Brazilian Journal of Medical and Biological Research (1999) 32: 1493-1497
ISSN 0100-879X

Application of isotope-selective non-dispersive infrared spectrometry for the evaluation of the ^{13}C-urea breath test: comparison with three concordant methods

L.G.V. Coelho[1], M. Reber[2], M.C.F. Passos[1], R.O.A. Aguiar[1], P.E. Casaes[1], M.L. Bueno[1], F.R. Yazaki[1], F.J. Castro[1], W.L.S. Vieira[1], J.M.M. Franco[1] and L.P. Castro[1]

[1]Serviço de Gastroenterologia, Nutrição, Cirurgia Geral e do Aparelho Digestivo (GEN-CAD), Hospital das Clínicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brasil
[2]Brisotop Representações Ltda., São Paulo, SP, Brasil

Abstract

The aim of this work was to compare the performance of isotope-selective non-dispersive infrared spectrometry (IRIS) for the ^{13}C-urea breath test with the combination of the ^{14}C-urea breath test (^{14}C-UBT), urease test and histologic examination for the diagnosis of *H. pylori* (HP) infection. Fifty-three duodenal ulcer patients were studied. All patients were submitted to gastroscopy to detect HP by the urease test, histologic examination and ^{14}C-UBT. To be included in the study the results of the 3 tests had to be concordant. Within one month after admission to the study the patients were submitted to IRIS with breath samples collected before and 30 min after the ingestion of 75 mg ^{13}C-urea dissolved in 200 ml of orange juice. The samples were mailed and analyzed 11.5 (4-21) days after collection. Data were analyzed statistically by the chi-square and Mann-Whitney test and by the Spearman correlation coefficient. Twenty-six patients were HP positive and 27 negative. There was 100% agreement between the IRIS results and the HP status determined by the other three methods. Using a cutoff value of delta-over-baseline (DOB) above 4.0 the IRIS showed a mean value of 19.38 (minimum = 4.2, maximum = 41.3, SD = 10.9) for HP-positive patients and a mean value of 0.88 (minimum = 0.10, maximum = 2.5, SD = 0.71) for negative patients. Using a cutoff value corresponding to 0.800% CO_2/weight (kg), the ^{14}C-UBT showed a mean value of 2.78 (minimum = 0.89, maximum = 5.22, SD = 1.18) in HP-positive patients. HP-negative patients showed a mean value of 0.37 (minimum = 0.13, maximum = 0.77, SD = 0.17). IRIS is a low-cost, easy to manage, highly sensitive and specific test for *H. pylori* detection. Storing and mailing the samples did not interfere with the performance of the test.

Correspondence
L.G.V. Coelho
Rua dos Otoni, 705/601
30150-270 Belo Horizonte, MG
Brasil
Fax: +55-31-222-4641
E-mail: lcoelho@gold.com.br

Research supported by FAPEMIG and CNPq. Publication supported by FAPESP.

Received November 20, 1998
Accepted July 28, 1999

Key words
- ^{13}C-urea breath test
- Infrared spectrometry

Introduction

Today, the causal relationship between gastric *Helicobacter pylori* infection and chronic gastritis and peptic ulcers is well established (1). Recently, this microorganism was considered as a carcinogenic agent (type I) by the World Health Organization for gastric cancer, and studies carried out since 1993 have suggested its role in gastric mucosa-associated lymphoid tissue lymphoma (2).

The diagnosis of its presence is regularly performed by endoscopic examination with the collection of gastric mucosa fragments for histological examination. This involves the use of various stains such as Giemsa stain, carbolfuchsin and others, microbiological tests (Gram and culture smears), or even colorimetric methods like the urease test which rely on the increased production of this enzyme by the microorganism. The diagnosis may even be performed through serological exams or by breath tests employing carbon^{13}- or carbon^{14}- labelled urea. These tests are based on the elevated production of urease. When the labelled urea is orally administered, the labelled CO_2, originating from the breakdown of this urea by the urease of the bacteria, can be detected in the air expired by infected individuals. These breath tests, due to their accuracy, simplicity and low cost, are universally accepted today as the gold standard for monitoring patients undergoing anti-*H. pylori* therapy. The breath tests employing ^{14}C-urea only require a liquid scintillation spectrometer which is easily available at most medium-sized health centers. The tests are inconvenient, however, because of their reliance on the use of a radioactive substance. The substance requires specialized personnel for handling, and should not be used in children and pregnant women. The recent development of instruments other than the mass spectrometer, which is of high cost and restricted availability, for tests using the stable, non-radioactive isotope ^{13}C has stimulated the wider use of this methodology in the diagnosis of the presence of *H. pylori* in the human stomach (3).

The present study aims to compare the ^{13}C-urea breath test with three other methods (histology examination, urease test, and ^{14}C-urea breath test) in patients with peptic ulcers.

Patients and Methods

Before participating in the study, all patients gave their written informed consent, and the study was approved by the Research Ethics Committee of UFMG University Hospital.

Fifty-three patients (30 men and 23 women) from the Peptic Ulcer Outpatient Clinic of the UFMG University Hospital, Belo Horizonte, were included. All patients had duodenal ulcers, including those admitted to the clinic for anti-*H. pylori* therapy, as well as those already submitted to eradication of the microorganism. All candidates for inclusion in the study underwent upper digestive endoscopy in addition to gastric biopsies to test for *H. pylori* by both the urease test and histological examination, and the microorganism was detected by hematoxylin and eosin and modified Giemsa staining. Next, the patients underwent the ^{14}C-urea breath test as previously described (4), with values above 0.800% CO_2/weight (kg) considered to be positive (5). For the objectives of this study, patients were considered to be *H. pylori* positive when they were positive to the three traditional tests performed (urease, histological examination, and ^{14}C-urea breath test), all performed within the preceding 30 days. Patients with three negative exam results during the same period were considered to be *H. pylori* negative. Patients were excluded from the study when they did not undergo all three tests, or when the tests gave conflicting results. Patients who used antimicrobial drugs within the preceding four weeks were also excluded.

^{13}C-urea breath test

The test was performed using the infrared isotope analyzer IRIS® (Wagner Analysen Technik, Bremen, Germany), which allows a precise determination of the two isotopes, $^{13}CO_2$ and $^{12}CO_2$. Two breath samples, taken respectively before and after the ingestion of ^{13}C-urea, are collected into 1.3-l bags and are presented together to the instrument. Sampling and analysis are fully automated. The following methodology was used: after an overnight fast, a sample of expired CO_2 air was taken, corresponding to time 0 (control), through inflation of a 1.3-l breath bag. Next, patients ingested 75 mg of ^{13}C-urea in 200 ml of orange juice without the addition of water or sugar. Another breath sample was taken 30 min after administration of the tracer. The two-bag samples obtained from each patient were stored at room temperature, packed and later shipped by conventional express air-mail to São Paulo, location of the equipment used in the study, where they were analyzed by one of the authors (MR). The results are presented as delta-over-baseline values (DOB) which indicate the change in the $^{13}CO_2/^{12}CO_2$ ratio brought about by the metabolic activity induced by the administration of the labeled urea. Positive test results were those with DOB values above 4‰, as indicated by the manufacturers and as recently validated (6).

Statistical analysis

All of the procedures were performed by the same investigators, who were unaware of the presence or absence of *H. pylori* in the examined patients. The homogeneity of the two groups studied was determined by the Mann-Whitney and chi-square tests, with the level of significance set at $P<0.05$. Spearman's coefficients were calculated to test the correlation between the results of the ^{13}C- and ^{14}C-urea breath tests.

Results

Table 1 shows the demographic characteristics of the two groups studied, demonstrating their homogeneity. The median interval between performance of the ^{13}C- and ^{14}C-urea breath tests in Belo Horizonte and their analysis in São Paulo was 11.5 days (minimum = 4, maximum = 21, SD = 5.84, CI 95% = 8-14).

There was 100% agreement between the results of the ^{13}C-urea breath tests and the *H. pylori* status determined by the combination of the urease test, histological examination and ^{14}C-urea breath tests.

Table 1 - Demographic characteristics of the 53 patients studied.

HP POS, *Helicobacter pylori* positive; HP NEG, *Helicobacter pylori* negative; NSAIDS, nonsteroidal anti-inflammatory drugs.

	HP POS	HP NEG	Total	P
Number	26	27	53	-
Mean age (years)	49	39	-	0.08
Sex (female/male)	13/13	10/17	23/30	0.65
Smoking (N)	12	8	20	0.78
Use of NSAIDS (N)	3	1	4	0.71
Median interval between ^{13}C- and ^{14}C-urea breath tests (days)	14	8	-	0.34

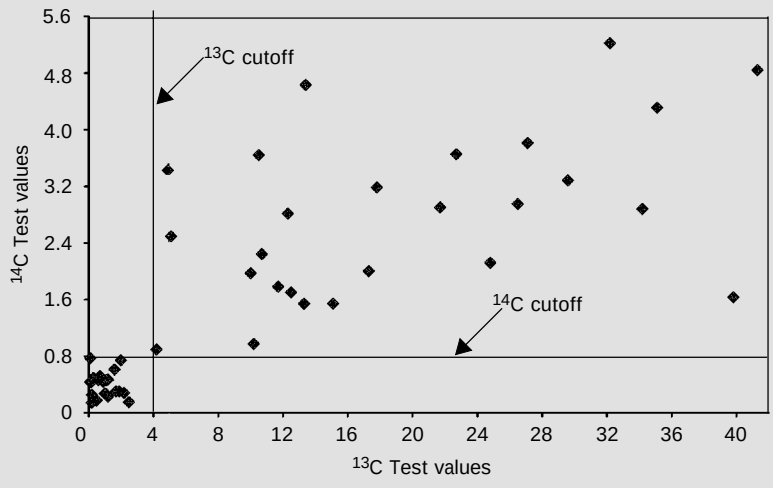

Figure 1 - Comparison of the results of the ^{13}C- and ^{14}C-urea breath tests using a cutoff of 4‰ DOB (delta-over-baseline) for the ^{13}C-urea breath test and a cutoff of 0.800% CO_2/weight (kg) for the ^{14}C-urea breath test for detection of *H. pylori* infection.

Figure 1 illustrates the results of the ^{13}C- and ^{14}C-urea breath tests, showing a 100% coincidence between the results of the two tests. With a cutoff point corresponding to a DOB value above 4.0, the ^{13}C-urea breath test had a median value of 19.38 (minimum = 4.2, maximum = 41.3, SD = 10.9, CI 95% = 14.95-23.81) for the positive patients and a median value of 0.88 (minimum = 0.10, maximum = 2.5, SD = 0.71, CI 95% = 0.60-1.17) for the negative patients. With a cutoff point corresponding to a value of 0.800% CO_2/weight (kg), the ^{14}C-urea breath test showed a median value of 2.78 for *H. pylori*-infected individuals (minimum = 0.89, maximum = 5.22, SD = 1.18, CI 95% = 2.30-3.26). The median value for non-infected individuals was 0.37 (minimum = 0.13, maximum = 0.77, SD = 0.17, CI 95% = 0.30-0.44). There was a significant correlation at the 0.01 level between the results of the two breath tests, with a Spearman's coefficient of 0.814.

Discussion

Various tests have been proposed for the diagnosis of *H. pylori* infection, all with some limitations. Among the invasive methods, culture of the microorganism, although considered 100% specific, results in up to 20% false-negative results which arise from problems in transport and in the conditions of the culturing environments involved. Moreover, the use of molecular biology techniques such as PCR is hampered by the eventual inhibition of Taq polymerase in some cases and the occurrence of false-positive results in others (7). Although useful, the two invasive methods most employed in daily practice, the urease test and histological examination, may be influenced by a number of variables related to the quality of the samples and the qualifications of the laboratory technician. Thus, the performance of multiple tests to obtain more precise results is recommended, especially with respect to clinical research.

Among the noninvasive tests, serology has been used, especially in the initial diagnosis of *H. pylori* infection in epidemiological surveys. However, the slow decline of the antibodies after eradication makes it difficult to determine the presence or absence of the microorganism in exams performed up to 6 months after treatment. In addition, it is necessary to collect serum before and after treatment (8). Finally, the ^{13}C- and ^{14}C-urea breath tests are coming into increasing use, with 90% sensitivity and specificity when compared with the invasive tests already described (1,9).

Our results show that the ^{13}C-urea breath test is 100% sensitive and specific when compared with the urease test, histological examination, and the ^{14}C-urea breath test, grouped together to give greater precision to the detection of the presence or absence of *H. pylori*.

The ^{13}C-urea breath test was described by Graham et al. (10) using a mass spectrometer. The basic principle of this test consists of the administration of ^{13}C-urea followed by the measurement of the $^{13}CO_2/^{12}CO_2$ ratio in the breath. An increase in the proportion of $^{13}CO_2$ indicates that the patient is infected. The equipment traditionally used to carry out the breath test with ^{13}C is the costly and scarcely available mass spectrometer. More recently, other alternative techniques have arisen, such as the non-dispersive infrared spectrometer (11-14), and in 1997 a laser prototype was described (15). In the non-dispersive infrared spectroscopy the gas sample is fed to the gas analyzer through an autosampler without any sample preparation. The absorption of infrared light in the measurement cell is compared to the specific absorption for $^{12}CO_2$ and $^{13}CO_2$. This is achieved by using reference gas cells filled with $^{12}CO_2$ and $^{13}CO_2$. After some corrections for cross-sensitivity, the $^{13}C/^{12}C$ isotope ratios can be measured with a reproducibility better than 0.3 delta ‰ over a wide

range of ratios. The non-dispersive infrared spectrometer equipment has the advantage of low cost and easy operation, requiring no helium. However, it requires a larger breath sample (± 500 ml) for analysis, a factor that makes the technique difficult to carry out in the examination of children and when samples have to be shipped over long distances for analysis. In contrast, the two other methods described require small samples (10 ml for the mass spectrometer and 2 ml for the laser analysis), facilitating the shipment of samples for analysis by mail. Nevertheless, in our study, the greater volume of breath required using the infrared equipment was not a problem when the samples were shipped to another center located 580 km away. Nor did sample storage for a period of 11.5 days lead to loss of air from the bags. This permitted us to ship the bags in lots containing the exams of several patients in addition to the duplicate analysis of each bag (1300 ml).

With the dissemination of the use of these breath tests in gastroenterology and nutrition, and especially in the diagnosis of *Helicobacter pylori* infection, it is hoped that in the short-term, new portable and low-cost equipment is placed on the world market for all to use.

References

1. Marshall BJ (1994). *Helicobacter pylori*. *American Journal of Gastroenterology*, 89 (Suppl 1): 116-128.
2. Wotherspoon AC, Doglioni C, Diss TC, Pan L, Moschini A, Boni M & Isaacson P (1993). Regression of primary low-grade B-cell gastric lymphoma of mucosa-associated lymphoid tissue type after eradication of *Helicobacter pylori*. *Lancet*, 342: 575-577.
3. Koletzko S, Haisch M, Seeboth I, Braden B, Hengels K, Koletzko B & Hering P (1995). Isotope-selective non dispersive infrared spectrometry for detection of *Helicobacter pylori* infection with ^{13}C-urea breath test. *Lancet*, 345: 961-962.
4. Coelho LGV, Chausson Y, Passos MCF, Sadala RU, Costa EL, Sabino CVS, Queiroz DMM, Mendes EN, Rocha GA, Oliveira CA, Lima Jr GF, Fernandes MLM & Castro LP (1990). Test respiratoire à l'urée marquée au carbone-14 pour le diagnostic de la colonization gastrique par *Helicobacter pylori*. *Gastroenterologie Clinique Biologie*, 14: 801-805.
5. Neves OF, Rocha Z, Coelho LGV, Chausson Y, Tófani P, Passos MCF, Aguiar ROA & Castro LP (1998). Uso de um método muito simples para a determinação e controle do ponto de corte do teste respiratório com 14C-uréia para a detecção de *Helicobacter pylori* no estômago humano. *Anais da 7ª Semana de Iniciação Científica da UFMG, 26-31/10/1998*. Pró Reitoria de Pesquisa, UFMG, Belo Horizonte.
6. Leodolter A, Domínguez-Muñoz JE, Von Arnim U, Manes G & Malferteiner P (1998). ^{13}C-Urea breath test for the diagnosis of *Helicobacter pylori* infection. A further simplification for clinical practice. *Canadian Journal of Gastroenterology*, 33: 267-270.
7. Lehn N & Mégraud F (1996). Diagnosis of *Helicobacter pylori*. *Current Opinion in Gastroenterology*, 12 (Suppl 1): 6-10.
8. Thijs JC, van Zweet AA, Meyer BC & Berrelkamp RJP (1994). Serology to monitor the efficacy of anti-*Helicobacter pylori* treatment. *European Journal of Gastroenterology and Hepatology*, 6: 579-583.
9. Atherton JC & Spiller RC (1994). The urea breath test for *Helicobacter pylori*. *Gut*, 35: 723-725.
10. Graham DY, Klein PD, Evans DG, Evans Jr DG, Albert LC, Opekun AR & Boutton TW (1987). *Campylobacter pylori* detected noninvasively by the ^{13}C-urea breath test. *Lancet*, 1: 1174-1177.
11. Hanauer G, Bethke T, Hermerschmidt M & Tuch K (1997). Detection of *Helicobacter mustelae* infection in ferrets with a ^{13}C-urea breath test using infrared spectrometry. *Gut*, 41 (Suppl 1): 120 (Abstract).
12. Yamamoto S, Kaneko H, Kajiwara M, Deng W, Mori S, Hayakawa T, Yamaguchi C, Uruma M, Yamashita K, Iyo T, Kusugami K & Mitsuma T (1997). Usefulness of an infrared spectrometer for monitoring of *Helicobacter pylori* infection with ^{13}C-urea breath test. *Gastroenterology*, 112 (Suppl): 335 (Abstract).
13. Kato M, Ohara S, Asaka M & Toyota T (1997). New, on the spot infrared analyser for ^{13}C-urea breath test: a simple, easy-to-use method for detecting *Helicobacter pylori* in the physician's office. *Gastroenterology*, 112 (Suppl): 169 (Abstract).
14. Hildebrand P & Beglinger C (1997). Nondispersive infrared spectrometry: a new method for detection of *Helicobacter pylori* infection with the ^{13}C-urea breath test. *Clinical Infectious Diseases*, 25: 1003-1005.
15. van der Hulst RWM (1997). Laser assisted ratio analyser-^{13}C-urea breath testing, a novel non-invasive system for the diagnosis of *H. pylori* infection: A prospective comparative diagnostic multicenter study. *Gut*, 41 (Suppl 1): 72 (Abstract).