F261

ANAL SPHINCTER DISRUPTION IN 32% OF VAGINAL DELIVERIES: PROSPECTIVE ULTRASOUND STUDY.

A H Sultan, M A Kamm, C N Hudson, C I Bartram.

St.Mark's and St.Bartholomew's Hospitals, London, UK.

The development of faecal incontinence in middle age has been previously attributed primarily to pelvic nerve damage during childbirth with subsequent progressive pelvic floor denervation. However, occult anatomical lesions may occur during childbirth. We have prospectively studied the incidence of damage to the anal sphincters during childbirth, using anal endosonography.

Methods: 134 women (113 vaginal delivery: 72 primiparous (A); 41 multiparous (B); 21 Caesarian section (C) were studied in the last 6 weeks of pregnancy and again 6 weeks post delivery. Anal endosonography was performed using a Bruel and Kjaer rotating 7 MHz probe, withdrawn down the anal canal at 1cm intervals to image the whole length of the sphincter. Anal manometry was performed using a 1cm stationary pull-through technique with an air-filled microballoon catheter.

Results: (A) All primiparous vaginal delivery patients had a normal sphincter pre delivery; post delivery 32% had an internal anal (IAS), 12.5% had an external anal sphincter (EAS) defect; a total of 32% developed a defect. Only three of these patients developed flatus incontinence. (B) 49% of multiparous patients had a preexisting lesion: IAS 46% and EAS 20%. IAS lesions worsened post delivery in 26% and 5% developed a new EAS lesion. 50% of patients with IAS defects had low resting pressures and 49% of EAS defects had low voluntary squeeze pressures. Caesarian sections were mainly on primiparous women: there were no defects pre or post delivery.

Conclusions: New defects occuring in both parts of the sphincter are common after vaginal delivery.Most are asymptomatic. The later development of incontinence is therefore likely to be due to the combination of occult defects with the progression of neuropathy.

F263

SYMPTOMATIC AND OBJECTIVE BENEFITS WITH BIOFEEDBACK FOR INTRACTABLE CONSTIPATION.

D Koutsomanis, M-C Lemieux, J E Lennard-Jones, M A Kamm, E Walker.

St. Mark's Hospital, City Road, London EC1V 2PS, UK.

There are few objective assessments of the benefit of biofeedback for intractable constipation. It is also not clear which type of patient benefits, and which symptoms improve. We have prospectively evaluated the success of treatment in patients with pelvic floor inco-ordination and slow colonic transit.

Methods: 30 consecutive patients with reduced bowel frequency or excessive need to strain or both, who had not responded to fibre or laxatives, entered the study. Week-long diaries were recorded pre- and 6 weeks post treatment to record bowel frequency, straining digitation, laxative use, pain, and well being. Proctography, pelvic floor relaxation (EMG and manometry) and transit time were measured pre treatment, and transit time remeasured post treatment. Biofeedback focussed on relaxing the pelvic floor, using visual EMG, and transmitting expulsive force to an intrarectal balloon. Patients had 2-6 45 minute sessions.

Results: 10 patients did not complete treatment. Of the remaining 20 patients: (i) 2 with slow transit only did not benefit. (ii) All 8 with pelvic inco-ordination only decreased time spent straining. (iii) Of 10 patients with pelvic inco-ordination and slow transit, 8 benefitted - 9 decreased straining (mean 30 v 8 mins, pre v post, $p < 0.05$), 3 reduced or stopped digitation. Mean proportion of retained markers significantly decreased (66% v 23%, $p < 0.02$). For all 18 with pelvic inco-ordination (i & ii) the bowel frequency increased (mean 4 v 6/week, pre v post, $p < 0.05$), straining time decreased (mean 29 v 11 min, $p < 0.01$) and straining episodes decreased (mean 5 v 2/week, $p < 0.01$). Pain was not improved in all groups.

Conclusions: Biofeedback improves bowel frequency and symptoms of straining, digitation and well being. It significantly reduces transit time when prolonged. Pain is not relieved. Patients with pelvic inco-ordination are most likely to benefit, although some with slow transit improve.

Helicobacter pylori F264–F271

F262

EFFECT OF NICOTINE ON RECTAL MUCUS AND MUCOSAL EICOSANOIDS

ED Srivastava, FJ Zijlstra, M Rhodes, F Fogg, M Copeman, HJ Samson, MAH Russell, M Van Blankenstein, JHP Wilson, A Allen, J Rhodes.*
Department of Gastroenterology, University Hospital of Wales, Cardiff, UK.*

Anglo-Dutch Nicotine Intestinal Study Group

Colitis is largely a disease of non-smokers and nicotine may have a beneficial effect on the disease. To examine the effect of nicotine on rectal mucosa 32 rabbits were allocated into 4 groups, controls and 3 treatment groups. Nicotine tartrate at low, medium and high doses was administered subcutaneously via an Alzet osmotic mini-pump in doses of 0.5, 1.2 and 2 mg/K/day respectively. After 14 days' treatment the rectum was removed and the visible adherent layer of mucus was measured using phase contrast microscopy on thick sections. Rectal mucosal synthesis of mucin and mucosal eicosanoids were also measured.

Serum nicotine concentrations (ng/ml) were 0.4±0.1, 3.5±0.4, 8.8±0.8, and 16.2±1.8 in control and treated groups respectively. Thickness of adherent mucus was reduced with low dose and increased with high dose nicotine (p 0.026 and 0.0001 respectively). Correlation coefficient for serum nicotine concentration and mucus thickness = 0.446, $p < 0.05$. Rectal mucin synthesis measured by ^{3}H-glucosamine incorporation was unchanged. All prostaglandin levels were significantly reduced by nicotine with inverse dose dependence - greatest inhibition with lowest dose.

Nicotine and possibly smoking may affect colitis by an action on mucosal eicosanoids and surface mucus thickness in the rectum and large bowel.

	Control	Low dose	Medium dose	High dose
Mucus thickness μ	36.0±2.0	*22.2±1.3	29.4±3.0	*44.7±2.8
Rectal mucin synthesis (dpm/g wet wt)	106,985	103,304	100,669	92,480
$F_2\alpha$	73.7	9.5*	23.6*	41.8*
PF $F_2\alpha$	13.6	2.7*	4.8*	7.1*
HHT (dpm/mg wet wt)	22.8	8.4*	14.8*	17.2*

* significant difference from control.

F264

SEVERITY OF GASTRITIS AND VIRULENCE OF *HELICOBACTER PYLORI*

N Figura, L Di Gregorio *, M Bugnoli **, D. Armellini **, E Esposito * , L Abate *, P E Bayeli * (introduced by J E Crabtree)
Istituto di Malattie Infettive, Università di Siena, 53100 Siena, Italy; Istituto di Patologica Medicina, University of Siena *; Sclavo Research Center, Siena **.

An association has been recently observed between the mucosal recognition of a 120 kDa protein of Helicobacter pylori (HP) and the severity of antral gastritis in dyspeptic patients. The aim of this study was to examine if an association exists between severe gastritis and putative virulence factors of HP: motility, urease activity and adhesion.

41 HP strains were tested in all: 13 strains were isolated from the antrum, 14 from the body, and 14 from the fundus of the stomach of 14 dyspeptic patients. Motility was tested in semi-solid brucella broth with 10% fetal calf serum (FCS). Strains were grown in brucella broth with 5% FCS at 150 oscillations per min for 72 hours. After centrifugation and filtration (0.22 μm) dilutions of broth culture were assayed for urease activity using a "urease test broth"; samples were read after incubation at 37°C for 60 min. The degree of adhesion of strains was assayed with Int 407 cells; after 3 hours incubation with 1.5 X 10^7 organisms/ml, cells were stained with acridine orange. The severity of gastritis was assessed histologically according to the "Sydney System" for all 41 biopsies.

No association was found between severe gastritis (SG, found in 8 biopsies, 4 from the antrum) and levels of urease (urease activity was detectable up to 1:64 dilution of filtrates for nearly all strains), motility (26 strains were motile, 4 associated with SG), and strong degree of adhesion (SDA, i.e. 100% of cells colonized with ≥ 50 organisms/cell; 30 strains showed SDA, 8 associated with SG). Similarly mild gastritis was not significantly associated with absence of motility, urease activity ≤ 1:64, and with mild or moderate degrees of adhesion. Thus severity of gastritis is not associated with virulence characteristics of H. pylori.

F265

ACUTE PERFORATED DUODENAL ULCER IS NOT ASSOCIATED WITH HELICOBACTER PYLORI INFECTION

D Reinbach, G Cruickshank, KEL McColl.
University Departments of Surgery, and Medicine and Therapeutics, Western Infirmary, Glasgow G11 6NT

Numerous studies have demonstrated that more than 95% of patients with chronic duodenal ulcer disease have Helicobacter pylori (HP) infection. We have examined the prevalence of HP in 61 patients (mean age = 52.1 y, range 18-85 y) presenting with acute perforated duodenal ulcer and compared this with age and sex matched control non-ulcer patients. HP status was assessed by serum anti-HP IgG (Helico-G kit, Porton) using a titre of 18 or less as negative. In our laboratory this has a specificity of 88% and sensitivity of 92%. Only 48% of the perforated duodenal ulcer patients were sero-positive and this was similar to the value of 51% in the non-ulcer controls. In 40 of the perforated duodenal ulcer patients ^{14}C-urea breath tests were also performed 4-10 weeks post surgery and this confirmed that only 45% were HP positive. None of these patients had received peroperative antibiotics capable of eradicating the infection.

The HP positive and HP negative perforated duodenal ulcer patients were similar with respect to NSAID usage (54%, 43%), smoking (70%, 80%), alcohol (35%, 40%), previously confirmed ulcer (19%, 23%), and age (55.6 y, 49.5 y).

In conclusion, the lack of association of perforated duodenal ulcer and HP infection indicates that perforated duodenal ulcer has a different pathogenesis from chronic duodenal ulcer disease. NSAID usage appears important.

F266

H PYLORI-RELATED HYPERGASTRINAEMIA IS DUE TO A SELECTIVE INCREASE IN THE G17 ANTRAL-DERIVED HORMONE

G Mulholland, JES Ardill, RS Chittajallu, KEL McColl.
University Department of Medicine and Therapeutics, Western Infirmary, Glasgow and Queen's University, Belfast.

Subjects with H pylori infection of the gastric antrum have an increased serum concentration of gastrin. This hormone circulates in two forms - G17 which is mainly of antral origin and rises in response to eating, and G34 which is present in the duodenum and shows little increase with eating. We have examined these different forms of gastrin basally and post-prandially in 13 duodenal ulcer patients before and one month following eradication of H pylori. The median basal G34 (pmol) was similar before (16, range 8 - 40) and after (16, range 7 - 36) eradication of H pylori. Likewise the 20 minute post-prandial G34 concentration was similar before (26, range 2 - 45) and after (16, range 7 - 26) eradication of the H pylori. However, eradication of H pylori lowered the median basal G17 (pmol) from 6 (< 2.4 - 25) to < 2.4 (< 2.4 - 15) ($p < 0.01$) and lowered the 20 minute post-prandial G17 from 43 (9 - 95) to 17 (< 2.4 - 52) ($p < 0.001$). HPLC analysis indicated that G17 and G34 were the only circulating forms of gastrin both before and after eradication of H pylori. In conclusion, the increased serum gastrin in H pylori infection is due to a selective increase in the G17 form of the hormone which is of antral origin and rises with eating. This explains why H pylori infection particularly raises post prandial gastrin concentrations.

F267

H. PYLORI ERADICATION: WHY DO IN VITRO ANTIBIOTIC SENSITIVITY TESTS HAVE POOR PREDICTIVE POWER

A W McKinlay, K Milne, N A G Mowat, I Gould
Aberdeen Royal Infirmary, Foresterhill, Aberdeen AB9 2ZB

Although in vitro sensitivity tests are an important step in formulating antibacterial treatment regimens they have proved disappointing in predicting the response of H. pylori to antimicrobials in the clinical setting. Most previous reports have measured Minimum Inhibitory Concentrations (MICs) as opposed to Minimum Bactericidal Concentrations (MBCs) because the latter are technically more difficult to perform with H. pylori.

We have adapted a technique using multiple well microtitre plates that simplifies MBC measurements for multiple strains of H. pylori.

Antibiotic	MBC(μmol/ml)	MIC(μmol/ml)	Group
Amoxycillin	0.001 - 0.25	0.16 - 0.5	
Metronidazole	0.25 - 16	0.25 - 2	A
Clarithromycin	0.002 - 0.031	0.002	
Cefaclor	0.125 - 128	0.031 - 0.25	
Cefixime	0.5 - >128	0.031 - 0.125	
Cefuroxime	0.5 - 128	0.031 - 0.125	
Ciprofloxacin	0.5 - 128	0.062 - 0.125	
Erythromycin	0.62 - 1	0.031 - 0.062	B
Mecillinam	1 - 128	0.008 - 0.5	
Olfloxacin	0.062 - >128	0.062 - 0.125	
TDB (Denol)	32 - 1024	8 - 128	
Temafloxacin	2 - 32	0.5 - 1	
Tetracycline	1 - 32	0.25 - 1	

Antibiotics in group A show good agreement between MBCs and MICs and also appear to be the most effective agents in vivo. In group B the MICs suggest that most strains will be sensitive but the MBCs are much higher implying that although growth is inhibited the organisms remain viable at high concentrations of the agents. This may explain why many of these agents are ineffective in vivo. We would suggest that MBCs, rather than MICs, should be used to assess the sensitivity of H. pylori in vitro.

F268

DOES THE SUCCESS OF ANTI-Helicobacter pylori (HP) THERAPY DEPEND ON A DIRECT ANTIMICROBIAL EFFECT OF TDB?

A W MCKINLAY, K MILNE, P W BRUNT, N A G MOWAT, I M GOULD.
Aberdeen Royal Infirmary, Foresterhill, Aberdeen.

It is usually assumed that Tripotassium-dicitrato-bismuthate (TDB) functions as an antimicrobial, in synergy with other antibiotics. Most studies have examined the bacteriostatic effects of the individual drugs, but the outcome of anti-HP therapy is more likely to depend on the combined bacteriocidal activity of the agents.

We have developed an in vitro test to look at the cidal activity of drug combinations against HP, permitting standard checkerboard titrations to be performed. The combined effects of two agents can be measured but also the contribution made by the individual antibiotics can be determined.

TDB + Tetracycline (Tc), and TDB + Amoxycillin (Am) produced little antibacterial activity at 4 and 8 hrs, but killing was complete by 24 hrs with any combination containing >0.0625 ug/ml of Am, or >8 ug/ml of Tc. The contribution from TDB was minimal, with viable bacteria present at concentrations of 64 - 128ug/ml.

In contrast,with Metronidazole (Mz) + TDB a bactericidal effect was seen at 8 hrs in any well containing >1 ug/ml Mz. Again the effect of TDB was minimal.

There was no evidence of synergy with any of the combinations tested, and TDB appeared to contribute little to the overall activity. Both Tc and Am were bactericidal at 24 hrs, but their speed of kill is slow. Mz had a more rapid onset of action against Hp, and this may explain why it has proved more effective in vivo.

Caution is required when extrapolating laboratory tests to the clinical situation. Our results suggest, however, that the success of combination therapy may not be due, primarily, to a direct antimicrobial effect of TDB.

F269

CHARACTERISATION OF CYTOTOXIN-ASSOCIATED PROTEINS OF HELICOBACTER PYLORI

M Bugnoli, D Armellini, R Rappuoli, Z-Y Xiang*, A Rossolini**, N Figura** (introduced by J.E. Crabtree). Sclavo Research Center, I-53100 Siena, Italy; Shanghai Hospital, Shanghai, China*; Istituto di Malattie Infettive, Siena**

Helicobacter pylori (HP) is not a clonal pathogen. Only certain strains are able to produce a toxic substance, a protein of ca. 130 kDa, which induces intracytoplasmic vacuolization on cells in culture. The aim of this study was to further characterise this cytotoxin-associated protein (p130) in concentrated broth culture filtrates (CBCF) and in whole cell suspension sonicates (WCSS) of HP strains by verifying whether p130 is shared by the HP strains tested and by determining its isoelectric point (pI).

CBCF and WCSS of 10 cytotoxic HP strains (CTHP) and 7 non-cytotoxic organisms (NCTHP) were separated by electrophoresis in 8% SDS-PAGE. Proteins transferred to nitrocellulose sheets were immunoblotted with sera from patients infected by CT and NCT HP strains and with a mouse polyclonal antibody raised against electroeluted p130 of a CTHP strain (CCUG 17874). CBCF and WCSS of this strain and of non-cytotoxic strain G21 were affinity purified on Affigel Blue. The most vacuolating fractions of the CTHP strain and the corresponding ones of G21 were analysed under NEPHGE conditions and incubated with the above mentioned sera. The reaction was visualised with anti-human and anti-mouse IgG conjugated with peroxidase.

p130 was only recognised by sera from patients infected by CTHP organisms and by the mouse anti-p130 antibody in CBCF and WCSS of cytotoxic HP strains alone. In addition, the mouse antibody neutralised intracytoplasmic vacuolization caused by CBCF and by live HP homologous strain. The average pI of p130 was 7.3. p130 was not present in CBCF and WCSS of non-cytotoxic organisms.

In conclusion, cytotoxic HP strains share an antigenically similar polypeptide of 130 kDa which is associated with intracytoplasmic vacuolization, and is mostly retained by HP organisms. The determination of the isoelectric point of p130 is a useful step in the purification of the toxic factor.

F270

ROLE OF HELICOBACTER PYLORI SEROLOGY IN SCREENING PRIOR TO DIRECT ACCESS ENDOSCOPY.

MA Mendall, PM Goggin, J M Marrero, N Molineaux, J Levy, P Kitchen, *S Badve, *C Corbishley, *C Finlayson, JD Maxwell and Professor TC Northfield.
Departments of Medicine and *Histopathology, St Georges Hospital Medical School, London, UK.

Background H. pylori is associated with 95% of duodenal ulcers and 80% of gastric ulcers, with most of the remainder being associated with NSAID use. A non-invasive means of diagnosing infection could be useful in screening subjects under the age of 45 (where the risk of gastric cancer is low) prior to endoscopy. Aims To assess the value of serology in screening prior to endoscopy. Methods The use of H. pylori serology in screening prior to endoscopy was assessed prospectively on 301 subjects directly referred by their primary care physician, following validation of the serology on 295 clinic referred subjects. The serology used was based on an acid glycine extract of H. pylori and is manufactured by Porton, Cambridge. It was validated against histology and biopsy urease (CLO) test. Results A cut-off point of 6.3, giving a sensitivity and specificity of 98% and 75% in the 295 clinic subjects, was selected as the most appropriate for screening. A screening policy of not endoscoping subjects under the age of 45 who were not taking NSAIDs or who had a serology titre of less than 6.3 units/ml was assessed prospectively on the 301 directly referred subjects. This would have missed only only one duodenal ulcer out of 19 peptic ulcers in the under 45s and would have saved 62/148(42%) of endoscopies in this age group. It would have been necessary to lower the cut-off point to 5.5 to pick up all pathology with a 35% saving in endoscopies. Conclusion H. pylori serology is valuable as a screening method prior to endoscopy.

F271

ACTIVATION OF HUMAN PERIPHERAL BLOOD NEUTROPHILS BY HELICOBACTER PYLORI IN VITRO.

GR Davies, TRJ Stevens, I Lawrenson, N Banatvala, M Powell, DS Rampton. Gastrointestinal Science Research Unit and Departments of Microbiology and Epidemiology, The London Hospital Medical College, London E1 1BB.

H.pylori (HP) infection causes chronic gastritis with neutrophil infiltration, and is associated with duodenal ulceration: the pathogenic mechanisms linking these events are unclear. We have previously shown enhanced reactive oxygen metabolite (ROM) production in HP-infected gastric biopsies using 75μM luminol-amplified chemiluminescence (CL), and have now investigated the possibility that stimulation of neutrophil ROM production by HP might explain this observation.

Methods Neutrophils were isolated from citrated venous blood of 8 healthy volunteers by dextran-sedimentation of erythrocytes, density gradient centrifugation of buffy coat and osmotic lysis of contaminating erythrocytes. HP was cultured in brucella broth + 1% calf serum, centrifuged and resuspended in PBS. Neutrophils (5 x 10^4/ml) were incubated with 1ml of luminol in a liquid scintillation counter for 5 minutes, or until a steady baseline CL response obtained. Washed, live or sonicated HP (10-100μL from stock containing 10^9 CFU/ml), or HP culture supernatant was then added. CL response was measured for a further 5 minutes and compared with buffer control or neutrophils stimulated with N-formyl-met-leu-phe (FMLP) ($5x10^{-9}$ to $5x10^{-7}$M) or phorbol 12-myristate 13-acetate (PMA) (5-100ng/ml).

Results ("CL index" = [stimulated CL - baseline CL] / baseline CL) Resting CL of neutrophils was increased by HP live organisms (median "CL index" 2.6 (quartile range 1.0 to 9.3), p = 0.002 compared to buffer control); HP sonicated organisms (2.1(0.9 to 3.5), p^2= 0.04); and HP culture supernatant (1.0(0.3 to 2.8), p = 0.01). The HP-induced CL response was biphasic and calcium-dependent. Preincubation with HP increased CL stimulated with FMLP (mean 20%, n=3) and PMA (35%, n=3). Bismuth subcitrate (1.2μg/ml) inhibited HP- and FMLP-stimulated neutrophil CL (-67%, -63% respectively, n=4); metronidazole (10μg/ml) inhibited HP-stimulated neutrophil CL (-41%, n = 3).

Conclusions i) H.pylori and its soluble products stimulate neutrophil ROM production *in vitro:* this phenomenon may be of pathogenic relevance *in vivo.* ii) The H.pylori-stimulated neutrophil response is similar to that produced by the chemotactic peptide FMLP. iii) Antioxidant properties may contribute to the effectiveness of metronidazole and bismuth in H.pylori-related human disease.

Colorectal neoplasia F272–F281

F272

FAECAL ALPHA-1-ANTITRYPSIN CONCENTRATION IN PATIENTS WITH COLORECTAL CANCER OR ADENOMATOUS POLYPS

[1]A Moran, [2]S Radley, [2]JP Neoptolemos, [3]AF Jones, [1]PA Asquith
[1]Department of Gastroenterology, East Birmingham Hospital; [2]Department of Surgery, University of Birmingham, Dudley Road Hospital; [3]Department of Clinical Chemistry, University of Birmingham, East Birmingham Hospital

Improvements in screening tests for colorectal cancer (CRC) are required. Alpha-1-antitrypsin (A1AT) measurement in faeces has been used as a test for protein losing enteropathy, but its value has not been examined in patients with colorectal neoplasia.

Using an improved extraction technique, faecal A1AT concentration in 24 patients with CRC, 10 patients with adenomatous polyps and 21 symptomatic controls (including patients with diverticular disease and peptic ulcer disease) was compared. All subjects underwent rigid sigmoidoscopy and double-contrast barium enema or colonoscopy before carrying out a 5 day faecal collection at home and without dietary restriction. Pooled samples were homogenised and lyophillized, A1AT was extracted from faeces with a cationic detergent and measured by radial immunodiffusion.

Patients with CRC had a higher dry weight concentration of faecal A1AT (3.4, 0.5-12.05 (median, range) vs 1.85, 0.5-3.4 mg g^{-1}; p=0.009 (Mann Whitney) and wet weight concentration (0.67, 0.1-2.21 vs 0.34, 0.07-0.71 mg g^{-1}; p=0.036) when compared with control subjects. There was no difference in dry or wet weight concentrations in the polyp group (2.3, 0.5-4.5 and 0.26, 0.11-0.88 mg g^{-1} respectively) compared with controls. 54% of CRC patients had a faecal A1AT concentration >3.4 mg g^{-1} dry weight and 46% >0.71 mg g^{-1} wet weight.

Preliminary results suggest that faecal A1AT excretion in patients with CRC requires further investigation, particularly with respect to a possible role in population screening.