Urea breath tests in the management of Helicobacter pylori infection

R P H Logan

Summary
The $^{13}$C-Urea breath test (UBT) is based on the simple principle that a solution of isotopically labelled urea will be rapidly hydrolysed by the abundantly expressed urease of $H$ pylori. The released $^{13}$CO$_2$ is absorbed across the mucus layer to the gastric mucosa and hence, via the systemic circulation, excreted in the expired breath. Distribution of urea throughout the stomach prevents sampling error and allows semiquantitative assessments of the extent of $H$ pylori infection.

Originally the $^{14}$C-UBT was complex, cumbersome and costly but, by simplifying the protocol and reducing the number of samples to be analysed, is now a much easier, quicker and cheaper test for detecting $H$ pylori. Although mass spectrometry is needed for analysis of exhaled $^{14}$CO$_2$, the use of the stable isotope, which is completely safe, provides advantages over the $^{13}$C-UBT using radioactive $^{13}$C-urea, such that it can be used in women and children and a user’s licence is not required. The widespread availability of scintigraphy for $^{14}$CO$_2$ analysis may make the $^{14}$C-UBT seem an attractive alternative to the $^{13}$C-UBT. However, there are no standard protocols for the $^{14}$C-UBT and although the methods are similar, several different cut off values are used which makes formal validation studies still necessary.

Both tests are easy to perform with minimum opportunity for observer variation or methodological error; they are very sensitive and specific tests and provide a clinical “gold standard” against which the accuracy of other tests can be validated. The $^{13}$C-UBT detects only current infection and as the sole method for assessing eradication. In addition, because the $^{13}$C-UBT can be performed repeatedly in the same subject, it can be used to monitor the effects of novel anti-$H$ pylori therapies and for epidemiological studies in children.

Introduction
Since the identification and subsequent isolation of $H$ pylori in 1983 a considerable body of evidence has accumulated showing that $H$ pylori is the principal cause of non-autoimmune gastritis and peptic ulcer. By 1994, the evidence was sufficiently strong for the National Institutes of Health (NIH) consensus conference in the United States to recommend eradication of $H$ pylori infection to prevent ulcer recurrence in all patients with documented peptic ulcer disease. The observation that eradication of $H$ pylori cures peptic ulcer disease and that screening for $H$ pylori infection may improve the diagnostic yield of endoscopy is leading to a major re-evaluation of the optimal management of patients with dyspepsia.

Prior to the first description of the $^{13}$C-Urea breath test (UBT), the diagnosis of $H$ pylori infection had usually been established by histology, culture or biopsy urease test or non-invasively by serology. Detection of $H$ pylori by ELISA serology reflects only previous exposure to $H$ pylori and may not indicate active or current infection. In addition because antibody titres can take up to six months to fall after successful treatment, ELISA tests cannot readily be used to assess the efficacy of new treatment regimens or novel antimicrobial agents specifically developed for the treatment of $H$ pylori infection. The development of the $^{14}$C-UBT as a simple, practical, highly accurate non-invasive test for $H$ pylori infection, is reflected in its increasingly important role in the management of dyspepsia. This review will consider some of the recent developments in the methodology and application of the $^{13}$C-UBT.

Principles of the $^{13}$C-UBT
The $^{13}$C-UBT exploits the copious amounts of urease produced by $H$ pylori which hydrolysates urea to form ammonia and soluble carbon dioxide which is expired in the exhaled breath. Labelling of urea with either isotope allows the $^{13}$CO$_2$ to be detected in the expired breath. $^{13}$C is always measured as a ratio of $^{13}$C to $^{12}$C ($\delta^{13}$CO$_2$, per mil), therefore the amount of excreted CO$_2$ does not need to be measured and a 10 ml tube of expired air suffices for analysis. When $H$ pylori is present, the relative amount of $^{13}$CO$_2$ increases considerably, and often exceeds that in the calibration standard, which is why results of the $^{13}$C-UBT are expressed as the “excess” $\delta^{13}$CO$_2$ per mil.

$^{14}$CO$_2$ is measured using scintigraphy which is simple and relatively cheap but may be more inconvenient, especially since its use is restricted in some European countries if stable isotopic equivalents are available. Although the radiation exposure of a single breath test is equivalent to only one day’s background dose, patients must still have the risks explained. Some patients with chronic lung disease may be unable to provide sufficient CO$_2$ to change the colour of the trapping solution, in which case the $^{13}$C-UBT provides a useful alternative. Most of the validation studies and assessments of new methodologies for the UBT have been done using $^{13}$C-UBT, although some reports have also studied the $^{14}$C-UBT.
Methodology

**Patient related factors**

Urease producing oropharyngeal bacteria may rarely cause false positive results if breath samples are taken within 10 minutes of urea administration; false negative 13/14C-UBT results will arise if tests are done in patients taking antibiotics, bismuth salts or more rarely taking proton pump inhibitors or sulfasalazine (which reduce the extent of antral *H pylori* infection). Patients should stop taking these drugs at least 10 days before undergoing a UBT.

Many 13C-UBT protocols require patients to fast for at least four hours but recently several groups have shown that fasting is no longer necessary if using the 13C-UBT and a protocol which includes a test meal. 5-7

**UBT related factors**

**Test meal**

The test meal delays gastric emptying to maximise the gastric residence time, and exposure of the organism, to labelled urea, thereby allowing the dose of isotope to be reduced and increasing the sensitivity of the test. Citric acid, which delays gastric emptying via pH, or nutrition supplements (for example, Pulmicore, or Calogen), which delay gastric emptying via lipid content, are often used as test meals for the UBT.

A citric acid test meal may decrease oral urease activity but at the same time increase the discrimination between positive and negative 13C-UBT values. 8-9 The explanation for the increased excess δ13CO2 excretion in infected patients with citric acid is unclear. However the additional source of [H+] contained in citric acid may enhance the extent of urea by providing a supplementary source of [H+] for the generation of carbamate and ammonium.

Another study suggested that citric acid, by increasing the extent of excess δ13CO2 excretion, was the optimal test meal for the 13C-UBT, but no results from uninfected patients were presented. 10 In addition, the extent to which citric acid increased the excess δ13CO2 excretion was difficult to judge as the breath test values are reported as proportional values above control (baseline) excess δ13CO2 values. However any test meal which is proved to delay gastric emptying can be used providing it is palatable (the poor palatability of citric acid improves with orange juice), cheap, has a long shelf half-life, does not impair the dispersal of 13C-urea or contribute in itself to excess δ13CO2 excretion.

**Quantity and formulation of urea**

Although the amount of 13C-urea now used is less than half that originally proposed by Graham, the quantity of substrate must be sufficient to saturate the enzyme. The specific activity of *H pylori* urease is similar to that of other bacteria but is produced over-abundantly and has a high binding affinity for urea. 11 With purified 13C-urea, the urease is fully saturated at the dose of 13C-urea (100 mg) currently used, in contrast to 13C-urea which should be used with cold urea to ensure full saturation of the enzyme. Lower doses of 14C-urea (75 mg) have been incorporated into several commercial kits (many which may have been incompletely validated) but may be associated with false negative results (Perri F, personal communication).

Without a test meal, 13/14C-urea in solution rapidly empties from the stomach, often within 10 minutes and breath samples taken after this initial period may give false negative results.

Recent reports have shown that a test meal is not needed if 13C-urea is given in a capsule specially coated to disintegrate within minutes of entering the stomach. Consequently there is no 14C-urea hydrolysis by oropharyngeal urease, so that in uninfected patients, the small increase in excess δ13CO2 excretion normally seen after swallowing a solution of 13C-urea, is almost completely abolished. 12 This may improve the accuracy of the test by reducing levels of excess δ13CO2 excretion in uninfected patients to almost zero. In addition without the hydrolysis from oropharyngeal urease, breath samples may be taken sooner, thereby reducing the time taken to perform the test. It is also possible that this approach will allow the quantity of isotope to be lowered, further reducing the overall cost of the test.

**Measurement of 13CO2 and analysis of results**

The major disadvantage to the 13C-UBT is the cost of 13CO2 analysis. Without the economies of scale and volume, purchase of a gas chromatography/isotope ratio mass spectrometry (GC/IRMS) unit is generally uneconomical, but samples can be sent for analysis by post to a commercial stable isotope laboratory. Although the cost of 13C detection by GC/IRMS has been falling, it is still the major factor limiting more widespread use of the 13C-UBT. Several alternative methods for the detection of 13CO2 have recently been described, including the use of laser or infra-red spectroscopy. 13-15 These new technologies are still at an early stage of development and studies of their accuracy are limited, but they promise to enhance considerably the use of the 13C-UBT, so that it can be done in most hospitals or specialist clinics.

The cut off value for the 13C-UBT was originally determined as 5.0 per mil based on the normal distribution of excess δ13CO2 values for *H pylori* negative subjects who have never been infected. However, a recent re-analysis of data from several large clinical trials of *H pylori* eradication have allowed construction of receiver operator characteristic curves (ROCs) to set the optimum cut off value according to sensitivity and specificity. On the basis of ROC analysis, a cut off value of 3.5 per mil excess δ13CO2 improves the sensitivity and specificity to 98.5% and 97.0%, respectively, although in clinical practice, and in contrast to the 13C-UBT, less than 0.1% of 13C-UBT results fall between 3.5–5 per mil. 16 17 The accuracy of the 13C-UBT is supported by well designed studies which have shown the 13C-UBT to have a sensitivity and specificity of >95%. Less encouraging results reported in early studies
Urea breath tests in the management of H pylori infection

S49

Results with the 13C-UBT are extremely rare and patients in whom eradication of H pylori can be used as the sole method is associated with resolution of histological gastritis and prevention of relapse of duodenal ulcer, easily and at an earlier stage than other tests for whom treatment has not been successful more patients successfully treated and detect those in being attempted. The test can clearly identify disease.20

Applications of the 13/14C-UBT

Both the 13C-UBT and 14C-UBT can be used to screen dyspeptic patients prior to endoscopy and to assess the efficacy of H pylori eradication therapy. However the 13C-UBT can also be used to detect infection in children, to measure suppression and clearance of infection in phase I and II trials, for epidemiological research and as a near patient test in primary care.

SCREENING BEFORE ENDOSCOPY

Several studies have suggested that non-invasive tests for H pylori can be used to screen young dyspeptic patients prior to endoscopy, although the most appropriate subsequent management strategy for these patients is as yet unclear and may vary from country to country.1 8 11 However a study from Glasgow which used the 13C-UBT to screen dyspeptic patients, found the prevalence of peptic ulcer in infected patients was 59%, with a positive predictive value high enough for it alone to be recommended as a screening test for ulcer disease.20

ASSESSING ERADICATION OF INFECTION

The 13C-UBT is the best method of following patients in whom eradication of H pylori is being attempted. The test can clearly identify patients successfully treated and detect those in whom treatment has not been successful more easily and at an earlier stage than other tests for H pylori. Because eradication of H pylori is associated with resolution of histological gastritis and prevention of relapse of duodenal ulcer, the 13/14C-UBT can be used as the sole method of follow up. Using well validated protocols the sensitivity and specificity of the 13C-UBT 4–6 weeks after the end of therapy has been found to be >95% and 96% respectively. There are fewer longer follow up studies, although, those using the European Standard 13C-UBT protocol have shown >99% concordance between a 13C-UBT and biopsy based tests at one year after treatment.21 Unfortunately other 13C-UBT studies from the United States with apparently minor protocol variations resulted in poorer sensitivity and specificity values when assessing eradication.

Applications of the 13C-UBT

ASSESSING SUPPRESSION AND RECURRENCE OF H PYLORI INFECTION

The 13C-UBT, as a semiquantitative measure of H pylori, can be used to monitor the suppression of H pylori by the fall in excretion of 13CO2. In single dose or short term treatment studies the extent of suppression can allow differences between anti-H pylori drugs to be rapidly and easily assessed. By performing serial 13C-UBTs the rate of recurrence of H pylori infection after different treatments can also be determined—for example, showing that, regardless of the preceding length of treatment with bismuth, H pylori infection recurs within days of finishing therapy.22

13C-UBT IN CHILDREN

The 13C-UBT is ideally suited for the determination of H pylori status in children, although until very recently there was a paucity of adequate data to validate its routine use.23 24 As the endogenous CO2 excretion by small children is much less than in adults, less urea is required and for children below the age of 8. Adult breath sampling is feasible for children over 3 years, but a mask may be required to collect expired breath samples from younger children.

Epidemiological research

A very important advantage of the 13C-UBT over the 13C-UBT is that it can be used for epidemiological studies, particularly in children, where in contrast to serological methods, the 13C-UBT detects active infection, rather than previous exposure. Recently several studies have been performed using the 13C-UBT in an attempt to document possible routes of transmission of H pylori between children.25 26

Conclusions

The 13C-UBT and 14C-UBT are very accurate tests for detecting H pylori infection with a sensitivity and specificity better than many other tests for H pylori. The 13C-UBT detects much lower levels of H pylori infection and by assessing the entire gastric mucosa avoids the risks of sampling error. The 13/14C-UBT is a practical and readily available test providing a “gold standard” against which other tests for H pylori can be compared. The recent development of encapsulated 13C urea and cheaper methods of 13C analysis allow the duration of the test and costs to be reduced, and may herald a more widespread clinical application of this useful test.

References

1 Logan RFA, Logan RPH. The management of Helicobacter pylori infection. PHLS Microbiol Dig 1997;14(suppl 1):34–8.


