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Evaluation of ^{13}C-urea breath test in the detection of *Helicobacter pylori* and in monitoring the effect of tripotassium dicitratobismuthate in non-ulcer dyspepsia

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Abstract

Sixty nine patients with non-ulcer dyspepsia have been studied with endoscopy, biopsy, quick urease (CLO) test, *Helicobacter pylori* culture, and the ^{13}C-urea breath test before and after treatment with tripotassium dicitratobismuthate (DeNol) two tablets twice daily for four weeks. Symptoms of non-ulcer dyspepsia were recorded using a standard questionnaire. Using *H pylori* culture as the gold standard, the sensitivity of the ^{13}C-urea breath test was 90%, the specificity 98·6%, and the accuracy 94·8% with a positive predictive value of 98·2% and a negative predictive value of 92·5%. Conversion rate from *H pylori* positive to negative status after treatment with tripotassium dicitratobismuthate was 17·9%. Symptoms of non-ulcer dyspepsia improved appreciably after treatment irrespective of *H pylori* status. The ^{13}C-urea breath test is an accurate research tool suitable for serial testing and population surveys.

Spiral organisms on the mucosa of the human stomach have been noted sporadically since the last century,[1-6] but interest was slight until 1983, when Marshall[7] reported the first successful culture of *Helicobacter pylori*. Many reports have shown a high prevalence of *H pylori* in patients with histological antral gastritis and duodenal ulcer, and a lower prevalence in gastric ulcer disease and in non-ulcer dyspepsia.[8-12] These observations have led to a lively discussion of the possible aetiological role of *H pylori* in these conditions.

Present methods for detecting *H pylori* comprise bacterial culture, urease tests, and histological examination of biopsy specimens; all of these are invasive and therefore not suitable for screening large populations or repeated testing of the same individuals. Serological tests are not invasive, but do not show unequivocally if active colonisation with *H pylori* is present. Two recent reports[13,14] suggest that the non-invasive ^{13}C- or ^{14}C-urea breath test provides a simple way to detect *H pylori* colonisation without endoscopy and biopsy. These breath tests exploit the high urease activity of *H pylori* in vivo and detect the CO_2 derived from labelled urea in expired breath.

The aim of the present study was twofold. Firstly, we have attempted to measure the sensitivity and specificity of the ^{13}C-urea breath test by comparison with culture of *H pylori* from endoscopic antral biopsy specimens because published data pertaining to this point are not abundant. Secondly, we investigated the incidence of *H pylori*, correlation with symptoms, the efficacy of clearing it with tripotassium dicitratobismuthate, and the rate of its recolonisation after clearance in a group of patients with non-ulcer dyspepsia.

Patients and methods

Consecutive outpatients aged 18–70 years referred for routine endoscopy because of ulcer-like symptoms (epigastric pain related to food and relieved by milk or antacids) were studied. Subjects were excluded if they had any endoscopically visible organic lesion of the upper alimentary tract, such as gastric or duodenal ulcer, cancer, macroscopic gastritis, duodenitis, or oesophagitis, or if they received drugs other than antacids during the previous two weeks. Patients with debilitating disease, previous gastric surgery, or renal insufficiency, or who were pregnant or breast feeding, or those who were unable to cooperate were also excluded. The study was approved by the Brent District Ethical Committee and all patients gave informed written consent.

ENDOSCOPY AND BIOPSY

Endoscopy was done one to five days before and within 48 hours of completing the treatment. Patients fasted for at least eight hours before the examination, which was performed with an Olympus GIF XQ10 fibreoptic panendoscope. Before reuse, the endoscope and the biopsy forceps were washed, cleaned, and soaked in glutaraldehyde solution (Cidex Solution* R, Arbrook, Inc, Arlington, Texas 76010) for 10 minutes and thoroughly washed again with sterilised water.

At endoscopy three antral biopsy specimens were taken for bacterial cultures, quick urease (CLO) test and a direct Gram stain.

CLO test

One specimen was used for the CLO test,[15] a commercially available quick urease test kit, which was kept for three hours at about 30°C and after that at room temperature for one day. The test was examined half an hour, three hours, and 24 hours after biopsy, and if a definite pink colour developed at any of these times the test

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was considered to be positive for *H pylori*.

Microbiology

The remaining two biopsy specimens were placed in thiol-containing transport medium, transferred to the microbiological laboratory within two hours, and processed on receipt. Each biopsy specimen was cultured on chocolate-blood agar to which lincomycin, colistin, amphotericin B, and trimethoprim (Oxoid SR 95 selective supplement) and 1% vitox (Oxoid SR 90) had been added. Plates were placed at 37°C in an anaerobic jar in which a microaerophilic atmosphere was maintained by means of a Microaerophilic System Envelope (BBL CampyPak) and incubated for up to seven days. Growth was recorded semiquantitatively (up to 50 colonies=light growth; 51–200 colonies= moderate growth; >200=heavy growth). Organisms were identified as *H pylori* on the basis of morphology and biochemical tests. Smears were also prepared from each biopsy specimen and stained by Gram's method. Smears showing typically spiral Gram negative organisms were reported as positive.

^{13}C-UREA BREATH TEST

The first ^{13}C-urea breath test was done within five days of the initial endoscopy, but before treatment with tripotassium dicitratobismuthate was started, and repeated within three days after the end of four weeks' treatment. Expired air was collected into 21 plastic bags fitted with a non-return inlet valve and a septum, through which gases were sampled over 5 s into 20 ml Vacutainers (Becton Dickinson, Rutherford, New Jersey, USA: Ref 606433). Duplicate baseline breath samples were taken from patients who had fasted overnight. Thereafter they swallowed 120 ml of a 31% (w/v) solution of nutrient-dense glucose polymer, which was made by diluting (Fortical, Cow & Gate Ltd, Tonbridge, Wiltshire) with distilled water. This was done to delay gastric emptying. Fifteen minutes later subjects swallowed 20 ml of water containing 250 mg ^{13}C-urea (99%, Tracer Technologies Inc, Somerville, MA 02145, USA). Breath samples were collected at 10, 20, 30, 40, 60, 80, 100, and 120 minutes after the dose. Samples were subsequently analysed within two weeks of collection using a Finnigan MAT Delta D dual-inlet multicollector isotope ratio mass spectrometer, fitted with an automated breath gas analysis system.[16] Total carbon dioxide production was measured by indirect calorimetry at standard time intervals relative to the ^{13}C sample. The instantaneous tracer production in the breath was then calculated from the product of the CO_2 production rate and the mole fraction of tracer $^{13}CO_2$ at each sampling time point. The breath test results were expressed as follows: (*a*) Atom Percentage Excess (APE) of the post-dose samples relative to the mean of the two pre-dose samples; (*b*) the cumulative percentage dose of tracer recovered in the breath. This was calculated as 100×the mole fraction of tracer derived ^{13}C in the expired CO_2. APE is conceptually equivalent to the specific activity of a radioactive tracer. Thus for each post-dose breath sample, the ^{13}C enrichment was calculated relative to the mean natural abundance of $^{13}CO_2$ in the pre-dose samples, expressed as APE relative to natural abundance. APE is analogous to specific activity as used to measure radioactive tracer enrichment, but using a stable isotope tracer, account must be taken of the natural abundance of the tracer isotope, so that APE is a measure of the mole fraction of tracer-derived CO_2 in the expired breath CO_2. The APE was calculated from the mass spectroscopic observations by standard methods.[17] The instantaneous rate of appearance of tracer in the breath is thus the product of APE/100 and the CO_2 production rate. The cumulative % dose of tracer recovered in the breath was obtained by integrating the instantaneous tracer production curve over the appropriate time period, and expressing this as a molar percentage of the ^{13}C-urea given.

In the absence of labelled urea, the Fortical meal results in a small increase in the ^{13}C content of the expired CO_2, presumably from maize-derived carbohydrate in the meal. This cannot be distinguished from $^{13}CO_2$ derived from labelled urea, and results in enrichments of about 0·005 APE after one to two hours, and an apparent % dose recovered of one to two after this time. The magnitude of this apparent % dose recovered depends on the ^{13}C content of the subject's diet as well as the ^{13}C content of the meal. To allow for the normal interindividual variation in baseline breath ^{13}C content, the cut off between positive and negative tests was set at 3% of the dose recovered after two hours. It might be possible to increase the sensitivity of the test to low levels of *H pylori* infection by using a meal with a lower ^{13}C content. Using the 3% cut off, no negative test had an APE >0·01 at 20 minutes, while in positive tests the APE ranged from >0·01 to 0·15 at 20 minutes.

CONDUCT OF STUDY

After the initial breath test the patients were treated with tripotassium dicitratobismuthate as DeNoltabs, two twice daily, given on an empty stomach 30 minutes before meals for four weeks; this dosage provides bismuth 480 mg/day. No other medication apart from an antacid (Gelusil tablets, 0·58 g aluminium-silicahydrate per tablet) was allowed. Before treatment patients were questioned in a standardised manner about five predefined symptoms: daytime or night time epigastric pain, nausea or vomiting, heartburn, and postprandial discomfort defined as fullness, belching, distension, or regurgitation occurring after a meal. The presence of each symptom was scored as: 0 absent; 1 recalled on direct questioning; 2 present, but not impairing activities; 3 interfering with daily work and life. Frequency of symptoms was scored as: 1 one or less day per week; 2 several times each week; 3 daily. The symptom scores of presence and frequency of each symptom were added for each patient giving a possible score ranging from 0 to 30. After completing four weeks' treatment, and before the second endoscopy which was performed within 48 hours after the end of treatment, patients were asked identical questions by

an investigator unaware of the patient's *H pylori* status. The second endoscopy with biopsies, and the second breath test were done on the same or on the following day, respectively. Patients with a negative breath test after four weeks' treatment with tripotassium dicitratobismuthate underwent a third breath test four weeks later.

Compliance with tripotassium dicitratobismuthate treatment was assessed by interview and by returned tablet count at the end of treatment: return tablet count below 10 tablets (out of 120 dispensed) was considered to indicate good compliance. Side effects were also inquired about at the second interview.

All completed tests independent of tripotassium dicitratobismuthate compliance were used for the analysis of the power of the ^{13}C-urea breath test, but analysis of the symptoms and of the efficacy of tripotassium dicitratobismuthate was only done on the patients who completed the treatment with it as well as the pre- and post-treatment endoscopies, biopsies, and breath tests.

Statistical analysis of the results was done using the U-test (Wilcoxon, Mann, and Whitney). Differences were considered significant at $p<0.05$.

Results

^{13}C-UREA BREATH TEST

Sixty nine patients, 35 men (mean age 38·7 years, range 18–70 years) and 34 women (mean age 45·3 years, range 23–68 years), entered the study; 65 completed all tests. The four dropouts were one woman who developed influenza at the time of follow up endoscopy, another woman who declined the second breath test, and two men who declined the second endoscopy. Thus 134 complete examinations were available for analysis of the breath test: 69 endoscopies, biopsy specimens with culture, CLO, and breath tests before treatment and 65 after treatment with tripotassium dicitratobismuthate.

A summary of all the test results is given in Table I.

COMPARISON OF ^{13}C-UREA BREATH TEST WITH H PYLORI CULTURE

Using bacterial culture as a gold standard *H pylori* was isolated from 60 patients either before or after treatment with tripotassium dicitratobismuthate. In 55 of these the ^{13}C-urea breath test was positive, but six had a false negative result (Table I). Five of the six false negative breath test results, however, were recorded immediately after four weeks' treatment with tripotassium dicitratobismuthate. In these patients the *H pylori* load was appreciably decreased, as shown by the other tests (Table II). Four weeks after the end of tripotassium dicitratobismuthate treatment the ^{13}C-urea breath test was positive again in four of these patients with false negative. The fifth patient declined the third breath test. In contrast, where *H pylori* culture was negative, taking the results as a whole in 74 patients, all but one had negative breath test results.

TABLE I *Comparison of all* H pylori *detection tests with culture results*

	134 complete examinations (69 patients)	
Culture	*60 positive*	*74 negative*
CLO test	54 (1 false positive)	80 (7 false negative)
Gram stain	51 (0 false positive)	83 (11 false negative)
Breath test	55 (1 false positive)	79 (6 false negative)

TABLE II *Results in five patients with false-negative ^{13}C-urea breath tests immediately after treatment*

Test	*Before tripotassium dicitratobismuthate (n)*	*After tripotassium dicitratobismuthate (n)*
Culture	Heavy (5)	Light (3) Moderate (1) Heavy (1)
Gram stain	Positive (5)	Negative (4) Positive (1)
CLO test	Positive at 30 min (5)	Positive at 3 h (1) Negative at 24 h (4)
Breath test	Positive (5)	Negative (5)

^{13}C-UREA BREATH TEST BEFORE TREATMENT

Breath tests recorded before treatment with tripotassium dicitratobismuthate correlated better with *H pylori* culture than those recorded after treatment. Thus, before treatment *H pylori* culture was positive in 34 and negative in 35 patients. Breath test was positive in 33 and negative in 36 patients, showing only one false negative result (Figure, Table III).

^{13}C-UREA BREATH TEST PARAMETERS

The sensitivity, specificity, accuracy, and negative and positive predictive values of the ^{13}C-urea breath test calculated on the basis of negative and positive cultures are given in Table III. Calculating the results of the breath test in different ways (cumulative % dose recovery after 120 minutes >3, or APE >0·01 at 40 minutes= positive breath test) gave exactly the same results.

COMPARISON OF CLO TEST WITH H PYLORI CULTURE

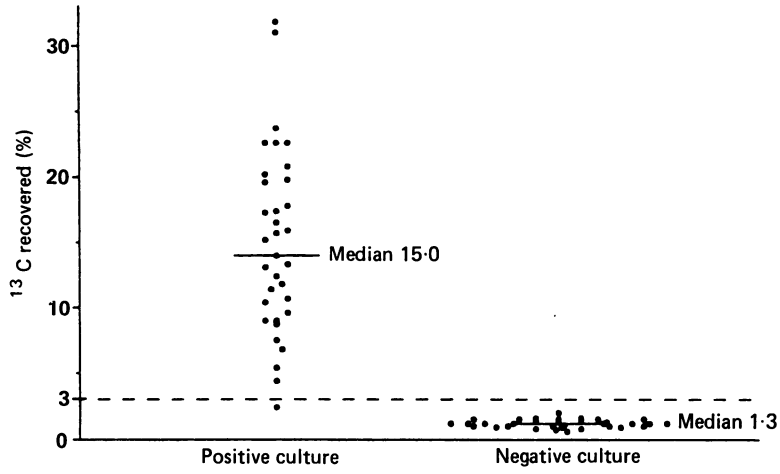
Fifty three of 60 *H pylori* positive patients had positive CLO tests, but seven had false negative results. Six of these were recorded after tripotassium dicitratobismuthate treatment. One CLO test was false positive. Thus in this study the CLO test had a sensitivity of 88·3%, specificity of 98·6%, and accuracy of 94·0% with a positive predictive value of 98·1% and a negative predictive value of 91·2%.

PREVALENCE OF H PYLORI IN NON-ULCER DYSPEPSIA

Based on the results of culture, *H pylori* was detected in 34 of 69 patients with non-ulcer

TABLE III *^{13}C-urea breath test*

	% Overall	*% Before tripotassium dicitratobismuthate*
Sensitivity	90·0	97·0
Specificity	98·6	100·0
Accuracy	94·8	98·5
Positive predictive value	98·2	100·0
Negative predictive value	92·5	97·2



Cumulative percentage dose recovery over two hours in all 69 patients before tripotassium dicitratobismuthate compared with H pylori *culture results.*

dyspepsia, giving a prevalence rate of 49·3%. Using the breath test, 33 *H pylori* positive patients were found, giving a prevalence rate of 47·8% – showing closely similar results with the two methods. The *H pylori* positive patients (n=28) were older (mean (SEM) 44·8 (2·12) years) than the rest (n=34; 39·1 (2·22) years; $p<0·05$).

EFFECT OF TRIPOTASSIUM DICITRATOBISMUTHATE ON H PYLORI STATUS

Thirty four of the 35 *H pylori* negative and 28 of the 34 *H pylori* positive patients complied with tripotassium dicitratobismuthate treatment and completed all the requirements of the trial protocol. Six (four men) *H pylori* positive patients (two did not attend for second endoscopy; three stopped tripotassium dicitratobismuthate treatment because of headache (one), nausea (one), or tiredness (one); one took only half the dose) and one *H pylori* negative patient (one woman did not attend for second endoscopy) were excluded from the analysis.

In the 28 positive patients all three tests became negative after tripotassium dicitratobismuthate treatment in only five (18%). All three tests became weakly positive in a further nine (32%) of compliant patients. In the remainder (n=14: 50%), results of the three tests remained unchanged despite four weeks' treatment with tripotassium dicitratobismuthate. ^{13}C-urea breath tests were positive again in four of the five negative patients who attended for the third breath test one month after completing treatment.

CORRELATION BETWEEN H PYLORI STATUS AND SYMPTOMS

There was no significant difference in the pretreatment mean (SEM) symptom score between the *H pylori* positive (n=28: score 18·4 (0·6)) and negative patients (n=34: score 20·1 (0·8)). The symptom score decreased significantly ($p<0·05$) in both groups of patients, but the mean final scores were almost identical (post-treatment mean (SEM) score *H pylori* positive 8·1 (1·0); negative 8·7 (1·0)).

Discussion

The present results suggest that the ^{13}C-urea breath test is a sensitive and highly specific method for non-invasive detection of *H pylori*. Presumably because of decreased numbers of *H pylori*, and therefore a lower quantity of urease, the ^{13}C-urea breath test was slightly less sensitive immediately after treatment with tripotassium dicitratobismuthate. Similar findings have been reported by Weil and colleagues.[18] We therefore recommend in future studies with drugs active against *H pylori* either repeating the test four weeks after the end of treatment, as was done in this study, or using the ^{13}C-urea breath test only a few weeks after completion of treatment. The ^{13}C-urea breath test compares well with the ^{14}C-urea breath test. Other workers using the ^{14}C-urea breath test have reported similar values for sensitivity and specificity[19 20] to those from this study. This is not surprising, as the ^{13}C and ^{14}C tests are conceptually similar. The great advantage, however, of the ^{13}C-urea breath test is the absence of radiation risk. This makes the ^{13}C-urea breath test suitable for serial studies and population surveys, or for studies in children and women who are at risk of, or are, pregnant. The ^{13}C test is particularly suitable for epidemiological investigations and for longterm follow up, essential for monitoring the response to treatment with helicobactericidal agents. Our data suggest that the ^{13}C-urea breath test may be simplified to only four breath samples: two baseline samples and single samples after 30 and 60 minutes.

Non-ulcer dyspepsia is generally diagnosed in patients with symptoms suggesting the presence of a peptic ulcer, but in whom no ulcer is found at endoscopy.[21] Little is known about the pathogenesis of this syndrome[22-25] and treatment is often ineffective.[25 26] Results of this study confirm previous reports indicating that *H pylori* is detected in about half the patients with non-ulcer dyspepsia,[10 13 27 28] and that the *H pylori* positive patients tend to be older.[27] The latter finding corresponds well with serological data, which show a progressively increasing incidence of *H pylori* antibodies with age.[29] We found that improvement of symptoms was independent of *H pylori* status. In a trial comparing bismuth, erythromycin, and placebo, McNulty *et al*[30] found symptomatic improvement in all three groups. There was a trend towards greater improvement of symptoms in those treated with bismuth, and in those cleared of *H pylori*, but these trends were not statistically significant. There was no placebo group in the present study, so it is possible that the symptomatic improvement recorded was due to some other action of tripotassium dicitratobismuthate, independent of its effect on *H pylori*. Other workers were also unable to find correlation between symptoms of non-ulcer dyspepsia and the presence of *H pylori*.[31-33] In contrast, Rokkas *et al*[34] reported appreciable symptomatic improvement in *H pylori* positive patients with non-ulcer dyspepsia treated with colloidal bismuth for eight weeks. The reasons for this discrepancy remain unclear.

Various clearance rates are reported by others using the same treatment scheme with tripotassium dicitratobismuthate 240 mg twice daily for four weeks.[35 36] In this study all detection methods were negative after treatment in only

18% of the initially positive patients, and it is now generally accepted that bismuth 480 mg/day produces poor clearance rates for *H pylori*. In those patients breath tests became positive again one month after treatment, suggesting either suppression, rather than eradication of *H pylori*, or rapid reinfection. Similar findings have been reported by others.[12 18] Restriction endonuclease DNA analysis[37] suggests that recrudescence of *H pylori*, rather than reinfection, causes the rapid return to positivity.

The results of this study show good accuracy and specificity of the ^{13}C-urea breath test, and suggest that the symptoms of non-ulcer dyspepsia do not correlate with *H pylori* status.

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