¹⁴C-urea breath test in *C. pylori* gastritis

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SUMMARY ¹⁴C-urea breath test was used to detect *Campylobacter pylori* colonisation in 129 consecutive non-ulcer dyspepsia patients. Fasting patients were given 3 µCi (110 kBq) of ¹⁴C-labelled urea after a test meal. Breath samples were collected at 10 minute intervals for 90 minutes and the C-14 activity was counted on a liquid scintillation analyser. Urea derived ¹⁴CO₂ appears in the exhaled breath of *Campylobacter pylori* culture positive individuals within 20-30 minutes. Likelihood analysis revealed a most favourable cut off level of [0.07% dose ¹⁴C-urea/mmol CO₂] multiplied by body weight at t=40 minutes, to separate culture positive from culture negative subjects. Using this upper limit of normal, a positive likelihood ratio of 50 and a negative likelihood ratio of 0.05 was calculated. Sensitivity of the test was 95% and specificity 98%. The ¹⁴C-urea breath test is a simple, sensitive and non-invasive test, that detects viable *C. pylori* microorganism and semiquantitatively assesses the bacterial load of *C. pylori* colonisation. Administration of a single dose of colloidal bismuth subcitrate resulted in a rapid decrease in ¹⁴CO₂ excretion, so this test can be used to confirm eradication of the bacterium in therapeutic trials without endoscopy, or need for culture.

There is increasing interest in the presence of *Campylobacter pylori* in gastric biopsies and their possible role in the aetiology of gastritis and peptic ulcer.¹⁻⁶ This spiral organism colonises the gastric antrum in 90-100% of patients with duodenal or gastric ulcer and in 50-70% of patients with non-ulcer dyspepsia.⁷⁻¹¹ *C. pylori* is also present in 20% of asymptomatic individuals.⁷ *C. pylori* colonisation can be established by culture or histological examination of antral mucosal biopsies. The use of endoscopy to obtain biopsies precludes large scale epidemiologic studies of this organism. We have previously reported that *C. pylori* has a very high urease activity.⁷ Intragastric hydrolysis of urea will result in the production of carbon dioxide and the generation of ammonia. We have utilised this urease activity to

develop a sensitive, inexpensive, non-invasive, reproducible breath test for *C. pylori* detection using a low dose ¹⁴C urea. This report compares this ¹⁴C-urea breath test with cultures of biopsies in 129 patients with non-ulcer dyspepsia.

Methods

PATIENTS

A total of 129 consecutive non-ulcer dyspepsia patients referred for upper gastrointestinal endoscopy entered the study. Patients with non-ulcer dyspepsia had epigastric discomfort after meals, a feeling of fullness, belching, bloating and/or abdominal distension. They had a normal physical examination, routine blood chemistry, abdominal ultrasound and upper endoscopy. Patients were not entered if they had used any medication other than antacids during the previous four weeks, nor if they had previous gastric surgery or malignancy. Informed consent was obtained before entry.

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STUDY PROTOCOL

Upper gastrointestinal endoscopy was carried out after an overnight fast. Two antral mucosal biopsy specimens were taken using a sterilised biopsy forceps, and were placed in 2 ml phosphate buffered saline at 4°C for bacteriological examination. Before the result was known, and within 48 hours after endoscopy, the ¹⁴C-urea breath test was done.

UREA BREATH TEST

The urea breath test was done after an overnight fast. Baseline samples of respiratory CO₂ were obtained. Patients first ingested a liquid test meal of Sustacal^R pudding (Mead Johnson, Evansville, IN) to decrease gastric emptying and 110 kBq ¹⁴C-urea (=3 µCi) (Amersham) mixed with 350 mg ¹²C-urea in 60 ml Fortimel^R (Nutricia) was then administered.

Breath samples were collected in an alkaline trapping solution at 10 minute intervals for 90 minutes. The trapping solution contained 2 mM ethanolic hyamine hydroxide and one drop of thymolphthalein, to indicate when complete neutralisation of the alkaline trapping solution had occurred.¹²⁻¹⁵

The C-14 activity of the breath samples was counted on a Packard 2000 CA TriCarb liquid scintillation analyser. The result was expressed as [% administered dose C-14/mmol of expired CO₂] multiplied by body weight in kilograms. This correction for body weight was used to account for endogenous CO₂ production.^{16, 17}

The reproducibility of the test was checked in seven subjects (three *C. pylori* culture negative and four culture positive) by repeating the study within 24 hours, as tested by:

$$s = \sqrt{\frac{\sum (\times 1 - \times 2)^2}{2n}}$$

in which $\times 1$ = first determination

$\times 2$ = second determination

n = number of paired samples

To determine the effect of the test meal on the recovery of ¹⁴CO₂, nine subjects (two *C. pylori* culture negative and seven *C. pylori* culture positive) were investigated twice within 24 hours, once with a test meal and once without test meal.

The effect of administration of a single dose of colloidal bismuth subcitrate was studied in seven patients with a positive test by retesting the next day, two hours after ingesting two tablets of colloidal bismuth subcitrate (each tablet containing 120 mg bismuth subcitrate).

BACTERIOLOGICAL ASSESSMENT

Biopsy specimens were inoculated within four hours of sampling by rubbing them over the surface of

Colombia agar (Oxoid, Ltd, London, United Kingdom), supplemented with 5% (vol/vol) horse blood and over agar (blood agar base no 2; Oxoid) containing 5% (vol/vol) horse blood and Skirrow selective supplement (Oxoid). All agars were incubated at 35°C under microaerobic conditions (CampyPak; BBL Microbiology Systems, Cockeysville, Md) for a total of seven days. Cultures were considered positive for *C. pylori* if one or more colonies of Gram negative, oxidase positive, catalase positive and urease positive spiral or curved rods were present. The bacteriologist was not aware of the histological results, the endoscopic findings or the results of the breath test. The bacterial load was assessed semiquantitatively using a standard dilution technique. Culture results were the 'gold standard'.

STATISTICAL ANALYSIS

Mean values (and standard deviation (SD)) of ¹⁴C-activity were calculated for each period of time. A ROC (Receiver Operating Characteristic) analysis was performed by increasing stepwise the cut-off value of the test, separating *C. pylori* positive from *C. pylori* culture negative subjects. Steps with an increment of 1 SD were taken, starting at the mean value of the *C. pylori* negative group up to the mean plus 5 SD.

Results

Urea breath test was carried out on 129 patients with non-ulcer dyspepsia. In patients with negative cultures ($n=53$) virtually no ¹⁴CO₂ excretion could be detected in the exhaled breath for at least 90 minutes. In *C. pylori* culture positive patients ($n=76$), a rapid rise in ¹⁴CO₂ excretion occurred within 20 minutes after ingestion of ¹⁴C-urea (Table 1, Fig. 1). The bacterial load assessed semiquantitatively in the antral biopsies, correlated well with the amount of ¹⁴CO₂ excreted. Patients with 3+ colonisation, could not be discriminated from those with 2+ colonisation, as the SEM of these two groups was overlapping. In these subjects with a high bacterial load, ¹⁴CO₂ excretion decreased after 60 minutes, possibly because of exhaustion of the ¹⁴C-urea substrate.

The reproducibility of the urea breath test was evaluated in seven individuals with ($n=4$) and without ($n=3$) *C. pylori* colonisation, by repeating the test within 24 hours. Reproducibility proved to be $[0.03\% \text{ dose } ^{14}\text{C/mmol excreted CO}_2] \times \text{weight (kg)}$.

Receiver operating characteristic curves were calculated for all points of the ¹⁴CO₂ production curve. Performance of the test was almost equally excellent for the 30, 40, 50, and 60 minutes values, while it decreased for the earlier and later values, mainly because of an increase in the false positive

Table 1 ^{14}C -urea breath activity during time

C pylori culture	Subjects (n)	Time (min) after ^{14}C -urea administration								
		10	20	30	40	50	60	70	80	90
Negative	53	0.035* (0.024)	0.023 (0.013)	0.023 (0.014)	0.025 (0.015)	0.026 (0.014)	0.026 (0.013)	0.030 (0.019)	0.031 (0.024)	0.032 (0.026)
Some colonies	6	0.062 (0.019)	0.047 (0.029)	0.052 (0.045)	0.067 (0.063)	0.085 (0.088)	0.085 (0.093)	0.103 (0.128)	0.118 (0.159)	0.128 (0.179)
1+	12	0.176 (0.104)	0.239 (0.167)	0.278 (0.183)	0.308 (0.208)	0.340 (0.247)	0.378 (0.324)	0.401 (0.395)	0.410 (0.436)	0.402 (0.424)
2+	33	0.265 (0.155)	0.443 (0.334)	0.505 (0.349)	0.541 (0.353)	0.557 (0.332)	0.563 (0.334)	0.563 (0.356)	0.519 (0.298)	0.475 (0.290)
3+	25	0.265 (0.186)	0.427 (0.331)	0.510 (0.368)	0.549 (0.391)	0.555 (0.373)	0.531 (0.339)	0.505 (0.302)	0.477 (0.265)	0.421 (0.230)

*Activity is expressed in [% dose ^{14}C -mmol excreted CO_2] $\times$ body weight (kg); mean value (SD) is given.

rate (Fig. 2). Based on this analysis a cut off value of [0.07% dose of ^{14}C -urea/mmole CO_2] multiplied by body weight at 40 minutes after ingestion of ^{14}C -urea was selected to separate the culture negative and culture positive individuals. Adopting this normal limit, the test combines a high positive and a low negative likelihood ratio, with a reasonable test time of 40 minutes.

From Table 2, the following parameters were calculated: sensitivity 95%, specificity 98%, positive

predictive value 99%, negative predictive value 93%, positive likelihood ratio 50, negative likelihood 0.05.

In the *C. pylori* negative group one false positive value was found ([0.08% dose ^{14}C -mmole CO_2] $\times$ weight (kg)), while four false negative values were found in the group of six subjects with 'some colonies'. No false negative results were obtained in the group with 1+, 2+, and 3+ colonisation.

The effect of the test meal on the recovery of $^{14}\text{CO}_2$ was studied in nine patients by repeating the test

Fig. 1 Recovery of $^{14}\text{CO}_2$ in *C. pylori* culture negative individuals and in individuals with some colonies, 1+, 2+, and 3+ colonies, assessed semiquantitatively in antral biopsies after administration of a test meal followed by 110 kBq ^{14}C urea.

Fig. 2 Receiver operating characteristic (ROC) analysis of C-14 urea breath test at various time intervals.

within 24 hours without the test meal. Figure 3 illustrates the effect of a test meal on the ¹⁴CO₂ excretion. There was no effect of the test meal on the ability of the breath test to identify *C pylori* positive individuals. When the breath test was carried out with a test meal, however, the resolution of the curves increased substantially. In both *C pylori* negative subjects, no significant effect was recorded. In *C pylori* positive subjects, the values obtained with a test meal exceeded those obtained without, at any time: 1.6 times at 30 minutes after starting the test to up to 5.5 times at 90 minutes (Fig. 3).

The effect of colloidal bismuth subcitrate was tested in seven patients by repeating the test two hours after the ingestion of 2 tablets colloidal bismuth subcitrate (Denol^R) (Fig. 4). The mean 40 minute value dropped from [0.57 to 0.28% dose ¹⁴C/mmol excreted CO₂] × weight (kg).

Discussion

At present the detection of *C pylori* requires a gastroduodenoscopy with antral biopsies for histological examination and bacterial culture. Assessment of the presence of *C pylori* by serological tests only is not reliable. Significantly raised antibody

Table 2 ¹⁴C-urea breath test in *C pylori* infection

	<i>C pylori</i> Culture positive	<i>C pylori</i> Culture negative
C-14 urea breath test +*	72	1
C-14 urea breath test -	4	52

*A positive test was defined as exhalation of over 0.07% of the dose of ¹⁴C per mmol exhaled CO₂, multiplied by body weight (kg) 40 minutes after ingestion of ¹⁴C-urea.

Fig. 3 Recovery of ¹⁴CO₂ in nine patients (seven patients *C pylori* culture positive) without and after a test meal, before ¹⁴C-urea is administered.

titres have been shown in *C pylori* colonised individuals,^{5 18-20} but these antibody titres cannot be used to confirm eradication of the organism because antibody titres decrease slowly after cure. Vaira *et al* described a decrease in IgG titres one week²¹ and three months²² after treatment with colloidal bismuth subcitrate but made no comment about culture results at that time. Although antibody titres may decrease considerably after successful treatment, raised titres have been found up to two years after resolution of the infection,^{18 23} unpublished observation.

Fig. 4 Recovery of ¹⁴CO₂ in seven *C pylori* culture positive patients, before and two hours after administration of a single dose of colloidal bismuth subcitrate.

For epidemiological studies, family studies, long-term follow up studies and therapeutic efficacy studies a non-invasive, accurate and non-expensive screening test would be desirable.

Graham and coworkers recently described a urea breath test for the detection of *C. pylori* colonisation using the non-radioactive stable nuclide ^{13}C and gas isotope ratio mass spectrometry.²⁴ A drawback of this method is the extremely high cost of ^{13}C -urea in comparison with ^{14}C -urea, a need for a mass spectrometer, as well as a limited sensitivity of this technique compared to radionuclide detection. This limited sensitivity may explain why Graham was not able to show a relationship between bacterial load and ^{13}C -urea breath test values. Bell²⁵ and Marshall²⁶ have described a ^{14}C -urea breath test. They reliably distinguished between patients with and without *C. pylori* colonisation but administered a four time higher dose of ^{14}C -urea (400 kBq) and accordingly higher concentrations of $^{14}\text{CO}_2$ recovery are measured in the exhaled breath. Bell administered a liquid meal before the breath test was started, in contrast with Marshall, but no influence of a meal on the results of the breath test nor correlation between the bacterial load and the amount of $^{14}\text{CO}_2$ exhaled was discussed in these papers.

In our study in a larger number of consecutive non-ulcer dyspepsia patients, while using a test meal and using only 110 kBq ^{14}C -urea, we were able to detect *C. pylori* colonisation and correlate the amount of $^{14}\text{CO}_2$ exhaled with the bacterial load. Patients with 3+ colonisation could be discriminated from those with 1+ colonisation, but not from those with 2+ colonisation. It may be that there is no significant difference between 2+ and 3+ colonisation, or that a difference could not be detected because insufficient substrate was administered.

The addition of a test meal increased the resolution of the curves, probably by increasing the retention time of ^{14}C -urea in the stomach. Because of the increased resolution of the curves when using a test meal, lower doses ^{14}C -urea can be used to reliably detect *C. pylori*. The test may be simplified by measuring only the 0 and 30 minute values of exhaled $^{14}\text{CO}_2$ as suggested by Marshall.²⁶ There would be no substantial loss of sensitivity or specificity with the simplified test. Colloidal bismuth subcitrate has an antibacterial action and the prompt effect of one dose colloidal bismuth subcitrate on the test result suggests that the ^{14}C -urea breath test may become a cost effective test to monitor treatment results.

Such a procedure would be helpful in epidemiological studies of *C. pylori* infection. The radiation dose of 110 kBq ^{14}C -urea is extremely low. In our opinion, the estimated whole body dose of less than 2 mrem (0.02 mSv) justifies the application of the ^{14}C -

urea breath test because of its low cost and high sensitivity compared to the ^{13}C -urea breath test.

In conclusion, the $^{14}\text{CO}_2$ -urea breath test is an inexpensive, simple, sensitive and non-invasive test, that detects *C. pylori* colonisation and semiquantitatively assesses the bacterial load of *C. pylori* colonisation. This test should be useful in carrying out epidemiological and therapeutic trials without endoscopy or need for culture.

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