal is recommended by several authors.¹⁻³ Solutions of glucose polymers and standardised commercial semiliquid meals have also been used.⁵⁻¹¹ Good results have been obtained by using a citric acid drink to inhibit gastric emptying.⁴⁻⁸ In the original paper by Graham *et al* the test meal composition was reported as being not important.⁷ However, the duration of the test, timing of breath sampling, and the accuracy of the method vary among different studies using different types of test meal.¹⁻¹¹ In the present study we aimed at identifying the optimal test meal or drink for rapid and accurate performance of the ¹³C-UBT for the detection of *H. pylori* infection. The accuracy of the optimised test was also calculated.

Methods

PATIENTS

Eighty patients (mean age 40, range 16–82 years, 41 men, 39 women) presenting for routine gastroscopy with dyspeptic symptoms gave their informed written consent for the study. The *H. pylori* status was investigated in all patients by: (a) the rapid urease test (HUT-Test, Astra GmbH, Germany)¹² on one biopsy sample from the antrum and one from the gastric body, (b) histology on two biopsy samples from the antrum and two from the body, and (c) culture on two biopsy samples from the antrum. Biopsy specimens for histological examination were fixed in formalin, embedded in paraffin wax, and stained with Giemsa stain for detection of *H. pylori*. Bacteria were cultured at 37°C in 5% O₂ for three days on Wilkins-Chalgren agar supplemented according to Skirrow (Anaerocult® C, Merck, Darmstadt, Germany). Diagnosis of *H. pylori* infection was based on either a positive culture or on a positive result in both the rapid urease test and histology. Patients had not received a compound containing bismuth, antibiotics, or a proton pump inhibitor in the four weeks before the study. None of the patients had a history of gastric surgery. The protocol was approved by the ethics committee of the University of Bonn.

METHODS

¹³C-UBT was performed in all patients after an overnight fast on three consecutive days. On each study day a different test meal was given in a randomised order: (1) 200 ml 0.1 N citric acid solution, with the addition of 25 mg saccharin as sweetener; (2) 250 ml of a standard semiliquid meal (Meritene®, Wander Pharma; 237 kcal; 5 g fat, 20 g protein, and 28 g carbohydrate; and (3) a semiliquid fatty meal consisting of 50 ml Ensure® (Abbott) and 50 ml Calogen® (SHS Pharma; 275 kcal; 26.7 g fat, 2 g protein, 6.7 g carbohydrate). The price of each of these meals per test was 0.25 DM

for the citric acid solution and about 6 DM for either of the two semiliquid meals. Ten minutes after ingestion of the test meal a baseline exhaled breath sample was collected in a CO₂ storage capsule (CEDIOX-System®, Topic AG, Lichtenstein) and thereafter 75 mg ¹³C-urea dissolved in 50 ml water was given orally (T₀). Further breath samples were taken at 15, 30, 45, and 60 minutes using the same CO₂ storage capsules. To facilitate the simultaneous study of several patients and to simplify the test, turning the patients on their sides was avoided and they stayed seated over the whole study period. Collected samples were analysed by isotope ratio mass spectrometry (CEDIOX®, Topic AG, Lichtenstein), with a quadrupole mass spectrometer (Balzers AG, Asslar, Germany). Once all three tests were done, patients were asked which was the most pleasant test meal or drink.

Results were expressed as delta (δ), defined as the ratio (r_i–r₀)/r₀ where r=¹³CO₂/¹²CO₂ (0=basal sample; i=15, 30, 45, 60 min). The result of the ¹³C-UBT is considered positive for *H. pylori* if maximal δ was greater than 4.0.

The within subject variability of the ¹³C-UBT in subjects with *H. pylori* infection was (mean (SEM)) 12.1 (2.4)% (unpublished data obtained by the daily performance of the ¹³C-UBT with citric acid as the test drink over three consecutive days in 20 *H. pylori* positive subjects).

ANALYSIS OF DATA

The maximal δ value (peak) provided the end point of the test. A curve was defined by spline interpolation of the δ values at the different sampling times, and the area under the curve was calculated. The time to the maximal value of this curve (T_{max}) was also measured. Results are expressed as means (SEM). Comparison between the three different test procedures was performed by repeated measures analysis of variance (ANOVA) with Bonferroni's correction for multiple comparisons. The sensitivity and specificity of the ¹³C-UBT according to the test meal used for the diagnosis of *H. pylori* infection was calculated.

Results

Forty eight patients (60%) were *H. pylori* positive and 32 (40%) *H. pylori* negative. Culture was positive in 33 of the 48 *H. pylori* positive patients, the rapid urease test was positive in 46 patients, and histology was positive in 43 patients. All *H. pylori* negative subjects had a negative result on culture, rapid urease test, and histology.

All three breath test procedures were well tolerated by the patients; the citric acid drink was, however, generally found to be the most pleasant. Figure 1 shows curves obtained in *H. pylori* positive patients by applying all three tests. The maximal δ value obtained with the citric acid drink was significantly higher than that obtained with Meritene® or Ensure®-Calogen® (Fig 2). Furthermore, the δ peak was obtained earlier with the citric acid drink than

Figure 1: Curves obtained with the three ¹³C-UBT procedures in the same patient population (n=48). On each ¹³C-UBT the same ¹³C-urea dose (75 mg) but different test meal was given. Results are expressed as means (SEM).

Figure 2: Maximal δ values (peaks) obtained with all three ¹³C-UBT procedures with different test meals. Results are expressed as means (SEM).

Figure 3: Time to δ peak for all three ¹³C-UBT procedures with different test meals. Results are expressed as means (SEM).

with the other two meals tested (Fig 3). In addition, the test performed with the citric acid drink produced the highest ¹³CO₂ recovery over 60 minutes (greatest area under the curve; Fig 4).

The Table shows the sensitivity of the ¹³C-UBT for the diagnosis of *H. pylori* infection according to the test meal used and the sampling time. The maximal δ value over 60 minutes was >4.0 (positive result) for all *H. pylori* positive patients with the citric acid drink as well as with the semiliquid fatty meal (sensitivity 100%), whereas two false negative results were obtained with Meritene[®] as the test meal (sensitivity 96%). However, if the increase of ¹³CO₂ production at 30 minutes was

Figure 4: Area under the curves over 60 minutes for all three ¹³C-UBT procedures with different test meals.

considered as the result of the test (as usually done routinely), all *H. pylori* positive patients had a positive test with citric acid (sensitivity 100%), three patients had a false negative result with Ensure[®]-Calogen[®] (sensitivity 94%), and 13 patients had a false negative result with Meritene[®] (sensitivity 73%). All *H. pylori* negative subjects had a negative result in the ¹³C-UBT with all three test meals (specificity 100%).

Discussion

Our study shows that the ¹³C-UBT performed with a solution of 0.1 N citric acid as test drink provides higher ¹³CO₂ recovery values and peaks recorded at earlier times than both a standard commercialised semiliquid meal (Meritene[®]) and a semiliquid fatty meal (Ensure[®]-Calogen[®]) in patients with *H. pylori* infection. As a result the duration of the test may be reduced without loss of sensitivity and specificity by using citric acid as the test drink. Furthermore, citric acid is inexpensive and palatable to patients.

Since the description of the ¹³C-UBT for the diagnosis of *H. pylori* infection,⁷ several modifications have been reported with the aim of simplifying and optimising the test.^{1,4,8,9} Most of them have evaluated different substrate doses as well as different times of sample collection. On the one hand, the dose of ¹³C-urea to be given must be high enough to prevent its rapid exhaustion; on the other, cost is a limiting factor. Doses up to 350 mg (5 mg/kg body weight) of substrate have been given and 100 mg is the recent recommended dose by a European Working party.² However, doses as low as 75 mg are as reliable as higher doses.⁵ Based on this finding 75 mg of ¹³C-urea was used in the present study.

The main goal for giving a test meal is to slow the gastric emptying of the substrate and thus allow a longer reaction time between ¹³C-urea and *H. pylori* urease. A lower gastric emptying is achieved by the semiliquid meals through their fatty content, whereas with the citric acid solution this effect is obtained by its low pH (3.0–3.5). We have recently found that the intraduodenal infusion of the citric acid solution used in the present study induces an

Sensitivity (% (95% CIs)) of the ^{13}C -UBT for the diagnosis of *H. pylori* infection according to the test meal used and the sampling time

Test meal	Sampling time (min)			
	15	30	45	60
Citric acid (CA)	100 (92.6–100)	100 (92.6–100)	100 (92.6–100)	100 (92.6–100)
Meritene (M)	56 (42.3–69.3)	73 (59.0–83.4)	88 (75.3–94.1)	96 (86.0–98.8)
Ensure/Calogen (EC)	90 (77.8–95.5)	94 (83.2–97.8)	100 (92.6–100)	100 (92.6–100)
Statistical comparison (binomial test)	CA v M: $p < 0.001$ CA v EC: $p < 0.05$ EC v M: $p < 0.001$	CA v M: $p < 0.001$ CA v EC: NS EC v M: $p < 0.02$	CA v M: $p < 0.05$ CA v EC: NS EC v M: $p < 0.05$	CA v M: NS CA v EC: NS EC v M: NS

inhibition of antral motility and a relaxation of the gastric fundus similar to that achieved by the intraduodenal perfusion of a fatty solution.¹³

The selection of the optimal sampling time not only depends on the quantity of substrate given, but also on the time needed for the hydrolysis of ^{13}C -urea by contact with the *H. pylori* urease. The test meal chosen for this purpose does, therefore, plays an important part.

A single sample at 30 or 60 minutes^{2 3 6} or multiple sample collections up to 90 minutes^{1 5 11} have been recommended by performing the test with different semiliquid meals. Three studies have been reported with citric acid solution as the test drink; and the collection of a single sample at 20 minutes⁴ or at 30 minutes^{8 9} has been recommended in these cases. In the present study we found that the time required to reach the maximal $^{13}\text{CO}_2$ exhalation was 30 (2) minutes when citric acid was given, 45 (2) minutes when a semiliquid fatty meal was used, and 53 (2) minutes when a standard commercial semiliquid meal was administered. Collection of a single sample at an earlier time (for example, 30 minutes) leads to a lower sensitivity. Therefore, a single post-prandial breath sample is adequate only if the optimal sampling time is identified. With the citric acid solution this time point was at 30 minutes, although all patients had already shown a positive result at 15 minutes, compared with 45 minutes in the best case when a semiliquid test meal was used.

Some authors recommend turning the patients on to each side, head down for several minutes after ingestion of the test meal and ^{13}C -urea to guarantee the contact of the substrate with the bacterial urease in the stomach.^{1 2} This seems to be unnecessary from the high diagnostic accuracy obtained in our study, in which we did not do this. This simplification of the test allows the simultaneous study of several patients.

The greater and earlier $^{13}\text{CO}_2$ recovery obtained with citric acid as the test drink may indicate an enhanced contact between

^{13}C -urea and *H. pylori* urease mediated by this solution. Other mechanisms such as alterations in the metabolism of the organism by citric acid cannot however be excluded.

The citric acid solution is about 24 times cheaper than any of the semiliquid meals. Furthermore, and contrary to the semiliquid fatty meal, the citric acid solution can easily be stored at 4°C for weeks.

In conclusion, citric acid as the test drink in the ^{13}C -UBT seems to be practical and accurate for detecting *H. pylori* infection. This test procedure is superior to the previously proposed semiliquid test meals in terms of $^{13}\text{CO}_2$ recovery, time requirement, and cost. Citric acid is, furthermore, palatable for patients. In clinical routine, sampling collection at times 0 and 30 minutes seems to be optimal and gives a high diagnostic accuracy.

This study was supported by a grant of the Faculty of Medicine, University of Bonn, Germany. We thank Mrs M Gelitzki and Mrs D Bammer for technical assistance.

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