Helicobacter pylori

HOW QUICKLY DOES HELICOBACTER PYLORI (H. PYLORI) RECUR AFTER TREATMENT? REH Logan, S Hardiman, PA Gummett, MM Walker, ON Kamin, HH Barron, HI Misiewicz, Parkside Helicobacter Study Group, Central Middlesex and St Mary's Hospitals, London.

Duodenal ulcer recurrence can be prevented by eradication of H. pylori: no evidence of infection at least one month after finishing treatment. The non-invasive 13C-urea breath test, free of sampling error and able to detect lower levels of active infection than endoscopical biopsy based methods or serology, is ideal for follow up after treatment. This study determines how soon H. pylori recurs after therapy thus providing a rational timing for assessment of eradication.

Patients needing H. pylori eradication were invited to enter the study. Before starting treatment H. pylori status was assessed by antral histology (H&E, and Gimenez stains), culture (micronaerophilic conditions for up to 10 days) and 13C-UBT (European standard protocol, positive result = excess 13CO2 > 5 per ml). The 13C-UBT was repeated immediately after finishing treatment and then at weekly intervals for 1 month (or until positive) and subsequently at 3, 6, and 12 months.

Forty-six patients (26 men, median age 45 y, range 19-67 y) with either active (n=10) or previous / recurrent (n=36) DU were studied. All patients had a positive 13C-UBT (mean ±SEM excess 13CO2 excretion = 26.8 ± (12.4) per ml) and either positive histology (n=42), or culture (n=40) before starting treatment. H. pylori was cleared in all patients: 13C-UBT negative immediately after finishing treatment (double / triple therapy for 1 or 2 weeks). In 17/46 patients eradication therapy failed: H. pylori recurred in 15/17 without symptom recurrence, (mean ±SEM excess 13CO2 excretion = 12.4 ± (6.7) per ml) at median 8 days (range 6-18d). In all 29 patients in whom H. pylori was successfully eradicated at one month mean (±SEM) excess 13CO2 excretion = 2.4 ±(0.9) per ml the breath test was negative at 2 weeks and thereafter (median t/2 = 4.6 months, range 1.0 - 7.2).

These results show that the 13C-UBT can detect recurrent H. pylori within days of finishing anti-H. pylori therapy, and suggest that eradication can be accurately diagnosed 2 weeks after the end of treatment.

DEFECTIVE ANTIGEN-SPECIFIC RESPONSES IN PATIENTS WITH GASTRIC H. PYLORI COLONIZATION. C N Shahi, X J Fan, J Huang, C J Smyth, A Chua, J McDevitt, D G Weir, P W N Keeling and D Kelleher Dept Gastroenterology, Immunology and Clinical Medicine, and Microbiology, St James's Hospital and Trinity College Dublin.

Helicobacter pylori (HP) is capable of colonizing the gastric mucosa, despite a significant humoral response, in 30-60% of healthy adults. In this study we have examined the cellular response to HP by measuring in vitro proliferative response of peripheral blood mononuclear cells to HP related antigens, purified protein derivative (PPD), whole cell inactivated E Coli antigens and phytohaemagglutinin (PHA) in 37 dyspeptic patients undergoing upper gastrointestinal endoscopy. A standard five day thymidine incorporation assay was used to measure HP antigen recognition (as determined in preliminary experiments) of 3pg/ml was utilized. HP status was determined by culture, histology and the rapid urease test on antral biopsy.

Patients with HP colonisation (HP+) had significantly lower proliferative responses relative to HP negative (HP-) individuals [1610±555 (n=21) vs 1946278 (n=16) cpm 3H-TdR incorporation, x±SEM, p<0.01]. There was no significant difference in proliferative responses to PPD [477±1613 vs 8109±2646, x±SEM, p. n] or to PHA [1041±2156 vs 950±1532]. We could not detect proliferative responses to E Coli antigens at significant levels in patients or controls. We also examined the secretion of T-cell cytokine γ-interferon (IFN) in these patients by ELISA. IFN secretion by PBMC in response to HP antigen was also lower in HP+ patients: 1475±595 (n=18) vs 123±3.2 (n=14) U/ml, p<0.02. However, neither spontaneous production nor responses to PHA were significantly different in the two groups. These findings suggest a defective peripheral blood T cell response to HP antigens in HP+ individuals. This might reflect an imbalance between mucosal and systemic immune responses in these individuals. Alternatively, these data might suggest prior encounter with and elimination of HP in HP- individuals due to recognition of specific epitopes. Finally, these findings could also reflect antigen-specific suppression of T cell proliferative responses in the peripheral circulation. These possibilities are under investigation.

TWENTY-FOUR HOUR HYPERPEPSINOGENAEMIA IN HELICOBACTER PYLORI-POSITIVE SUBJECTS IS ABOLISHED BY ERADICATION OF THE INFECTION. A G Fraser, E J Prewett, R E Pounder, I M Samloff. University Department of Medicine, Royal Free Hospital School of Medicine, London NW3, UK, and University of California School of Medicine, Los Angeles, California, USA.

H. pylori infection is associated with hyperpepsinoaemia, but the effect of eradication on 24-h profiles of plasma peptinogen I (PG I) and PG II were not known.

METHODS: 24-h plasma PG I and PG II concentrations were determined in 8 healthy subjects with antibody to H. pylori, before and 4-6 weeks and 20-24 weeks after treatment with tripledose diclitaride, bismuth, amoxicillin and metronidazole.

RESULTS: Therapy was successful in the 5 subjects with active infection. Antral chronic active gastritis, which was moderate to severe before treatment, had resolved by the time of follow-up biopsy 20-24 weeks post-treatment.

Median integrated 24-h plasma PG I and PG II (ug.h./L).

<table>
<thead>
<tr>
<th>Group</th>
<th>Median PG I</th>
<th>Median PG II</th>
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<tbody>
<tr>
<td>Before</td>
<td>2288</td>
<td>1811</td>
</tr>
<tr>
<td>4-6 wks</td>
<td>157</td>
<td>151</td>
</tr>
<tr>
<td>20-24 wks</td>
<td>157.5</td>
<td>157.24</td>
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*p < 0.05 compared with before treatment.

In the 5 subjects with active infection, PG I and PG II fell significantly by 4-6 weeks after successful eradication. The decrease in PG II was proportionally greater than the decrease in PG I (58 vs. 27%), and the median fasting (8000ng) PG I:PG II ratio rose from 7.05 to 11.3. There was no indication of hourly median PG I concentration throughout the 24-h period, although some individuals did show a delayed meal-associated increase. In the 3 subjects without active H. pylori infection, pretreatment plasma PG I and PG II values found in the H. pylori-infected subjects after successful treatment, and did not change in response to therapy.

CONCLUSION: H. pylori infection is associated with reversible 24-h hyperpepsinoaemia.
W13

EXPRESSION OF 120,000 M , PROTEIN AND CYTOTOXICITY IN HELICOBACTER PYLORI

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Two characteristics of Helicobacter pylori which have been associated with peptic ulceration are immune recognition by mucosal IgA of a 120,000 M protein (p120) and vacuolating cytotoxic (cytotoxin-positive) activity. We have previously shown that p120 is expressed by antrum biopsies of a variety of strains. Only some strains of H. pylori express p120, suggesting that p120 may be involved in pathogenicity. To determine the presence of p120 in the cytotoxin-negative (cytotoxin-negative) strain, we have used lectins as defined cytotoxin potential, binding), and concanavalin A (mannose binding) lectins. We have used 5 neoglycoproteins (Ng), each bearing one lectin carbohydrate epitope, to detect H. pylori surface lectins in this study. The Ng gal-galNac-Human serum albumin (HSA) and galNac-BSA were conjugated to biotin and then purified by gel filtration. They were subsequently confirmed to bind PNA and VVA by ELISA. Gold labelled Fucoase-Bovine serum albumin (BSA), galNac-BSA and mannose-BSA were incubated with 100 pl (20 pg/ml) conjugated Ng. The unbond Ng were then washed off and the bound biotin-labelled Ng were identified by using avidin-biotin complex/peroxidase and O-phenylene diamine in citric acid buffer containing H2O2. The bound gold-labelled Ng were identified by silver enhancement method. The controls were performed by replacing the Ng by unconjugated HSA or BSA followed by washing and then further incubation with the same pre-labelled anti-hSA or anti-HSA antibody. The peroxidase activity was similarly identified as before.

Gal-galNac-HSA, galNac-HSA and mannose-BSA bound to H. pylori surface proteins but not the Fucose-BSA or glucNac-BSA. The controls were negative. These results indicate that H. pylori possess surface lectins that bind to gal-galNac, galNac and mannose. These lectins probably enhance their colonisation in the stomach and account for their gastric tropism.

W14

H. PYLORI LECTINS DETECTED BY NEOGLYCOPROTEINS.


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Gastric epithelial surface glycoconjugates were found previously to bind Peanut agglutinin (PNA, gal-galNac binding), Vicia villosa agglutinin (VVA, galNac binding) Ulex europaeus I (fucose binding), Limulus polyphemus (glicNac binding) and concanavalin A (mannose binding) lectins. We have used 5 neoglycoproteins (Ng), each bearing one lectin carbohydrate epitope, to detect H. pylori surface lectins in this study. The Ng gal-galNac-Human serum albumin (HSA) and galNac-BSA were conjugated to biotin and then purified by gel filtration. They were subsequently confirmed to bind PNA and VVA by ELISA. Gold labelled Fucoase-Bovine serum albumin (BSA), galNac-BSA and mannose-BSA were incubated with 100 pl (20 pg/ml) conjugated Ng. The unbond Ng were then washed off and the bound biotin-labelled Ng were identified by using avidin-biotin complex/peroxidase and O-phenylene diamine in citric acid buffer containing H2O2. The bound gold-labelled Ng were identified by silver enhancement method. The controls were performed by replacing the Ng by unconjugated HSA or BSA followed by washing and then further incubation with the same pre-labelled anti-HSA or anti-BSA antibody. The peroxidase activity was similarly identified as before.

Gal-galNac-HSA, galNac-HSA and mannose-BSA bound to H. pylori surface proteins but not the Fucose-BSA or glucNac-BSA. The controls were negative. These results indicate that H. pylori possess surface lectins that bind to gal-galNac, galNac and mannose. These lectins probably enhance their colonisation in the stomach and account for their gastric tropism.

W15

HELCOBACTER PYLORI AND REACTIVE OXYGEN METABOLITE PRODUCTION IN DUODENAL ULCER DISEASE


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We have previously shown that production of reactive oxygen metabolites (ROMs) in duodenal ulcer patients is enhanced in H. pylori positive, compared to negative, non-ultrastral gastritis. The aim of this study was to assess the influence of gastric H. pylori infection on the production of ROMs in human duodenal ulcer disease.

Methods Endoscopy biopsies were taken from the edge of duodenal ulcers (DU), areas of maximal inflammation in patients with severe erosive duodenitis (ED), and from controls without evidence of either duodenal mucosa. ROM production was assessed using 75 μM luminol-amplified chemiluminescence (CL). H. pylori were detected in antral biopsies using an immunoblotting technique. We found no labelled biotin-labelled p120.

Results CL was enhanced in biopsies from DU (median CL after subtraction of background 15.9 ± 10 phonom/min (95% confidence interval 4.9 to 47.9), n = 24) and ED (7.0 (0.0 to 88.4), n = 13) compared to control (0.4 (0.9 to 1.1), n = 21) (P < 0.001, Kruskal-Wallis). There was no difference in CL between the ED and DU groups. In the 20% of patients with DU and ED who were H. pylori negative, CL (-0.1 (8.9 to 7.2), n = 8) although not different to control, was much lower than in patients with the same macroscopic lesions who were H. pylori positive (24.2 (6.7 to 62.9), n = 29) (P = 0.004). In the control group, duodenal CL was not affected by H. pylori status (HP positive 0.5 (1.1 to 1.5), n = 10); HP negative 0.4 (1.7 to 1.7), n = 11) (P = NS).

Conclusions 1 Duodenal mucosa from patients with erosive duodenitis or duodenal ulcer produces excess reactive oxygen metabolites only in the presence of antral H. pylori infection. 2 A pathogenic role for reactive oxygen metabolism production in duodenal ulcer disease is likely to be restricted to H. pylori-related cases. 3 In the majority of patients with antral H. pylori infection who develop duodenal ulceration, the infection is likely to act synergistically with other pathogenic factors, stimulating reactive oxygen metabolism production in duodenal mucosa.

W16

PREVALENCe OF HELICOBACTER PYLORI (Hl) INFECrION AMONGST ATTENDANTS AT THE AUTUMN BSG MEETING 1991: "BARE HANDED" ENDOSCOPY A RISK FACTOR FOR HP ACQUISITION

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Diagnostic testing for Hp infection by 13C breath test was offered by a pharmaceutical company at the Autumn 1991 meeting of the BSG. 144 attendants were tested and 115 returned an anonymously completed questionnaire giving details of their test result, age, sex, professional status, endoscopy service, use of gloves and past medical history of dyspepsia and proved upper GI disease.

Respondents were 63 doctors (61 male, mean age ± SD 43.0 ± 8.9), 37 nurses (all female, 42.4 ± 8.6) and 15 non-clinical respondents (7 male, 42.4 ± 12.3). Positive tests for Hp infection were found in 25 of 63 doctors (40% of 37 nurses and only 15 non-clinical respondents (P<0.05 cf doctors and nurses, Fisher's exact test). Life table analysis did not show a significant increase in probability of infection with age, length of clinical or endoscopic service. However, Endoscopy Unit staff who always wore gloves had a lower rate of infection (8 Hp +ve of 34) than did staff who had at some time worked "bare-handed" (27 Hp +ve of 57) P<0.05 (Chi square test). Overall, Hp +ve respondents were more likely to suffer from dyspepsia (21 of 39) than Hp -ve respondents (21 of 76) P<0.01 (Chi square test). Three Hp +ve respondents and one Hp -ve respondent had a past medical history of DU (NS, Fisher's exact test).

In this small survey both doctors and nurses have a greater prevalence of Hp infection than the non-clinical group. Acquisition is probably early in the career as prevalence does not increase with length of service. Always wearing gloves is associated with a lower rate of infection. Infection with Hp is associated with a greater rate of dyspepsia but not in proven cases of ulcer disease, but the survey may have been too small to show this.