RESISTANCE TO HYDROXYUREA IN BONE MARROW STROMAL CELLS

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Objectives: We examined the effect of hydroxyurea (HU) on genes that may be involved in the hypomethylating activity of HU, as well as the expression of HU transporters. Methods: We compared the expression of genes that may be involved in the hypomethylating activity of HU in bone marrow stromal cells (BMSC) from patients with myelodysplastic syndrome (MDS) and healthy individuals. Results: We found that the expression of genes that may be involved in the hypomethylating activity of HU is increased in BMSC from patients with MDS. Conclusions: These findings suggest that BMSC from patients with MDS are more sensitive to the hypomethylating activity of HU than healthy individuals.

O: Oral presentations

1A:20 HELICOBACTER PYLORI RESISTANCE TO MACROLIDES. CONFIRMATION OF POINT MUTATION AND DETECTION BY PCR-RFLP

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Resistance of Helicobacter pylori to macrolides is a major cause of treatment failure of eradication therapies. A princeps work based on U.S. strains showed that this resistance was due to a point mutation on the gene coding for the 23S ribosomal RNA of H. pylori. Our goal was to extend this work using European strains, to test the consequence of this mutation on erythromycin binding to H. pylori ribosomes, and to find a quick method to detect this mutation. The reference strain CIP 101260 and 7 pairs of H. pylori strains were used, the parent strain being susceptible to macrolides, and a second strain having acquired resistance in vivo during clarithromycin treatment. The identity of the strains was confirmed by RAPD using 2 primers. The MIC values were measured by E test and agar dilution method. Amplification was carried out on the peptidyl transferase loop region of the gene coding for the 23S RNA using appropriate primers in order to confirm the presence of the mutations 2058, 2059 and 2611 previously described. The amplified products were sequenced. Erythromycin binding was tested on purified ribosomes isolated from one pair of strains using 14C labelled erythromycin.

A point mutation was found on resistant strains at position 2058 (3 cases) and 2059 (4 cases) but never on the opposite DNA fragment of domain V (for the gene. The mutation was A→G in all except one case (A→C) in position 2058. Erythromycin binding increased in a dose dependent manner for the susceptible strain but not for the resistant one. Using BsaI and BbSI restriction enzymes on the amplified products, we could confirm the mutations in positions 2058 and 2059, respectively.

In conclusion, the same mutations as previously found were present in European strains. This mutation correlates with a decreased uptake of macrolide by ribosomes. Finally the mutation could be detected without sequencing by performing PCR-RFLP using appropriate restriction enzymes.

1C:06 DOES HELICOBACTER PYLORI INFECTION HAVE A ROLE IN PERNICIOUS ANEMIA?

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Previous studies indicate that patients with pernicious anemia (PA) are infected with H. pylori more frequently than age- and sex-matched controls. However, Hp infection may be present prior to the development of PA and gradually become absent due to the development of the associated type A atrophic gastritis. To determine whether evidence of Hp infection may disappear during the course of the illness, we studied 47 PA patients (mean age 58.2 y and 36.2% male) prospectively. The diagnosis of PA was established by either: i) abnormal Schilling test corrected by intrinsic factor (IF); ii) lack of IF in stimulated gastric juice or iii) the combination of low B12 levels and serum anti-IF antibodies. Sera were available for a mean of 5.9 ± 0.5 years and ELISAs were used to detect Hp-specific serum IgG or IgA. In total, 12 (25.5%) patients showed evidence of Hp infection. Comparison of the 12 Hp-seropositive (Hp+) and 35 Hp seronegative (Hp−) PA patients showed that the former group had significantly lower gastrin (mean 686 vs 1293 pg/ml, p = 0.045) and higher pepsinogen A levels (mean 27.1 vs 11.6 pg/ml, p = 0.05), factors consistent with less severe atrophic gastritis. The 35 Hp− patients were followed for a total of 204 person-years; none showed seroconversion. The 12 Hp+ were followed for a total of 63 person-years, (mean 5.25 years); during this time, 4 (33%) of the patients seroconverted. Thus, among patients with PA, the seroconversion rate was greater than 6% per year. Those who seroconverted were younger (52 vs 69 y, p = 0.06) and had lower pepsinogen A levels (9 vs 29, p = 0.057) than those remaining positive. These data are consistent with the hypothesis that Hp-infection precedes at least some cases of PA and that the infection reverses with time; and presumably with progressive gastritis.

This pattern may be especially associated with a subset of PA characterized by less severe gastrin and pepsinogen abnormalities in infected persons. The development of atrophic gastritis as part of PA leads to gradual elimination of the organism and failure to detect a past Hp infection. Prospective studies, in the pre-PA stage of gastritis are needed to clarify the putative role of Hp in causing PA.

1C:13 HELICOBACTER INFECTION AND THE RISK OF MYOCARDIAL INFARCTION

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Certain infections including those caused by helicobacters, may increase the risk of myocardial diseases. Our purpose was to analyse the association of helicobacter infection with myocardial infarction in the general population.

Methods: A health examination survey was carried out in 12 Finnish cohorts during 1973–76 with a follow-up until the end of 1985. 3471 men, aged 45–64 years, participated in the baseline survey. During the follow-up a new or fatal non-myocardial infarction was observed in 276 subjects who were free of cardiovascular disease at baseline and in 165 subjects who had cardiovascular disease at baseline. A nested case-control study based on these cases and two matched control subjects was performed. A total of 842 controls were matched for age, sex and municipality and cardiovascular disease at baseline. Serum samples taken at baseline were analyzed for helicobacter IgG and IgA antibodies.

Results: 79% of the subjects with cardiovascular disease at baseline and myocardial infarction during the follow-up had elevated titers of helicobacter IgG antibodies, versus 77% in their control subjects, which resulted in a relative risk (RR) of 1.16; 95% confidence interval (CI) 0.73–1.86. Of those who initially did not have cardiovascular disease but had first myocardial infarction during the follow-up, 83% had IgG antibodies at the baseline, versus 78% in the control subjects; RR 1.37; (95% CI 0.94–2.00). Overall IgG positivity of the 441 subjects with myocardial infarction was 82%, versus 78% in the control subjects, RR 1.29 (95% CI 0.96–1.73). The respective figures for IgA antibodies were 68% and 67%, RR 1.05 (95% CI 0.82–1.34).

Conclusion: The present data indicate a possible although statistically nonsignificant association between the risk of myocardial infarction and helicobacter infection as measured by IgG positivity. However, this possible risk seems to be small.

2A:07 AN INTERNATIONAL ASSOCIATION BETWEEN PREVALENCE OF INFECTION WITH CagA POSITIVE STRAINS OF H. PYLORI AND MORTALITY FROM GASTRIC CANCER

P.M. Webb1, D. Forman, D. Newell2, A. Cavaccia3, J.E. Crabtree and the Eurogast Study Group. University of Leeds & St. James’s Hospital, Leeds, UK; 1 University of Queensland, Brisbane, Australia; 2 Central Vet Labs, Weybridge, UK; 3 IRIS, Siena, Italy

Purpose: There has been considerable recent interest in whether individuals infected with H. pylori, expressing the cagA gene product, have an increased risk of gastric cancer in comparison with those infected with cagA negative strains. This question has been investigated in the Eurogast study in which we have previously demonstrated an international correlation between gastric cancer mortality rates and H. pylori infection prevalence.

Methods: A total of 2839 serum samples collected in 1990–2 from approximately 50 men and 50 women in the age groups 25–34 and 55–64 years from each of the 17 Eurogast study centres, were analysed in a single laboratory for the presence of IgG antibodies to the CagA protein by an ELISA using as antigen a purified recombinant fragment of CagA. Regression analyses were then carried out in which the prevalence of CagA seropositivity was modelled on the cumulative (0–74 yrs) gastric cancer mortality rate for each population in the mid-1980’s.

Results: There was a statistically significant association (p = 0.01) between the prevalence of individuals with CagA seropositivity in each population and the mortality rate from gastric cancer. The regression coefficient (2.76) was higher than that for the effect of H. pylori seropositivity (1.72) although the latter was of similar significance (p = 0.002). Based on these data, the relative risk of gastric cancer associated with CagA positive H. pylori infection would be 15.8 in comparison with a risk of 5.6 associated with H. pylori per se.
2B:05 THE 13C-UREA BREATH TEST FOR THE DIAGNOSIS OF HELICOBACTER PYLORI INFECTION IN CHILDREN


Background. The 13C-Urea Breath Test (13C-UBT) is a simple non-invasive highly accurate test for the detection of Helicobacter (H.) pylori infection in adults. Although the use of 13C-labelled urea renders this test absolutely safe and thus undoubtedly suitable for the detection of H. pylori infection in children, as yet a standardized 13C-UBT protocol for children has not been formulated. In particular we have no information on the three fundamental components of the 13C-UBT: the number of and time intervals for breath sample collection, the appropriate test meals to delay gastric emptying and doses of 13C-Urea. Aim. The aim of our study was to evaluate the accuracy of the 13C-UBT in children using different types of test meals, doses of 13C-Urea and breath sampling intervals. Methods. 98 children, recruited in our study (51 males, 47 females; age yrs) range 2-16, mean ± SE: 10.1 ± 0.3; body surface area (m²) range 0.5-1.7, mean ± SE: 1.2 ± 0.03) underwent routine upper GI endoscopy. 3 antral and 2 corpus-fundus biopsy specimens were taken for histological examination for the presence of H. pylori infection (Haematoxylin/Eosin; GEMSA) and the quick Urease-test was performed. The 13C-UBT was performed in each child after undergoing endoscopy, and was then repeated within three days modifying the test meal or the dose of the 13C-Urea. 62 children were given a fatty test meal, Pulmocare (Abbott) 100 ml, and two different doses of 13C-Urea, 100 and 50 mg respectively. 36 children were given the same dose of 13C-Urea, 50 mg, but two different types of test meal, Pulmocare 100 ml and 10 ml at 10% of Pulycose (polymer of glucose) respectively. Breath samples were collected every 10 minutes for 60 minutes and analyzed by an Automated Breath 13C Analyzer (ABCA Europa Scientific). The “gold standard” for the detection of H. pylori infection was defined as a concordant result on histology and quick urease-test. The cut-off value was calculated taking the mean of H. pylori +ve and 44 H. pylori -ve.

According to the “gold standard” 48 children were considered H. pylori +ve and 44 H. pylori -ve.

2A:07 COST-IMPACT OF CLARITHROMYCIN PLUS OMEPRAZOLE COMPARED TO TRADITIONAL THERAPIES FOR TREATMENT OF H. PYLORI ASSOCIATED DUODENAL ULCERS

A. Sonnenberg, VA Medical Center, Albuquerque, NM; The Gastrointestinal Utilization Trial Study Group

Introduction: The NIH Consensus Development Conference recommended a comprehensive economic analysis of the impact of treating or not treating H. pylori (HP) associated ulcers. Patients were enrolled in a multicenter (n = 123), controlled clinical trial to determine cost savings of eradicating HP with clarithromycin plus omeprazole (C + O) versus conventional anti-ulcer therapy (omeprazole (O) or ranitidine (R) alone).

Methods: Adult patients with HP and active duodenal ulcer were randomized to double-blind treatment (Rx): 1) C 500 mg TID + O 40 mg

2B:07 ABERRANT EXPRESSION OF GLAND-TYPE GASTRIC MUCIN IN THE SURFACE EPITHELUM OF H. PYLORI-INFECTED PATIENTS

J.C. Byrd, P. Yan, C. Yunker, H. Schroepper, R.S. Bressler. Henry Ford Health Sci. Ctr., Detroit, MI, USA

Mucins are high-M, glycoproteins that protect the gastric epithelium. Three mucin genes, MUC5, MUC6, and MUC1, are expressed in normal stomach. Our aim was to compare expression of gastric mucin genes in HP+ patients vs uninfected (HP-) individuals. Gastric biopsies obtained at routine endoscopy (29 HP+ and 33 HP- by histology and CLO) were examined by immunohistochemistry for mucin gene expression in surface mucous cells and mucous neck glands.

In HP-negative specimens, MUC6 was expressed only in mucous glands, but in 21/29 HP+ specimens, there was also staining of surface mucous cells. Expression of MUC5 mucin (surface but not glands) and

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% of patients with staining of surface mucus cells

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MUC1 (surface and glands) was unaffected by HP infection. Carbohydrates recognized by anti-LeX (Galβ1,4(Fuco2)GlcNAc) and by ConA (after IO5/NaBH4) are normally found only in mucous glands, but were present in surface mucous cells in 16/28 and 16/23 of HP+ tissues. Surface LeX (Fucos2Galβ1,3(Fuco4)GlcNAc) was present in 26/27 HP+ and 18/30 HP-neg tissues, consistent with a role for LeX in HP colonization. Mucins purified from gastric aspirates of 5 HP+ patients bound more anti-MUC6 (0.32 ± 0.04 vs 0.09 ± 0.07 A405/25 ng, by ELISA) and anti-LeX (1.31 ± 0.30 vs 0.05 ± 0.03), than mucins from 5 HP-neg patients, suggesting erosion of the altered surface mucin layer.

Conclusion: In HP+ patients, the type of mucin that is normally limited to mucous glands is also expressed in surface mucous cells. Abrupt expression of MUC6 might disrupt the protective surface mucin layer, which could be important in the pathogenesis of HP-associated diseases.

3B:59 THE COMPLETE DNA SEQUENCE OF THE HELICOBACTER PYLORI GENOME

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The DNA sequence of a bacterial genome can be of immense value, especially in cases of important pathogens such as Helicobacter pylori. Here we present the complete annotated sequence of a representative H. pylori strain (KE26695). This strain was chosen for four reasons: (i) it colonizes piglets and elicits immune and inflammatory responses; (ii) it is of the toxicogenic and Cag+ (putatively more virulent) type; (iii) its ~40 kb cag region is not interrupted by rearrangement, unlike certain other Cag+ strains; (iv) it is transformable, and thus amenable to mutational tests of gene function. The genome sequence was determined using a random library of small insert plasmid clones, essentially as in our sequencing of the Haemophilus influenzae and the Mycoplasma genitalium genomes (Science 269, 496–512, 1995; Science 270, 397–403, 1995). In the initial random phase, we achieved 7.5 fold coverage by generating 24028 sequences with an average read length of 551 bases. We also sequenced the ends of 500 large fragment lambda phage clones to create a scaffold that helped ensure correct assembly. The availability of this sequence should greatly speed many areas of H. pylori research, including characterization of colonization mechanisms, virulence traits and host interactions; analyses of H. pylori population structure and genome evolution; and development of new potent therapies against H. pylori and associated gastrointestinal diseases.

4B:06 SERO-CONVERSION AND SERO-REVERSION IN IgG ANTIBODIES TO H. PYLORI: AN 11-YEAR FOLLOW-UP OF 2,523 RANDOMLY SELECTED DANES

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Purpose: To assess the 11-year sero-conversion and sero-reversion rate in IgG antibodies to H. pylori in adults. Methods: In 1983, 3,589 Danes aged 30–60 years entered a population-based cohort study. After 11 years, 2,656 participants attended a follow-up examination. A total of 2,523 matching pairs of sera were eligible for this study. IgG antibodies against H. pylori were measured with an in-house ELISA assay. Cut-off points for seronegativity and seropositivity were set at ≤100 ELISA units (EU) and ≥400 EU, respectively. Participants who were IgG seronegative or IgG border-line at study entry and IgG seropositive at follow-up were regarded as sero-converters. All sero-converters had at least a four-fold increase in base-line IgG antibody levels between study entry and follow-up. Sero-reverters were those who were IgG seropositive at study entry and showed at least a four-fold decrease in IgG antibody levels at follow-up. Results: Seroconversion for IgG antibodies to H. pylori was seen in 24.7% [23.0–26.4] in 1983 and 24.5% [22.8–26.2] in 1994. A total of 36 participants sero-converted within the observation period. The cumulated 11-year sero-conversion rate (incidence of H. pylori infection) was 18.9 [12.8–25.0] per 1,000 persons at risk. Sero-reconversion was seen in 42 [m/f: 17/25] participants. The cumulated 11-year sero-reversion rate was 67.4 [47.7–87.1] per 1,000 persons at risk. Conclusions: The incidence of H. pylori infection is low in adults. Elimination of H. pylori infection seems to outbalance acquisition of the infection in adults in developed parts of the world.