
Vaccination is a potentially cost-effective approach to the prevention and treatment of Helicobacter pylori-induced chronic disease. Since Helicobacter pylori causes a superficial infection of the gastric epithelium, the principal mediator of effective immunity should be IgA antibody stimulated by mucosal presentation of antigen and secreted into the mucus gel. A strong rationale exists for selection of urease as a vaccine candidate. The ureA and ureB genes of H. pylori were cloned in E. coli, and the multimeric urease apoenzyme expressed and purified as a soluble protein of baculoviral urease (550kDa) having 12 nm particulate structure. Manufacturing, quality control, stability and toxicological tests were performed in accordance with regulatory guidelines for clinical products. Given by the oral or intragastric route (but not by the parenteral route) to mice, 4 weekly doses of ≥ 5 μg apoenzyme protected up to 100% of mice against challenge with virulent H. felis. Protection was antigen dose-dependent and required coadministration of a mucosal adjuvant, such as LT or an atonic LT mutant, which was effective at doses of ≤ 500 ng. Immunized mice became transiently colonized with H. felis after challenge, but cleared their gastric infections within 2-7 days, whereas unimmunized controls remained persistently infected. Clearance of gastric infection was effected by the rapid recruitment of IgA antibody secreting cells (ASC) and anti-urease ASC into gastric mucosa and by the secretion of anti-urease IgA into gastric mucus. Immunization resulted in long-lasting serum and secretory anti-urease antibody responses, detectable for >7 months. Mice chronically infected with H. felis mounted serum IgG anti-urease responses but failed to develop serum or secretory IgA, whereas artificially immunized mice developed strong IgA responses—providing an important clue to the protein-specific mechanism of immune evasion by Helicobacter and to the mode of action of a urease vaccine. Assessed by cytokine responses in gastric explant cultures, infection was characterized principally by a Th1 response, whereas oral immunization activated Th2 pathways. Mice with protective immunity followed for up to 7 months showed persistent lymphoid aggregates and large numbers of IgA+ ASC in the gastric antrum and corpus. To investigate therapeutic immunization, the vaccine was administered orally with LT to Balb/c or outbred mice with chronic H. felis gastritis. Immunization resulted in clearance of the infection in up to 90% of treated mice. Clearance was associated with recruitment of IgA+ and anti-urease ASC into the gastric mucosa. Studies are underway to confirm prophylactic and therapeutic activities of the urease vaccine against H. pylori infection in mice, monkeys and cats, and a Phase 1 clinical trial is underway to assess tolerability of the vaccine in humans.

HELICOBACTER PYLORI INDUCED GASTRITIS IN THE DOMESTIC CAT. J.G. Fox, M. Batchelder, R.P. Marini, L. Yan, L. Handt, X. Li, B. Shames, A. Hayward, J. Campbell, J.C. Murphy. Division of Comparative Medicine, MIT, Cambridge, MA, USA

Helicobacter pylori has been cultured from the inflamed gastric mucosa of naturally infected cats, the lesions in the H. pylori infected cat stomach mimic many of the features seen in humans. To determine whether H. pylori negative specific pathogen free cats with normal gastric mucosa were susceptible to colonization and whether gastritis developed after infections, four H. pylori negative cats treated with cimetidine were orally dosed 3 times with 3 ml (1.5x10^6 CFU/ml) of H. pylori on alternate days. All 4 cats became persistently colonized as determined by gastric culture and PCR during serial gastric biopsies and necropsy at 7 months p.i. The two control cats did not have H. pylori isolated, nor was gastric tissue positive by PCR, one cat had a few focal lymphocytic aggregates in the body submucosa, whereas the second cat had normal gastric mucosa. All 4 H. pylori infected cats had multifocal gastritis, consisting of lymphoid aggregates plus multiple large lymphoid nodules, which were most noticeable in the antral mucosa. In addition, one H. pylori infected cat had a moderate diffuse infiltration of polymorphonuclear leukocytes in the cardia, pyloric and submucosal regions of the antrum. H. pylori like organisms were focally distributed in glandular crypts of the antrum. H. pylori infected cats had an increase in IgG H. pylori serum antibody over baseline. The H. pylori isolated from the 4 experimentally infected cats had identical RFLP patterns specific for the fla A gene to that of the inoculating strain. H. pylori readily colonizes the cat stomach and produces a persistent gastritis.

Introduction: The non-invasive methods of diagnosing H. pylori infection include breath tests and antral biopsy. The methods for H. pylori infection can take several days to return. Recently a rapid whole blood test has become available (Helisal®-Cortec Systems). The purpose of this study is to test if this test can give a diagnosis of H. pylori infection within 10 minutes. The accuracy of this test needs independent validation before it can be recommended to change clinical practice.

Methods: Patient's H. pylori status was evaluated by histology (2 antral and 2 corpus biopsies), culture (1 antral biopsy), rapid urease test (1 antral biopsy) and 14C carbon breath test. The patient was defined as H. pylori positive if two or more tests gave a positive result and negative if all tests gave a negative result. The H. pylori status was indeterminate if only one test was positive. The results of the rapid blood test (RBT) were compared to this gold standard.

Results: 11 patients took part in the study (median age 47, range 21-71 years, 55 males) with 2 subjects giving indeterminate results. Of the 59 gold standard H. pylori positive patients the RBT gave 5 false-negative results (sensitivity 92%: CI 81-97%). In 53 gold standard H. pylori negative patients the RBT gave 4 false positive results (sensitivity 92%: CI 82-98%).

Conclusion: The RBT diagnoses H. pylori infection with good accuracy. As this test gives a diagnosis within 10 minutes it should prove useful in clinical practice especially in primary care.

A CITRIC ACID SOLUTION IS AN OPTIMAL TEST MEAL IN THE 14C-UREA BREATH TEST (UBT) FOR THE DIAGNOSIS OF H. PYLORI INFECTION


14C-UBT is the most accurate noninvasive method for the detection of H. pylori infection. Different test meals have been used with the purpose to slow gastric emptying and to enhance the accuracy of the test. Aim of this study was to establish the most valuable meal modification to optimize the accuracy and practicability of the 14C-UBT. Material and Methods. 18 H. pylori positive subjects (age range 25-61 years, 15 male, 3 female) were studied three consecutive days. H. pylori infection had been previously proven in all of them by urease rapid test and histology of antral biopsies. On each study day, 75 mg of 13C-urea were given 10 min after administration of either 200 ml of Mertine®, 200 ml of Ensure® (Calgon® 1:1), or 200 ml of citric acid 0.1 N in a randomized order. The 14CO2/12CO2 ratio in a basal breath sample was compared with that of samples collected at 15, 30, 45 and 60 min after the administration of 13C-urea. The ratio increase at each time (Di) was corrected according to the weight of the patient (nD). A positive test is defined by a maximal nD (peak)>0.25. Statistical analyses were performed by repeated measures one-way ANOVA with the Bonferroni’s correction for multiple comparisons. Results: Peak and T-peak (peak time, minutes) and results are shown in the table (mean±SEM).

<table>
<thead>
<tr>
<th>Mertine®</th>
<th>Ensure® (Calgon®)</th>
<th>Citric acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak</td>
<td>T-peak</td>
<td></td>
</tr>
<tr>
<td>0.05±0.02</td>
<td>1.0±0.1</td>
<td>1.49±0.15</td>
</tr>
<tr>
<td>T-peak</td>
<td></td>
<td></td>
</tr>
<tr>
<td>53±3</td>
<td>45±3</td>
<td>30±3</td>
</tr>
</tbody>
</table>

Sensitivity of 14C-UBT with the different test meals depended on the sampling time, but at time T-peak sensitivity was 100% for all three test procedures. Conclusions: Administration of citric acid as test meal in the 14C-UBT provides a higher and earlier peak for discrimination between H. pylori positive and negative subjects. If other test meals are used, the time interval between breath sampling must be longer than 30 min (between 45 and 60 min) in order to maintain a high sensitivity.

Aim: The study was designed to validate a simplified 13C-Urea breath test (UBT) and a newly developed biopsy urease test (BUT) for the detection of Helicobacter pylori (HP) infection. In addition, the hypothesis was tested that the 13CO2/12CO2 ratio and the reaction velocity of the UBT correlate significantly with the density of HP as well as with the severity of gastritis.

Methods: 70 dyspeptic patients with unknown HP status were enrolled to the study. Patients were investigated clinically and endoscopically with taking of 4 antrum and 4 body biopsies, which were assessed for HP infection by means of an urease test (HUT test: separate analysis of antrum and body biopsies), culture and histology (HE and Warthin-Starry stains). In addition, a simplified 13C-Urea breath test (75 mg 13C-Urea, measurement of the 13CO2/12CO2 ratio before and 30 min after urea intake) was performed.

Results: 47 patients proved to be HP positive as judged from histology. In 46 patients, HP infection was also detected with the BUT, culture and UBT (sensitivity: 97.9%; specificity: 99.0%). In one patient with focal and minimal colonization of HP on histology, all the other methods failed to detect bacterial colonization. In 33 patients all four methods did not indicate bacterial colonization (specificity of BUT and UBT: 100%; sensitivity: 99.0%). The 13CO2 excess as well as the reaction velocity of the BUT were significantly correlated with the histologically visible degree of HP infection (r=0.87; BUT antrum: r=0.82; BUT corpus: r=0.87; as well as with grade of UBT: r=0.69; BUT antrum: 0.66; BUT corpus: r=0.61) and activity of gastritis (UFP: r=0.71; BUT antrum: 0.79; BUT corpus: 0.62) (p<0.0001).

Conclusions: The simplified urea breath test used in this study and the new biopsy urease test (HUT test) are highly sensitive and specific with regard to diagnosis of H. pylori infection. In addition, the 13CO2 excess and the reaction velocity of the BUT allow a rough prediction of grade and activity of gastritis.


Identification of Helicobacter pylori DNA by polymerase chain reaction (PCR) as a new sensitive tool for detection of the organism. PCR assay for H. pylori in gastric juice may show the organism’s overall presence in the whole stomach. In this study, we compared the detectability limits of the PCR assay in gastric juice with biopsy specimens using the agarose gel electrophoresis and Southern hybridization of H. pylori-specific DNA sequences. The PCR assay results were compared with those from culture, ELISA assay and 13C-urea breath test. Endoscopic examination and H. pylori PCR assay were undertaken in total 124 patients with peptic ulcer or chronic gastritis. Gastric juice and biopsy specimens from the antrum and the corpus were taken from these patients. After neutralization and DNA extraction, H. pylori DNA was amplified with the primer (Valentine et al. J Clin Microbiol 1991;29:909-95), which was homologous to a portion of the 1.9-kb fragment of chromosomal DNA of H. pylori. In comparison of PCR assay in gastric juice with biopsy specimens (90 examinations), both positive in 34(38%) and 36(40%), both negative in 37(41%) and 30(33%), gastric juice positive but biopsy negative in 10(11%) and 12(13%), gastric juice negative but biopsy positive in 10(11%) and 12(13%), and vice versa in 9(10%) and 12(13%), when detected by electrophoresis and hybridization, respectively, showing equivalent detection rates in the PCR assay. PCR-positive samples of gastric juice evaluated by electrophoresis and hybridization coincided with positive samples in 56% of culture, 59% of tissue IgA antibody identification(ELISA), 94% of serum IgG antibody (ELISA) and 77% of 13C-urea breath test; PCR-negative samples coincided with negative samples in 96% of culture, 81% of tissue IgA antibody and 98% of serum IgG assessments and 93% of 13C-urea breath test evaluations. Detection of H. pylori in gastric juice has potential advantage for examining H. pylori infection in the whole stomach.