ORIGINAL ARTICLE

^{14}C-Urea Breath Test: A Useful Non-Invasive Test in the Diagnosis of *Helicobacter Pylori* Infection

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Summary

Sixty-three breath samples were collected from patients who underwent a ^{14}C-urea breath test. Following ingestion of a radiolabelled ^{14}C-labelled urea solution, breath samples containing ^{14}C-labelled carbon dioxide were trapped in a solution containing hyamine hydroxide. Samples were then counted in a liquid scintillation counter. Breath samples were collected at 2, 15, 20, 25 and 30 minutes following ingestion of the urea solution.

The presence or absence of *Helicobacter pylori* (HP) infection was determined on the basis of endoscopic biopsy tests which included culture, histological examination, rapid urease test and a gram stain of a fresh tissue smear. Thirty-two HP positive and 31 HP negative samples were collected. The mean counts at 15, 20, 25 and 30 minute time points were: 4413, 4458, 4109 and 3795 dpm respectively for the positive samples and 1275, 877, 690 and 565 dpm respectively for the negative samples. Based on a cutoff value (mean of the negative samples + 3 standard deviations) for every time point, HP positive and negative samples could be clearly differentiated giving a sensitivity and specificity of 100%. The ^{14}C-urea breath test is a reliable and convenient diagnostic test for *H.pylori.*

Key Words: Helicobacter pylori, Urease, Urea breath test

Introduction

Helicobacter pylori (HP) infection is perhaps the most widespread bacterial infection in the world today. Its importance and role in peptic diseases and in carcinoma of the stomach is increasingly recognized[1,2]. The organism was first discovered on histological examination of gastric biopsies[3] and subsequently cultured after prolonged incubation in enriched culture media. Other tests have subsequently been developed and include testing gastric biopsies for urease activity and examining for the organism on a gram stain of a fresh tissue smear. Endoscopic biopsy tests are well established methods of diagnosis.

The radiolabelled carbon urea breath test (UBT) is a novel non-invasive method of diagnosis which is based on the fact that HP produces large amounts of the

enzyme urease which breaks down a radiolabelled carbon urea test meal; the tagged $C0_2$ being then exhaled via the breath of patients, trapped, analyzed and quantitated. Urease is not produced by mammalian tissue and HP is unique in that it is the only bacterium colonizing the upper gastrointestinal tract which produces urease[4].

The use of a breath test to detect gastric urease was first reported by Graham *et al*[5] using a radiolabelled ^{13}C-urea test meal. Both ^{13}C- and ^{14}C labelled breath tests have now been developed and established for use, in clinical practice.

In our study we have established the use of a ^{14}C-urea breath test following the protocol described by Marshall[6] and compared it with proven methods of diagnosis in our laboratory viz, culture, histology, urease tests, and Gram's stain of a fresh tissue smear.

Patients and Methods

Patients were selected from those who underwent gastroscopy for dyspepsia. Patients with previous gastric surgery and who had recently taken bismuth compounds, antibiotics or any other drug that may interfere with the HP status were excluded.

These patients had gastric biopsies performed and the samples were tested for urease activity using a rapid urease test in the endoscopy suite and sent for culture and histological examination. These tests have been described and evaluated previously in our laboratory and have been found to be reliable and accurate in diagnosing HP infection[7]. A positive diagnosis of HP infection was made when culture was positive or when histology and one other test was positive. A negative diagnosis of HP was made when all tests performed were negative.

Patients underwent the UBT on the same day of the gastroscopy. Patients rinsed their mouths thoroughly with water and then swallowed 185 kBq (5 uCi) of [^{14}C]-urea (Amersham, UK) dissolved in 20 ml of water in a single swallow. Immediately after swallowing the urea solution, patients rinsed their mouths again. Patients blew through a disposable drinking straw into a chamber containing desiccant (silica gel) and the exhaled CO_2 was trapped in a solution containing 1 mmol of hyamine hydroxide diluted to 2.5 ml with methanol; 20 µl of 0.1% methanolic thymolphthalein was added as a pH indicator (Fig. 1). Patients blew into this solution until the colour changed from blue to colourless. Samples at 2, 15, 20, 25 and 30 min after ingestion of the [^{14}C]- urea were collected. Scintillation fluid (10 ml) was then added and the trapped [^{14}C]-CO_2 was counted in a liquid scintillation counter (Tri-Carb, Packard, Model 2500 TR). The scintillation cocktail was made up of 0.4% 2,5-diphenyl-oxazole and 0.01% (1,4-bis-[4-methyl-5-phenyl-2-oxazolyl] benzene) in toluene. Radioactivity was expressed as disintegrations per minute (dpm) and reflected the amount of ^{14}C exhaled in the breath of patients.

The microbiology, histopathology and UBT results were evaluated by independent investigators who were blind to each others results until final analysis.

The study was approved by the Radiation Safety subcommittee of the University Hospital, Kuala Lumpur.

Results

Sixty-three samples, 32 positive and 31 negative for HP, were collected. The results of the tests at the various time points are shown graphically in Figure 2. The mean counts (dpm) of the samples collected and the cut-off values are shown in Table I. Breath test results from the 31 negative samples were used to define the negative (normal) range. For any time point, normal was defined as a dpm value which falls below the mean + 3 standard deviations (SD) for this group of patients. The percentage of HP negative samples which were included in this normal range gave the specificity of the test while the percentage of HP positive samples which had values above the normal, defined the sensitivity of the test.

Results from the 2 min. time point ranged from 1995 to 16163 dpm with a mean of 6587 dpm for the negative samples and ranged from 4369 dpm to 49158 dpm with a mean of 13730 dpm for positve samples. A marked overlap in the counts was observed at this time point, between HP positive and negative samples.

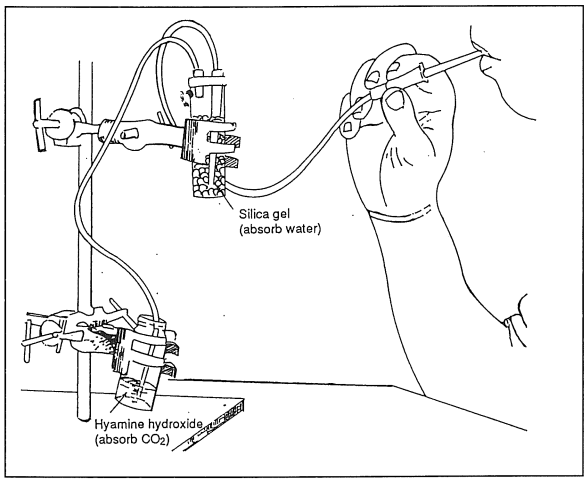

Fig. 1: Diagram showing apparatus used to perform the 14C-urea breath test

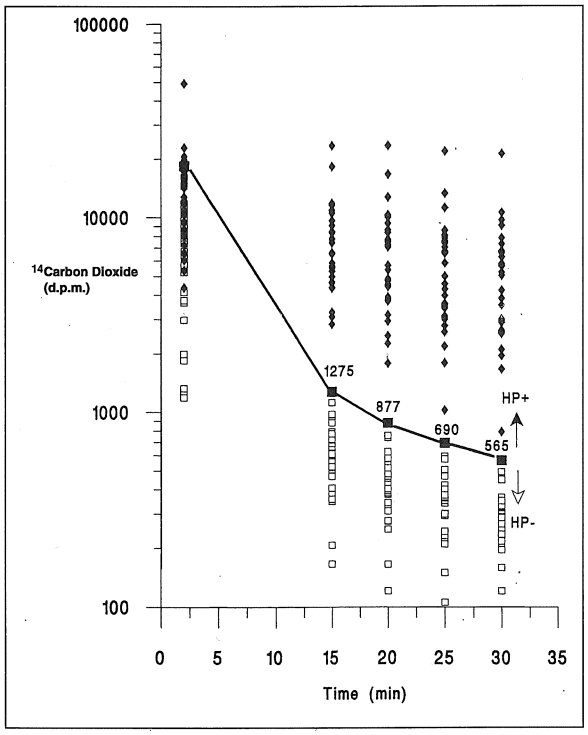

Fig. 2: Scintillation counts of HP positive and negative patients at various time points

Based on a cut-off value of the mean of HP negative+ 3SD the following values were obtained: 15 min.– 1275 dpm, 20 min.– 877 dpm, 25 min.– 690 dpm and 30 min.– 565 dpm. All positive patients had values markedly above the cutoff between 15-30 minutes. Similarly all negative patients had values clearly below the cutoff at all these time points. The sensitivity and specificity of the test was 100% at these time points.

Table I
Mean counts and cut-off values between 2 and 30 minutes after ingestion of ^{14}C-urea.

	Mean counts (dpm)				
Time (min)	2	15	20	25	30
HP pos (n=32)	13730	7979	7021	6083	5226
HP neg (n=31)	6587	605	433	339	295
HP neg + 3SD	18431	1275	877	690	565

Discussion

The UBT is an extremely useful method of diagnosing HP infection. Its non-invasive nature is a clear advantage where repeated tests are required to be performed on patients for instance to monitor response to treatment or in the setting of a field survey where many tests need to be performed over a short period of time. In contrast to serology where antibodies may persist for an indefinite period after eradication of HP, UBTs detect only current infection and are thus helpful in following patients after treatment.

^{13}C and ^{14}C UBT have both been performed and reported by other workers[5,8,9]. The ^{13}C UBT possesses the advantage of utilising a non-radioactive isotope. However, about 1.1% of all carbon in nature is ^{13}C and to ensure that exhaled ^{13}C is not naturally occurring, a relatively large dose of ^{13}C labelled urea substrate is necessary. Furthermore, ^{13}C needs to be measured using a mass spectrometer, an instrument which is not widely available.

The ^{14}C test on the other hand is simple to perform although it carries a small inherent risk of radioactivity.

The liquid scintillation counter is available in most service laboratories in hospitals and the actual measured risk of radioactivity used in our protocol is insignificant[6,10].

The urea solution that is dispensed to patients is odourless and has no distaste as the amount of urea used is small. All patients tolerated the solution well. The method of trapping the breath is also simple and convenient for patients to follow.

We have followed the protocol established by Marshall and colleagues[6] where several modifications have been carried out on previously described methods. This included dispensation with a test meal which would normally prolong the excretion of ^{14}C in the breath. This has the advantage of a shorter breath collection time as well as ease of preparation of the test solution. The only drawback with the urea solution is that some urea is hydrolyzed as it comes into contact with bacterial urease in the oral cavity. Readings of early samples are therefore falsely high and should be disregarded.

As was noted in our experience, counts at the 2 minute time point was excessively high and there was marked overlap in the counts between HP positive and negative patients.

We have shown in our tests that the ^{14}C UBT gives reliable and accurate results from the 15 minute time point upwards. For practical purposes any of these time points can be selected for single breath sample collection for the diagnosis of HP.

In our experience, the ^{14}C urea breath test shows a high degree of accuracy in the diagnosis of HP infection. It is easy and rapid to perform and is an invaluable non-invasive test in the diagnosis of HP infection.

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