**Hepatitis C** W1–W8

**W1**

Gut 1995;36 (Suppl 1) A1

**IGM ANTI-HCV TESTING MAY REDUCE NEED FOR PCR TESTING.**


Conventional serology for Hepatitis C Virus (HCV) by enzyme-immunoassay (ELISA) provides evidence of infection, past or present, but does not indicate infectivity. Recombinant immunoblot assay (RIBA) correlates better than ELISA with the presence of HCV genome by the polymerase chain reaction (PCR) which currently tends to be the "gold-standard" for infectivity. However, both RIBA and PCR are slow, subjective, labour intensive and therefore expensive. Infection with HIV and viral hepatitis is strongly associated with HCV, with CD4+ lymphocytes being the main target of severe viral infections. The appearance of IgM antibodies provides an indication of acute infection, but persistently low levels are often found in chronicity.

**AIM:** To evaluate a new test for IgM anti-HCV (Abbott Diagnostics, Weisbaden, Germany) on 42 anti-HCV positive recipients of anti-D immunoglobulin, in comparison with "in house" PCR and Roche Amplicor PCR.

**RESULTS:** Of the 42 patients, 29 (69%) were positive for IgM anti-HCV and by both PCR methods. Five patients were negative for IgM anti-HCV and by PCR. In 7 cases PCR was positive but IgM anti-HCV was not found. In one case IgM was positive and PCR was negative but the patient had been treated with interferon. Thus there was a 100% positive correlation between the presence of IgM anti-HCV and the presence of HCV genome in untreated chronic HCV infected patients (X²=11.04, p<0.001) but not necessarily the reverse. Conclusion: We conclude that IgM anti-HCV testing (at approximately one-third of the cost of PCR) may provide an economical aid to minimising the need for a significant proportion of PCR testing in chronic HCV infection.

**W2**

LYMPHOCYTE PHENOTYPES IN CHRONIC HEPATITIS C INFECTION: A CORRELATION WITH DISEASE ACTIVITY.

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Academic Department of Medicine, *University Department of Histopathology and University Department of Immunology, The Royal Free Hospital, Pond St, London, NW3 2 QG.*

To clarify the pathogenesis of hepatitis C (HCV) infection, we have phenotyped the inflammatory cells in liver biopsy (13) or liver extract (6) specimens of 19 patients with chronic hepatitis C infection by immunohistochemical methods. All patients were anti-HCV positive by 2nd generation RIBA assay or PCR for HCV RNA. Seven patients had histological mild hepatitis; 6 active hepatitis and 6 end stage liver disease necessitating liver transplantation.

In the parenchyma T lymphocytes were more prevalent than B lymphocytes in all stages of disease (mean count 3.39±2.33 cells per 10 microns² vs. 0.03±0.05, t=6.15, p<0.01). CD4 positive lymphocytes increased in active vs. mild disease (0.85±0.58 cells per 10 microns² vs. 0.24±0.38, t=2.22, p<0.06), conversely CD8 positive cells fell slightly in these groups (0.98±0.12 vs. 1.62±0.96, t=1.81, p=0.1). No changes in the number of cytotoxic granule containing (TiaI positive) or CD11b positive cells were seen in active vs. mild disease. Tissue macrophages were predominantly of the antigen presenting (62% RFD1 positive) rather than phagocytic (25% RFD7 positive) or suppressor type (15% both RFD1 and RFD7 positive) in all stages of disease.

Lymphoid aggregates were predominantly composed of CD8 positive rather than CD4 cells in histological mild disease (5.18±2.59 vs. 0.3), but in active disease CD4 positive cells predominated (3.1±2.46 vs. 12.5±7.32). B lymphocytes were infrequently found.

Our results suggest that histological active disease is associated with an increase in CD4 but not CD8 positive cells within the hepatic parenchyma, and therefore questions the role of CD8 positive cytotoxic lymphocytes in the hepatoctye injury of chronic hepatitis C infection.

**W3**

MANAGEMENT OF HEPATITIS C VIRUS (HCV) INFECTION IN PATIENTS WITH COAGULATION DISORDERS.


Liver Unit, Haemophilia Unit and Dept of Pathology*, Queen Elizabeth Hospital, Edgbaston, Birmingham, UK.

HCV is a common cause of chronic liver disease in patients with coagulation disorders. We describe a combined medical/surgical approach to the management of chronic HCV infection in these patients.

Case records of the 393 adults attending the haemophilia unit at Queen Elizabeth Hospital were reviewed. Patients who had ever received cryoprecipitate or factor concentrate were invited to attend a joint liver/hematology clinic. Following counselling, liver biopsy was offered to HCV RIBA positive patients fulfilling the following criteria: age>70yrs, no inhibitors to factor VIII/IX and CD4>200/mm³ if HIV positive. Liver biopsies using a Menghini technique without ultrasound guidance were performed following factor infusion to 100% levels. Patients remained in hospital overnight and factor levels were maintained at >50% levels over 3 days. The biopsy was assessed using a modified Knodell Histological Activity Index (HAI, range 0-13, 13 is worst). Patients with HAI≥6 were followed up every 6 months with a view to re-biopsy in 5 years to assess progression.

273/393 patients (69.5%) have received cryoprecipitate or concentrate treatment. To date, 173 have been tested for HCV by RIBA and 131 (75.7%) are positive (122M:9F, median age 34yrs) including 56 who are also HIV antibody positive. In RIBA positive patients, AST was elevated in 75% (median 48iu, range 9-310) and ALT was elevated in 80% (median 54.iu, range 7-249). Of 30 RIBA positive patients tested for HCV by PCR, 29 (96.7%) were serum PCR positive. To date, 28 patients, including 4 HIV positives, have been biopsied with no complications. The median HAI was 5 (range 0-12). Cirrhosis was present in 1 patient who also had a heavy alcohol intake. 11 patients had a HAI≥6 and were offered IFN treatment. To date, 5 have completed treatment with sustained biochemical response int2.

Conclusions: The majority of patients who had had factor/cryoprecipitate infusions are RIBA positive. Most RIBA positive patients have abnormal biochemistry and are PCR positive. Liver biopsy with adequate factor cover and overnight admission is safe. Histological changes tend to be mild.

**W4**

HEPATITIS C INFECTION AND ALPHA-INTERFERON THERAPY IN PATIENTS WITH NORMAL TRANSMISASES - A PILOT STUDY

CJ Healey, S. Read, J. Kurtz, KA Fleming and RWG Chapman

Dept of Gastro., Oxford Regional PHLS, John Radcliffe Hospital, and Nuffield Dept. of Pathol, University of Oxford, Oxford UK

Introduction Many HCV cases have normal liver function (LFTs) but may have significant liver pathology. This pilot study used RT-PCR to identify HCV infection and examine response to α-interferon (INF) in patients with normal LFTs. Methods Patients who were anti-HCV +ve and HCV RNA +ve were offered liver biopsy. Histology was compared (stage and histological activity index, HAI) between patients with persistently normal LFTs(NOR) and those with abnormal LFTs(AB). Cases with normal LFTs who had chronic hepatitis were treated with INF, 3 MU 3x/week for 24wks. PCR was performed at 0, 12, 24, 36 and 48 wks.

**Results** A Comparison of histology between two groups

<table>
<thead>
<tr>
<th>LFTs (mean HAI)</th>
<th>NO</th>
<th>AB</th>
<th>CR</th>
<th>*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td>123.2</td>
<td>72.5</td>
<td>60.3</td>
<td>4</td>
</tr>
<tr>
<td>AST</td>
<td>49.5</td>
<td>50.5</td>
<td>49.5</td>
<td>3</td>
</tr>
<tr>
<td>ALT:AST</td>
<td>2.5</td>
<td>1.4</td>
<td>1.2</td>
<td>2</td>
</tr>
<tr>
<td>HAI</td>
<td>2.5</td>
<td>1.4</td>
<td>1.2</td>
<td>2</td>
</tr>
</tbody>
</table>

C=Control, *No=Normal, Min=Minimal hepatitis, Cirrhosis

14 patients with normal LFTs commenced INF.

b) Response to INF therapy in patients with normal LFTs

<table>
<thead>
<tr>
<th>Stage</th>
<th>Post therapy</th>
<th>%+ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100%</td>
<td>50%</td>
</tr>
<tr>
<td>2</td>
<td>50%</td>
<td>25%</td>
</tr>
</tbody>
</table>

In 10 patients who have finished treatment (8 completed, 2 withdrawals), only 2 have remained HCV RNA negative (>3mths post therapy).

Conclusions Histological change is common patients with normal LFTs. Response to INF can be measured by RT-PCR in patients with normal LFTs. The majority of such INF treated patients have virologic relapse within 6 months of therapy. INF does not appear in this pilot study to be more effective in patients with normal LFTs.
**LIVER HISTOLOGY IN PATIENTS WITH HEPATITIS C (HCV) AND NORMAL TRANSMISSION LEVELS.**

AJ Stanley, GH Hayward, J Paris, and Ph Simmonds, PC Hayes Centre for Liver and Digestive Disorders, Departments of Pathology and Virology, Royal Infirmary of Edinburgh.

Serum transaminase levels are commonly used indicators of hepatic damage in HCV infection. The aim of this study was to assess the relationship between liver histology in patients with HCV infection and serum transaminase levels.

**Methods:** Laparoscopic liver biopsy specimens from 100 consecutive patients (74 male), with chronic HCV infection were examined by one pathologist. In all, with respect to transmission level, serotype and route of infection. Each specimen was graded according to the Edinburgh Scoring System; this includes fatty change, inflammation, fibrosis and cirrhosis. Serotype was assessed by an ELISA for type specific antibodies.

**Results:** All patients had abnormal liver histology, although 15 patients had normal transaminase levels. There was no statistical significant difference between the number of patients with severe liver damage and a normal serum transaminase, and those with comparative damage in the whole population. Similarly, there was no significant difference in the numbers of intravenous drug users and haemophiliacs in the two groups. HCV serotype 1 was the most common viral serotype in both groups.

**Conclusion:** 15% of our total population had normal transaminase levels, of whom 31% had evidence of severe histological changes. Liver biopsy is essential in HCV infected patients to assess hepatic pathology, both to guide therapy and aid prognosis.

<table>
<thead>
<tr>
<th>HISTOLOGY</th>
<th>All patients (%)</th>
<th>Patients with normal transaminase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very mild</td>
<td>11</td>
<td>21</td>
</tr>
<tr>
<td>Mild</td>
<td>29</td>
<td>43</td>
</tr>
<tr>
<td>Moderate</td>
<td>33</td>
<td>14</td>
</tr>
<tr>
<td>Severe</td>
<td>27</td>
<td>21</td>
</tr>
</tbody>
</table>

**Cytokines, Inflammation and Hepatitis C Infection.**

**Methods:** The pathogenesis of hepatic damage in chronic hepatitis C virus (HCV) infection is unclear, with evidence supporting both cytopathic and immunopathic mechanisms. Cytokines, proteins playing a pivotal role in many inflammatory conditions, have been demonstrated at increased levels in the serum and liver of patients with both acute and chronic liver disease. This study investigates the expression of messenger RNA (mRNA) specific for three cytokines, interleukin-1α, interleukin-6 and tumour necrosis factor-α, together with mRNA for the interleukin-6 receptor, by radioactive in situ hybridisation in liver tissue from 25 patients with chronic HCV.

Expression of interleukin-1α and tumour necrosis factor-α was demonstrated in 84% and 76% of biopsies respectively. Although no expression of interleukin-6 was detected, receptor to interleukin-6 was present in 44% of biopsies. Autoradiographic signal was located over mononuclear cells, Kupffer cells, hepatocytes and bile duct epithelial cells. The specificity of the technique was confirmed by the use of appropriate controls.

A direct relationship was found between expression of interleukin-1α mRNA and inflammatory activity in biopsies, as assessed by Scheuer's scoring system (Spearman's Rank Order Correlation: correlation coefficient 0.817, p < 0.005).

Cytokines, in particular interleukin-1α appear to play a role in mediating the hepatic inflammation seen in chronic HCV infection, suggesting that immunopathic mechanisms are important in the pathogenesis of this disease.

**TREATMENT OF ACUTE HEPATITIS C WITH ALPHA INTERFERON IN HYPOGAMMAGLOBULINAEMIA - THE UK GAMMAGARD OUTBREAK.**

Cl Healey, J Watson, M Durridge, N Snowdon, J Christie, S Wong, S Read, J Kurz, KA Fleming, H Chapel and RWG Chapman, Departments of Gastro., Immunol., and Nuffield Dept. of Pathology, Oxford Regional PHLS, John Radcliffe Hospital, Oxford, UK

**Introduction.** Intravenous immunoglobulin (IVIG) is established therapy for antibody deficiencies. Despite anti-HCV screening, 36 UK patients were treated with IVIG containing HCV (Gammagard). The majority of them have developed acute HCV (genotype 1a). HCV infection in hypogammaglobulinic patients appears to run a severe course. Treatment of HCV with alpha-interferon (IFN) has been shown to be effective in acute HCV.

**Methods.** HCV infection was confirmed by PCR. Patients with documented HCV infection were offered treatment with IFN. Therapy was administered as 6 Mega Units(MU), SC, 3x weekly for 6 mths. Results-Data from 9 UK centres is available for 34/36 cases of contaminated IVIG exposure. 5 patients were PCR -ve, 3 declined therapy and 8 were considered inappropriate for treatment (5 malignancies, 1 lung disease, 1 granulomatous liver disease, 1 depression). One case had previous HCV cirrhosis and has had a liver graft. 17/29(59%) HCV infected patients commenced treatment with IFN (13-6 MU 3x weekly, 4-2 reduced doses) within 8 months of exposure (range 2-8 mths). 4 treated patients required dose reduction (2 thrombocytopenia, 2 severe lethargy) and 4 failed to finish 6 months (2 intolerant to interferon, 2 concurrent illness). 3/17 patients are still undergoing therapy and 4/17 patients who terminated therapy early are still HCV +ve. 10/17 patients have completed 6 months IFN, all had normal transaminases at cessation. However 1/6 tested was still HCV PCR +ve. 3/10 have relapsed within two months.

**Summary:** IFN in acute HCV amongst hypogammaglobulinemic patients results in rapid biochemical improvement plus viral loss in the sera. However side effects were marked. 3 patients have relapsed rapidly despite normal LFTs and clearance of viraemia during therapy though 3 patients are showing a sustained response.
LAPAROSCOPIC BILIARY AND GASTRIC BYPASS - A USEFUL ADJUNCT IN THE MANAGEMENT OF CARCINOMA OF THE PANCREAS
M. Rhodes, L. Nathanson, G. Fielding. University Department of Surgery, Royal Brisbane Hospital, Brisbane, Australia.

Over 90% of patients with inoperable carcinoma of the pancreas are successfully palliated by ERCP and stent insertion. Management of the residual 10% of patients often involves a laparotomy which is difficult to justify when median survival of these patients is only 150 days. Laparoscopic biliary and gastric bypass offers a less invasive alternative than open surgery with shorter hospital stay and more rapid return to normal activity.

Between August 1991 and March 1994, 16 patients (median age 69 years, range 31-85) underwent laparoscopic bypass surgery. The indications for surgery were gastric outlet obstruction at initial presentation (n=4), blocked biliary stent (n=8) and metastatic tumour at laparoscopy (n=4). Surgery took the form of choledocjejunostomy (n=7), gastroenterostomy (n=5), both procedures (n=3) and failed operation (n=1).

Operative duration was 75 minutes (range 45-190) and hospital stay 4 days (range 3-33) and all apart from two patients were discharged from hospital in 7 days or less. Morbidity occurred in 2 patients (13%) in the form of a CVA and delayed gastric emptying. Median survival in ten patients who have died is 201 days (range 20 - 525).

Laparoscopic biliary and gastric bypass is possible in the majority of patients in whom endoscopic stenting has failed and in those who subsequently develop gastric outlet obstruction. Hospital stay is shorter than after open surgery and recovery more rapid.

CRITERIA FOR ERCP IN THE LAPAROSCOPIC ERA
GSM Robertson, C Jagger, PRV Johnson, BJ Rathbone, ACB Wicks, DM Lloyd, PS Veitch. Department of Surgery, Clinical Sciences Building, Leicester Royal Infirmary LE2 7LX.

The technical difficulties encountered during laparoscopic exploration of the common bile duct (CBD) have prompted a fundamental reappraisal in recent years of the identification and management of CBD stones. This study attempted to refine the preoperative selection of patients at risk of CBD stones by reviewing the indications for ERCP in 317 patients with in situ gallbladders and gallstones.

Abnormalities justifying ERCP were found in 210 patients (66%) of whom 178 had CBD stones. There were significant differences between this group and the 107 patients with normal ducts.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Normal</th>
<th>Abnormal</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal LFTs</td>
<td>69</td>
<td>57</td>
<td>p=0.0005</td>
</tr>
<tr>
<td>All LFTs abnormal</td>
<td>36</td>
<td>125</td>
<td>p=0.0001</td>
</tr>
<tr>
<td>Jaundice</td>
<td>36</td>
<td>125</td>
<td>p=0.0001</td>
</tr>
<tr>
<td>Pancreatitis</td>
<td>28</td>
<td>27</td>
<td>p=0.0031</td>
</tr>
<tr>
<td>Bilirubin (mean)</td>
<td>29.1</td>
<td>58.3</td>
<td>p=0.0001</td>
</tr>
<tr>
<td>rGT (mean)</td>
<td>155.1</td>
<td>258.4</td>
<td>p=0.0012</td>
</tr>
<tr>
<td>ALP (mean)</td>
<td>200.3</td>
<td>379.8</td>
<td>p=0.0001</td>
</tr>
<tr>
<td>Albinon</td>
<td>39.4</td>
<td>35.8</td>
<td>p=0.0001</td>
</tr>
<tr>
<td>Dilated CBD on US</td>
<td>32</td>
<td>168</td>
<td>p=0.0001</td>
</tr>
<tr>
<td>CBD stones on US</td>
<td>7</td>
<td>74</td>
<td>p=0.0001</td>
</tr>
</tbody>
</table>

(Bold figures signify a greater number than would be expected.)

Logistic regression produced a model using 6 factors with a specificity of 75% and a sensitivity of 89% for CBD abnormalities. Past pancreatitis or elevation of individual LFTs were not predictive factors.

The use of such a model rather than individual criteria would improve the selection of patients for preoperative ERCP optimising its role in the laparoscopic era.
WILL LAPAROSCOPIC BILIARY BYPASS REPLACE ENDOSCOPIC STENTING FOR MALIGNANT OBSTRUCTIVE JAUNDICE?
B.R. England, P.R. Yarnasky, P.B. Cotton, Department of Radiology and Division of Gastroenterology, Duke University Medical Center, Durham, North Carolina, U.S.

Laparoscopic cholecystectomy (CCE) may be an alternative to biliary stenting to palliate malignant biliary obstruction. To be suitable for CCE, obstruction must be distal to the cystic duct origin (ideally >1 cm).

Our aim was to determine the eligibility for CCE in patients with malignant obstruction.

METHODS: We studied consecutive patients with malignant obstruction who had ERCP in 1992. Radiographs were reviewed to determine if patients might be eligible for CCE, i.e., non-hilar lesions and/or hilar surgery. Films were also reviewed to determine if cystic duct or gallbladder filling was noted. If filling occurred, the proximal extent of the stricture to the cystic duct origin was recorded.

RESULTS: 121 patients with malignant obstruction had ERCP. 40 (33%) had previous biliary surgery and 11 (9%) had hilar tumours. There were 70 patients (58%) potentially eligible for laparoscopic CCE. 7 patients had failed cholangiograms and 3 could not be assessed. Of the remaining 60, 29 had no filling of the cystic duct or GB. In 31, with filling, the stricture was >1 cm from the cystic duct origin in 11. All 31 "normal" patients had GB or cystic duct filling.

CONCLUSION: Based on criteria listed, less than 10% (11/121) of patients with malignant obstruction were candidates for CCE. Laparoscopic CCE will not be an alternative to endoscopic stenting in the majority of patients with malignant obstructive jaundice.

ENDOSCOPIC BILIARY ENDOPROSTHESIS FOR THE MANAGEMENT OF BILE DUCT STONES IN HIGH RISK GROUPS: A TRIAL, RANDOMISED, CONTROLLED STUDY
Chopra F., Peters R., O‘Toole PA, Williams S., Gimson AES, Longmore M, Westaby D. Chelsea & Westminster Hospital, London;#Walton Hospital, Liverpool;#Institute of Liver Studies, King's College School of Medicine & Dentistry, London

The use of an endoprosthesis for management of bile duct stones is controversial. To investigate the efficacy and safety of endoprosthesis insertion (BE) to endoscopic duct clearance (DC) in the elderly (>70 years) or chronically ill with symptomatic bile duct stones.

Eighty-four patients were randomised to either BE insertion (n=42, 7F Pigtail prosthesis; <0.75 cm sphincterotomy) or DC (n=42, Standard sphincterotomy; balloon and/or basket + mechanical lithotripsy). Drainage was achieved in 41 (98%) BE group after one session (one patient required 2 sessions). Duct clearance was achieved in 23 (55%) at first attempt, rising to 33 (80%) after a median 2 sessions (range 2-4). Eight (20%) in DC group required a prosthesis for long term drainage. Fewer early complications were observed in the BE group but this failed to reach significance (3% vs 8 patients respectively; p>0.1). During follow-up of 3-31 months (median 17), 9 (24%) BE group and 6 (14%) DC group patients had biliary complications. Using Kaplan-Meier analysis 76% BE and 90% DC group were free of complication at 20 months (p=0.05). There were 1 DC with only 1 (BE) related to biliary complications.

Endoprosthesis insertion proved a safe and effective means of bile duct drainage and represents an alternative to duct clearance. The significant risk of cholangitis should restrict endoprosthesis insertion as a definitive treatment to highly selected cases.

ERCP-RELATED PANCREATITIS: THE DANGER OF LEAVING AN OBSTRUCTED BILE DUCT
A.C. de Beauchamp, K. Palmer, D.C. Carter
University Department of Surgery, Royal Infirmary, Edinburgh EH3 9YW

Pancreatitis following ERCP is a recognised complication, more common after therapeutic ERCP or pancreatic duct injection. We report 14 cases of ERCP-related pancreatitis observed over a five year period with 4 deaths (29% mortality). The diagnosis of pancreatitis was based on an elevated serum amylase in the presence of clinical or radiographic evidence. The ERCP was performed under elective conditions for suspected choledocholithiasis, with antibiotic prophylaxis, by experienced endoscopists. Pre-ERCP serum bilirubin was above the upper limit of normal (17 μmol/l) in 9 patients (mean of 62 (range 22-97) μmol/l). Gallstones were confirmed in 12 patients, with choledocholithiasis at the time of ERCP in 10 patients. These 10 patients underwent endoscopic sphincterotomy and bile duct clearance by basket or balloon catheter methods, with successful duct clearance in 7 patients. In the remaining 3 patients, the stones were considered 'small' by the operator and deemed able to pass through the sphincterotomy. Pancreatic duct injection was inadvertently performed in 6 patients.

Comparing the 4 patients who died with the 10 survivors, there was no significant difference in age (65±7.5 years vs 55±3.0, p=0.20 Mann-Whitney U test), sex (p=0.15), pre-ERCP bilirubin (62±13 μmol/l vs 61±7, p=0.89), the presence of choledocholithiasis (p=0.15), successful duct clearance (p=0.76) or pancreatogram (p=0.74). However, in the 4 patients who died, the serum bilirubin continued to rise on the first and second day post-ERCP (suggesting an obstructed bile duct free from stone impaction or oedema of the ampulla of Vater) whereas in the 10 patients who survived, the serum bilirubin level fell post-ERCP (p<0.001). The predominant presenting symptom of ERCP-related pancreatitis with an obstructed biliary tree post-ERCP in this small series was a lethal combination. Monitoring of serum bilirubin in ERCP-related pancreatitis may allow early surgical duct decompression in this high risk group
ENDOSCOPIC STENTING ALONE AND STENTING WITH RADIODERAY IN NON-RESECTABLE CHOLANGIOCARCINOMA: A RETROSPECTIVE COMPARISON.

T E Bowling, A R W Hatfield, M F Spittle, J Solano. Departments of Gastroenterology and Radiotherapy, The Middlesex Hospital, London W1N 8AA, UK.

Between 1988-1993 145 patients with suspected cholangiocarcinoma were stented at ERCP for malignant strictures. Of the 60 with histologically proven non-resectable tumour, 31 received external beam radiotherapy (30 Gy in 10 fractions) followed by brachytherapy (60 Gy) using iridium 192 inside the stent. The remaining 29 patients were considered for radiotherapy but, mainly for geographic reasons, this was not pursued. These formed the stenting only control group.

The two groups were well matched in age, sex and type of stricture distribution. 5 patients (2 control, 3 DXT) are still alive (follow-up 8-19 months). Of those who died, survival was similar in the control and radiotherapy groups (median 8 and 11 mo). There was a significantly greater number of stent changes in the radiotherapy group compared to the control group (1.9 ± 0.9 per patient - p < 0.005).

Type I stent (med; range) Control (n=11) DXT (n=8) P value

<table>
<thead>
<tr>
<th>Age</th>
<th>64 (46-88)</th>
<th>65 (58-76)</th>
<th>NS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival from diagnosis (mo)</td>
<td>7.5 (1-29)</td>
<td>9.2 (5-75)</td>
<td>NS</td>
</tr>
<tr>
<td>Patients surviving &gt; 1 year</td>
<td>2</td>
<td>2</td>
<td>NS</td>
</tr>
<tr>
<td>Hospital stay (days)</td>
<td>7.0 (3-26)</td>
<td>50 (23-77)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Type II, III (med; range) Control (n=18) DXT (n=23) P value

<table>
<thead>
<tr>
<th>Age</th>
<th>65 (45-85)</th>
<th>60 (35-77)</th>
<th>NS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival from diagnosis (mo)</td>
<td>9.5 (2-19)</td>
<td>12 (4-34)</td>
<td>NS</td>
</tr>
<tr>
<td>Patients surviving &gt; 1 year</td>
<td>6</td>
<td>6</td>
<td>NS</td>
</tr>
<tr>
<td>Hospital stay (days)</td>
<td>18 (3-85)</td>
<td>38 (1-140)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Radiotherapy does not appear to significantly influence survival in either types I or II,III cholangiocarcinoma. There was a wide range of survival in both groups, with about one third of all patients surviving over 1 yr. The cost and increased hospital stay make radiotherapy unsuitable for routine use.

CRYPT PROLIFERATION AND REPRODUCTION IN CARCINOGEN OR EPIDEMIC GROWTH FACTOR (EGF) TREATED RAT COLON

HS Park, RA Goodlad, CY Lee, D Ahn3, & NA Wright.
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1Dept. Medicine, Denver VA Med. Ctr., Denver, Co., USA.

Epidermal growth factor (EGF) is a potent stimulant of intestinal cell proliferation. It has been postulated that EGF may influence the proliferative gut activity. Colon cancer is known to have a relatively high cell production rate (CCPR) and crypt size were therefore studied in a multifactorial experiment using EGF, the carcinogenic dimethylhydrazine (DMH) and EGF + DMH together. In addition, colonic crypt fission, a poorly understood process of crypt reproduction was also quantified.

Orally fed male F344 rats were: (1) injected with EDTA (control) weekly for 16 weeks, (2) treated with DMH (20mg/kg, subcutaneous, weekly for 16 weeks) or (3) infused with EGF (by osmotic mini pump, 300 µg/kg/day for 7 days at week 24), (4) treated with DMH and then infused with EGF.

25 weeks after the first injection, the rats were injected with vincristine to arrest cells entering metaphase and the colonic crypt microdissected. Crypt area, fission index (FI), and crypt cell production rate (CCPR) were then measured.

Two way analysis of variance showed that the crypt fission indices were elevated by DMH (p<0.001) and decreased by EGF (p<0.004). There was no interaction between EGF and DMH. Crypt area was increased by EGF (p=0.019) as was CCPR (p<0.001). A small effect of DMH was also observed. There was no interaction between EGF and DMH.

Increased colonic CCCPRs induced by EGF result in increased crypt size.

It is concluded the crypt fission as well as cell proliferation can play a major role in colonic adaptation and that the primary response of colonic epithelial cells to the carcinogenic occurs via the by crypt fission route and the two processes, cell proliferation and crypt fission, may be independently regulated.

The presence of apoptosis was determined by DNA gel electrophoresis.

RESULTS: Gel electrophoresis demonstrated the DNA ladder characteristic of apoptosis in the detached cells but not cells from the monolayer. Flow cytometric analysis of cell particles released into the incubating medium immediately after exposure to 1 mM H2O2 for 1 h revealed cell fragments only. However, flow cytometric analysis of the number of particles released into the incubating medium between 8 and 24 h after exposure to 1 mM H2O2 revealed an increase in 2.1 ± 0.6 fold compared to control (n = 3, P < 0.014).

CONCLUSION: H2O2 induces two distinct death pathways in HT-29 cells. PARP inhibition prevents the immediate cell death process in HT-29 monolayers but has no effect on delayed cell death leading to apoptosis. (Funded by a BDF project grant).

HYDROGEN PEROXIDE INDUCES APOPTOSIS IN HT-29 CELLS; A PROCESS WHICH IS NOT PREVENTED BY INHIBITION OF POLY-ADP-RIBOSE POLYMERASE (PARP)

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BACKGROUND: Oxidants, including H2O2, cause cell death in inflammation. We have shown previously that H2O2 induces immediate cell death (necrosis) in HT-29 cells; a death process which is prevented by inhibition of PARP (GUT 1994;35:S46). PARP is a nuclear enzyme which is activated by H2O2-induced DNA damage causing NAD and ATP consumption sufficient to cause cell death. The objective of this study was to determine whether H2O2, induced another form of cell death called apoptosis in HT-29 cells and 2) whether apoptosis was prevented by PARP inhibition.

METHODS: Cells were damaged by H2O2 with or without the PARP inhibitor 3-aminoazonobenzine (3-ABA). It has been shown previously that HT-29 cells undergoing apoptosis detach from the monolayer (EMBO J 1993;12:3679). Therefore cellular detachment into the incubating medium was quantified by flow cytometry and used to measure apoptosis. Cell fragments were distinguished from whole cells by comparison with HT-29 cells brought into suspension by trypsinization. The presence of apoptosis was determined by DNA gel electrophoresis.

RESULTS: Gel electrophoresis demonstrated a DNA ladder characteristic of apoptosis in the detached cells but not cells from the monolayer. Flow cytometric analysis of cell particles released into the incubating medium immediately after exposure to 1 mM H2O2 for 1 h revealed cell fragments only. However, flow cytometric analysis of the number of particles released into the incubating medium between 8 and 24 h after exposure to 1 mM H2O2 revealed an increase in 2.1 ± 0.6 fold compared to control (n = 3, P < 0.014).

1 mM 3-ABA had no effect on this delayed detachment process. (This concentration of 3-ABA prevents immediate cell death in the monolayer caused by exposure to 1 mM H2O2 for 30 min.)

CONCLUSION: H2O2 induces two distinct death pathways in HT-29 cells. PARP inhibition prevents the immediate cell death process in HT-29 monolayers but has no effect on delayed cell death leading to apoptosis. (Funded by a BDF project grant.)

Cell biology W18-26

DISTRIBUTION OF THE EGF-RECEPTOR IN THE NORMAL HUMAN GASTROINTESTINAL TRACT. T. McGrattery, N.A. Wright, A. Hanby, S. Gschmeisser, L.P. Peiffer, R.J. Playford Departments of Gastroenterology, Hammersmith Hospital, London W12 ONN & Hershey Medical Centre, Hershey, PA, USA.

Background: It is clear that luminal epidermal growth factor (EGF) stimulates repair of the damaged bowel. Its significance in maintaining normal gut growth remains uncertain. If EGF is important in maintaining normal gut growth, the EGF receptor (EGF-R) should be present on the apical (luminal) surface in addition to the basolateral surface. We therefore examined the distribution of the EGF-R in the human gastrointestinal tract using a combination of immunohistochemistry, electron microscopy and Western blotting.

Methods: Fresh endoscopic biopsies were obtained from the oesophagus, stomach, small intestine and colon of 20 normal subjects. The distribution of the EGF-R was determined using immunohistochemistry and immunogold staining (for electron microscopy) using both monoclonal and polyclonal antibodies. Additional samples were used for brush border preparations followed by Western blotting for EGF-R.

Results: The EGF-R was present in abundant quantities throughout the normal gastrointestinal tract. Immunostaining of the oesophagus showed circumferential EGF-R positivity in the cells of the basal portions of the stratified squamous epithelium but surface cells were EGF-R negative. In the rest of the bowel, immunostaining localised the receptor to the basolateral surface with the apical membranes being consistently negative. The quality of brush border preparations was confirmed by increase in apically located alkaline phosphatase and concomitant decrease in basolateral located sodium-potassium ATPase activity. Western blotting demonstrated a 170kDa protein in whole tissue homogenates but not in the brush border vesicle preparations.

Conclusions: As the EGF-R is located only on the basolateral surface, the major role of EGF in the lumen of the GI tract is likely to be to stimulate repair rather than to maintain normal gut growth.
A SPECIFIC ROLE FOR SODIUM BUTYRATE IN GROWTH ARREST OF COLON CANCER CELLS.

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The differentiative effect of sodium butyrate on colorectal cancer cells has been attributed to its effects on DNA methylation and histone acetylation and is therefore considered to be non-specific, although DNA motifs have been reported that confer butyrate-inducibility on heterologous promoters. By searching the promoters of butyrate-inducible genes for common sequence motifs, we have defined several classes of putative butyrate response elements (BRE) and have identified three in the promoter region of GADD153, a Growth Arrest and DNA Damage inducible factor. It is a member of the CEBP family of bZIP transcription factors, with a cell lineage-specific and differentiation-dependent pattern of expression in gut epithelial cells.

Using RNase protection assays and GADD153 antibodies, we have found that gadd153 expression is induced at least 10-fold in colon carcinoma (T-84) cells in response to butyrate at 5 mM. The transcriptional activation is seen at butyrate concentrations of as low as 0.1 mM, occurs within 5 min of butyrate addition and shows two peaks: one at 30 min after treatment and a second after 72 h. Using oligodeoxynucleotides containing the putative BRE motif present in the gadd153 promoter, we now show at least two of these BREs bind factors present only in T-84 cellular extracts prepared from sodium butyrate-treated cells and that a factor present only in the non-butyrate-treated cells appears to be removed from one BRE.

In the light of health experts' advice to increase dietary soluble fibre/carbohydrate consumption, the finding that butyrate specifically induces a gene whose product is involved in growth arrest, differentiation and apoptosis is highly significant since it provides a molecular rationale for the differentiative effects of sodium butyrate.

K-RAS MUTATIONS IN HUMAN COLONIC ABERRANT CRYPTS.

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Enlarged, darker, and slightly bulging crypts have been identified on the methylene-blue stained mucosal surface of human colon. They are usually observed in clusters referred to as aberrant crypt foci (ACF). ACF show several morphological and biochemical alterations. Furthermore K-ras mutations have been recently demonstrated. Thus ACF might represent precursor lesions of colon cancer. The present study was undertaken in order to evaluate whether and at what extent K-ras and p53 gene mutations were present in ACF. Specimens of mucosa from 5 patients with colon cancer were collected after surgery and immediately observed after staining with methylene-blue, under a stereomicroscope. A total of 14 ACF along with 5 samples of normal mucosa and 5 tumours were frozen in liquid nitrogen and then analyzed for K-ras and p53 mutations. Non radioactive single strand conformation polymorphisms (SSCP) analyses for K-ras exon 1 and p53 exons 5 through 8 detected no mutation. However, using allele-specific polymerase chain reaction (ASPCR), mutations in codon 12 of K-ras gene were found in 8 of 14 (57%) ACF. In two cases the same K-ras mutation was found in the tumour and in at least one ACF of the same patient, in other case mutations were different. In one patient both tumour and ACF were wild type (wt), and in another case the tumour was wt and 3 of 4 ACF showed mutations. The only ACF with no dysplasia at histology had no K-ras mutation. These data suggest that K-ras mutations are frequent in human colon ACF, suggesting a role for some of them in the early stages of colorectal carcinogenesis.

QUALITATIVE AND QUANTITATIVE ALTERATIONS OF E-CADHERIN DURING COLORECTAL TUMOURGENESIS: IN VIVO AND IN VITRO.

J.A. Jankowski, S. Kirkland, K. Henderson, F. Bedford, A. Ashworth, Y.S. Kim, M. Pignatelli, N.A. Wright. ICRF, London, Colorectal Cancer Program, University of California, San Francisco, GI Research Laboratory, VAMC, SF, CA.

Study of the molecular events in colorectal tumourogenesis requires that a satisfactory human model is available to mimic the heterogeneity of tumours. We studied colorectal specimens together with four colonies of the human colorectal cancer cell line HCA-7 which had progressive abnormalities in vivo in xenografted mice: colony 1 and 3 (were poorly differentiated and invasive), colony 6 (moderately differentiated, slow growth), colony 30 (well differentiated). Colony 1 grew fastest both in monolayers and in agarose whereas colonies 3, 6 and 30 exhibited progressively decreasing growth.

Molecular changes were studied by Western blot, nucleic acid analyses as well as PCR and gene cloning. The expression of the tyrosine receptor c-erbB3 and the adhesion molecule E-cadherin were correlated with tissue differentiation.

Assessment of genomic perturbations revealed that all colon specimens expressed DCC but none expressed truncated APC or K-ras point mutations indicating that other genetic anomalies may account for their different biological behaviour. p53 mutations were detected in equal numbers in all the HCA-7 colonies.

E-cadherin transcripts were maximally expressed in colonies 6 and 30 indicating a close relationship with the protein levels. There were no truncated transcripts to suggest 'exon-skipping' and there was also no gene amplification to account for these differential E-cadherin levels. Colonies 1 and 30 had multiple mutations of the E-cadherin gene in functional binding domains. Interestingly these same colonies were most easily disaggregated by anti-E-cadherin antibodies. Intriguingly in colony 1 there was also a paradoxical increase of P-cadherin levels compared with all other colonies.

By degenerate PCR we have identified a novel cadherin transcript, with 20% amino-acid homology to E-cadherin, which is correlated with an invasive phenotype.

In conclusion this cell line has allowed detailed investigation of the qualitative and quantitative changes of the cadherin family involved in the generation of heterogeneity within colorectal tumours.

MICROSATELLITE INSTABILITY IN SPORADIC COLORECTAL CANCER DETECTED BY FLUORESCENT PCR

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We report here a fluorescent PCR technique which improves the assessment of microsatellite instability, a phenomenon first described in hereditary non-polyposis colorectal cancers and in "sporadic" colorectal cancers, where it was associated with good prognosis. This assay, which combines accurate allele sizing with improved data display, was used with 11 microsatellites to investigate 54 sporadic colorectal adenocarcinomas. We found 12/54 (22%) cancers exhibited some degree of microsatellite instability. For the tumours showing microsatellite instability results were obtained for a minimum of 8 markers and there appears to be two main groups emerging with some tumours exhibiting microsatellite instability at many markers (at least 63% of markers affected) and others showing instability at a lower frequency (up to 37% of markers affected). Patients with a tumour that showed microsatellite instability had a significantly better prognosis (p=0.03). 42% of the patients who had a tumour showing microsatellite instability had a synchronous and/or metachronous colorectal tumour (compared to 7% of patients whose tumour did not show microsatellite instability). Microsatellite instability assays may identify a subgroup of patients who require closer clinical surveillance due to increased risk of further tumours but who may also benefit from less aggressive therapy due to an improved prognosis.
NUCLEAR p53 OVEREXPRESSION IN DUKES' STAGE B AND C COLORECTAL CANCER: CLINICO-PATHOLOGICAL CORRELATION AND PROGNOSTIC SIGNIFICANCE. R.Fante, L.Lossi, C.Di Gregorio, L.Roncucci and M.Ponz de Leon. Deparment of Internal Medicine and Departmentally Morphological and Forensic Medical Sciences, University of Modena, Italy.

Overexpression of p53 protein was studied in tumour specimens of 150 patients selected from the files of a Colorectal Cancer Register between 1984 and 1986. Ninety-two had Dukes' stage B, 58 stage C adenocarcinoma (M 75, F 75). p53 was assessed via monoclonal antibody PAb 1801 on archival material. Nuclear staining of neoplastic cells was evaluated in two tumour microsections subdivided into three groups: 1) negative 2) low expressors (1-20% stained nuclei) 3) high expressors (>20% stained nuclei). p53 status was related to major clinical features of patients, to histomorphological variables and to 5-year cancer-specific survival. These relations were performed considering low expressor together with negative or high expressor tumours alternatively. Nuclear p53 expression was found in 71 (50 high, 21 low expressors) out of 150 tumours (47.3%). There was no relation between p53 status and age or sex of patients, tumour site, size, pattern of growth, extent of fibrosis, lymphocytic response and amount of mucin. No difference was found in p53 expression between Dukes' stage B and C tumours. Patients with p53 positive or negative neoplasia had similar overall survival either in the whole series or within stage B or C. In colorectal cancer patients, survival was not influenced by p53 status. p53 negative rectal cancer patients seemed to have a better prognosis (borderline significance). These findings seem to suggest that p53 nuclear overexpression may indicate different in the biology of colonic and rectal cancer, but it does not seem to be a useful prognostic indicator.

9-CIS RETINIC ACID DIFFERENTIALLY MODIFIES VITAMIN D3 ACTION IN TWO HUMAN COLORECTAL CANCER CELL LINES. K.E. Kennedy, M.S. Langman, S. Williams, Department of Medicine, The University of Birmingham, Birmingham, UK.

Epidemiological, animal & in vitro studies indicate that 2,3(25)-vitamin D3 (1,25D3) protects against colorectal cancer. Post-translational synthesis of vitamin D3 analogues, which exert minimal calcemic effects, are promising antiproliferative agents. D3 action is mediated via its high affinity nuclear receptor (VDR) which regulates target gene expression by binding to DNA as a homodimer or as a heterodimer with other members of the nuclear receptor superfamily. 9-cisRA modifies D3 action in vitro but these effects have not been studied in the colon. We have previously demonstrated expression of mRNAs for VDR and RXRα in both neoplastic & normal human colonic mucosa. We investigated whether 9-cisRA modifies D3 action in two D3 responsive colon cancer cell lines (HT-29 and Caco-2). In these studies cells were treated with D3, its analogue, EB1089, or 9-cisRA at concentrations of 1x10^{-6} to 1x10^{-10} M. Northern analysis of receptor expression was performed. Growth response to 72hrs of treatment was assessed by [3H]-thyminidine uptake. Hormone responsiveness of the classical D3 target gene, 24-hydroxylase, was assessed by mRNA expression and enzyme activity by HPLC analysis after incubation of cells with [3H]-25(OH)D3. Both cell lines express identical receptor mRNAs to those shown in human tissues. In HT-29 cells a dose dependent (1x10^{-8} to 1x10^{-10} M) anti-proliferative response to D3 and EB1089 was seen, but 9-cisRA (1x10^{-10} M) increased [3H]-thyminidine uptake by 40%. Co-treatment with D3 & 9-cisRA resulted in a dose dependent blockade of the anti-proliferative D3 response. 24-hydroxylase mRNA was induced maximally (100%) by 1x10^{-8} M D3, 9-cisRA caused minor induction (5%) & co-treatment with D3 & 9-cisRA resulted in synthetic induction of gene expression. 24-hydroxylase mRNA responses were confirmed by enzyme activity assays. In Caco-2 cells a dose dependent (1x10^{-9} to 1x10^{-11} M) anti-proliferative response to D3, EB1089 & 9-cisRA was seen. Co-treatment with D3 & 9-cisRA resulted in an additive anti-proliferative effect. 24-hydroxylase mRNA was induced only 10% of the levels in HT-29 cells after 48hr treatment with D3, 1x10^{-8} M 9-cisRA caused no induction of the 24-hydroxylase gene when used either alone or as co-treatment with D3. Enzyme activity studies confirmed these responses. These data demonstrate complex cell specific interactions between 9-cisRA and D3 in HT-29 & Caco-2 cells. We propose that colonic tumour cell responses to D3 are variable & dependent on the specific target gene activated, relative D3 & 9-cisRA concentrations & the availability of VDR and RXR.

BODY COMPOSITION AT THE BEDSIDE: S. Ghosh, S.J. Cowen, W.J. Hannan, A. Ferguson. Department of Medicine & Department of Medical Physics, University of Edinburgh.

Analysis of body composition provides measurements of lean body mass (LBM) and body fat but is infrequently used in clinical practice as trained personnel and often sophisticated equipment are required. Bioelectrical analysis (BIA) has been recently used for its measurement but trained Medical Physics personnel and dedicated software are required. User-friendly BIA machines are popular in sports medicine and nutrition/clinics, but have yet to find a place in clinical practice.

We have evaluated a hand-held, inexpensive, menu-driven BIA machine which can be operated by untrained personnel in a clinical setting.

114 subjects were recruited including (a) 58 (24M/34F) healthy teenage volunteers (median age 15y); (b) 14 (9M/5F) healthy volunteers (median age 52y); (c) 17 (10F) patients with eating disorders (median age 26y); (d) 7 (3M/4F) patients with chronic pancreatitis (median age 47y); (e) 18 (15M/3F) patients with EBD (median age 39y). Bioelectrical Impedance was measured both by the hand-held machine (Bodytlink 1500, Bodylink Ltd, Isle of Man, UK) and the standard in-house machine (R.L. Systems Inc, model 101, Detroit, MI, USA). LBM was also measured by dual energy X-ray absorptiometry (DEXA) using a Hologic QDR-1000W scanner (Hologic Inc, Waltham, MA, USA) which is an accurate method of estimating LBM.

The hand-held machine could be operated by untrained personnel and gave results quicker than the in-house BIA or DEXA. Both resistance and impedance measured by the in-house BIA correlated very well with impedance measured by the hand-held BIA (r=0.9690±0.0001). The in subjects of different age groups. Specific prediction equations derived for estimating disorder patients or EBD patients, when applied to the impedance obtained by the hand-held machine, actually worsened the correlation with LBM measured by DEXA. Percent LBM measured by the hand-held BIA did not correlate very well with body mass index (r=0.065) or percent ideal body weight (r=0.26). Percent LBM was significantly greater in men than in women (p<0.001).

The hand-held BIA machine which can be operated by untrained personnel measures LBM as accurately as in-lab, sophisticated, expensive systems. Important information on body composition can readily be obtained in a clinical setting.


Reports of manganese (Mn) toxicity in patients on total parenteral nutrition (TPN) have prompted the commercial withdrawal of at least one trace element preparation. Parkinsonism, psychosis, and cholestasis in infants are described, with resolution on removal of Mn from TPN solutions. We measured Mn levels in patients on home parenteral nutrition (HPN) and looked for evidence of Mn toxicity. Methods: Serum and packed red cell Mn were measured in 30 adult patients on HPN. Red cell Mn was corrected for haematocrit to obtain a value for whole blood manganese. Serum liver function tests were also measured.

Patients: Mean duration of HPN was 4 years (range 3 months to 14 years), and proportion of nutritional requirements received parenterally varied widely. Patients received a median of 0.375 of Mn in each feed. The recommended daily Mn requirement is 17 umol.

Results: 25 patients had an elevated whole blood Mn (Normal < 210 nmol/l), of whom 7 patients had levels within the toxic range (> 360 nmol/l). Serum Mn levels were concordant. Liver function tests were normal in 12 patients. There were mild elevations of alkaline phosphatase and gamma glutamyl transferase in 11 patients, and levels more than twice the upper limit of normal in 7 patients. These levels were stable and not associated with clinical evidence of cholestatic liver disease. There was no correlation between blood manganese levels and cholestasis. No patient had extrapyramidal signs or parkinsonism.

Conclusion: Clinically relevant cholestatic liver disease is uncommon in adult patients on long-term home parenteral nutrition. Despite conservative Mn administration, toxic blood manganese levels were frequently observed but were unassociated with increased incidence of cholestasis or extra pyramidal disorders.
GLYCERYL TRINITRATE (GTN) DOES NOT REDUCE THROMBOPHLEBITIS DURING FULL INTRAVENOUS NUTRITION THROUGH A PERIPHERAL VEIN.

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IS DIARRHOEA COMPLICATING INTRAGASTRIC ENTERAL FEEDING CAUSED BY AN ABNORMAL PEPTIDE Y RESPONSE?

T. E. Bowling, S R Bloom, D B A Silk. Department of Gastroenterology, Central Middlesex Hospital, London, UK.

Diarrhoea is a common complication of enteral feeding. In recent in vivo human studies we have demonstrated a secretion of water and electrolytes in the ascending colon during intragastric but not intraduodenal enteral feeding. The cause of this secretion is likely to be neurohumoral in origin.

In vivo segmental colonic perfusion studies were undertaken in 24 healthy volunteers using an established technique, during which either a low load (1.39 kcal/min; 8.75 mgN/min) or a high load (4.2 kcal/min; 26.1 mgN/min) polymeric enteral diet was infused intragastrically or intraduodenally (n=6 each group). Before and at hourly intervals during these studies serum was taken for estimations of neurotensin (NT), pancreatic glucagon (PG), Peptide YY and VIP.

Ausc col water flow PYY (pmol/l) 
(P(m/hr;Median,range) (Median,range)

Fasting Feeding Fasting Feeding

Gastric +56 -174 13.3 15.0 NS
low load (6-72) -130 -30 (7-21) (11-18)
Gastric +120 -126 13.0 14.6 NS
high load (96-150) -250 +60 (7-19) (6-24)
Duodenal +40 +30 10.0 21.7 <.05
low load (50-132) -12 +125 (6-14) (17-34)
Duodenal +90 -72 12.5 44.1 <.05
high load (50-132) -150 =6 (17-19) (27-74)

* = net absorption — = net secretion

There were no changes in NT, PG or VIP levels either between fasting and feeding, or between the gastric and duodenal groups.

PYY is known to stimulate intestinal absorption. The absence of a rise during intragastric feeding may be important in the underlying mechanisms of enteral feeding induced colonic secretion and hence enteral feeding related diarrhoea.

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DID THE ADDITION OF GLUTAMINE TO INTRAVENOUS NUTRITION (IVN) PREVENT IMMUNOSUPPRESSION FOLLOWING MAJOR SURGERY?

J. May, W32

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W34

LECTINS WHICH BIND N-ACETYLGLALACTOSAMINE STIMULATE CHOLECYSTOKININ (CCK) RELEASE AND PANCREATIC PROTEIN SECRETION IN RATS. Jordinson M, Calan J, Royal Postgraduate Medical School, London W12 0NN

Lectins are present in the normal diet and bind to specific carbohydrate motifs. We found that soybean lectin(SBL) strongly releases CCK, and now ask which other dietary lectins have this effect. SBL and peanut(PNL) lectin bind to motifs including N-acetylgalactosaminogalactosamine (galNAc). Wheatgerm lectin(WGL) binds to N-acetylglucosamine, whereas the broad bean lectin(BBL) binds to manose and glucose residues. SBL, 84g strongly stimulated CCK release when given with soybean trypsin inhibitor (SBTI); 0.16mg and protein in the form of cooked soya flour (CSF); 44mg.

METHODS Rats were anaesthetised with halothane. 15 min collections of bile/pancatic were returned to the duodenum after retaining 20 ml for protein assay. Lectins, SBTI and CSF were instilled into the duodenum in the above amounts. Results are expressed as 1 h integrated protein response. Jugular vein blood was collected for CCK radioimmunooassay.

RESULTS protein response CCK(pmol/l)

(ng/h) before after

CSF 0.2±0.3 0.5±0.1 0.3±0.1
CSF+SBTI 0.7±0.2 0.4±0.01 0.8±0.3
CSF+SBL+PNL 2.0±0.2* 0.4±0.1 9.8±1.0*
CSF+SBL+WGL 0.6±0.1 0.3±0.1 0.7±0.2
CSF+SBL+BBL 0.4±0.4 0.2±0.2 0.3±0.1

Mean±SEM, * indicates P<0.05

CSF had no effect but CSF+SBTI slightly elevated CCK release and pancreatic secretion. PNL strongly stimulated CCK release and pancreatic secretion. PNL had the same effect as SBL which also binds to galNAc. WGL and BBL had no effect.

CONCLUSION: Only the lectins which bind to galNAc release CCK. Intrestingly lectins which bind this motif also have the greatest effect on intestinal cells. Lectin which bind galNAc might cause disturbances of organs such as the pancreas which are stimulated by CCK.

The high rate of glutamine utilisation by lymphocytes and macrophages in the small bowel suggests that glutamine provision may be important for the maintenance of immune function, particularly when the body is stressed after major surgery.

A prospective randomised study was performed in 18 patients undergoing major resections for gastrointestinal malignancy. IVN was commenced on the day of surgery and continued for a mean of 9 days post-operatively. Patients were randomised to receive either glutamine-supplemented IVN, or a standard preparation with equivalent protein content (0.2g/kg). Lymphocyte subset numbers and natural killer (NK) cell cytolytic activity were measured before operation, and on post-operative days 2, 7 and 14.

Results: When the results at day 2 were compared to pre-operative levels, there was no significant difference in NK cell function or lymphocyte subsets between the two groups at 2, 7 and 14 days. However, there was significant depression in both total lymphocyte count and subsets CD3, CD19 and CD8 (all p<0.05) in the group receiving standard IVN, which was not observed in the group receiving glutamine supplemented IVN.

Conclusion: This study suggests that there may be an important role of glutamine in the preservation of immune function in the first 48 hours following major surgery.

Patients were well matched in terms of age and sex, and side and site of vein. There was no significant difference in the incidence, or the median onset of IVR between the two groups.

This study suggests that the GTN patch may be of little benefit in the prophylaxis of IVR during full IVN through a peripheral vein.

Lectins which bind N-acetylgalactosamine stimulate cholecystokinin (CCK) release and pancreatic protein secretion in rats. Jordinson M, Calan J, Royal Postgraduate Medical School, London W12 0NN

Lectins are present in the normal diet and bind to specific carbohydrate motifs. We found that soybean lectin(SBL) strongly releases CCK, and now ask which other dietary lectins have this effect. SBL and peanut(PNL) lectin bind to motifs including N-acetylglactosaminogalactosamine (galNAc). Wheatgerm lectin(WGL) binds to N-acetylglucosamine, whereas the broad bean lectin(BBL) binds to manose and glucose residues. SBL, 84g strongly stimulated CCK release when given with soybean trypsin inhibitor (SBTI); 0.16mg and protein in the form of cooked soya flour (CSF); 44mg.
THE INFLUENCE OF NON-DIGESTIBLE CARBOHYDRATES ON ENTEROGLUCAGON SECRETION AND MUCOSAL CELL PROLIFERATION IN THE RAT. JM Gee, W Lee-Flingas, GW Wortley and IT Johnson, Institute of Food Research, Norwich Laboratory, Norwich Research Park, Colney, Norwich, NR4 7UA.

Enteroglucagon (EG) is derived from proglucagon and secreted by the L-cells of the distal small intestine and colon. High plasma levels of EG occur in experimental and disease conditions associated with small intestinal mucosal hyperplasia, which implies that it may function as a trophic hormone for the proximal gut. Certain types of dietary fibre increase both plasma EG and crypt cell proliferation in the rat, but the mechanisms are unknown. We investigated the relative importance of the solubility, viscosity and fermentability of non-absorbed carbohydrates as determinants of plasma EG and mucosal cell proliferation in the distal ileum of rats.

Rats were fed semi-synthetic diets containing insoluble, non-fermentable cellulose (controls) or one of three test carbohydrates with defined properties. Guar gum, which is soluble, viscous and highly fermentable, caused a sustained rise in plasma EG (2-3 fold higher than controls; p < 0.001). The hypothesis that this was due to delayed nutrient absorption was disproved by the fact that under the same conditions carboxymethylcellulose, which is a viscous but non-fermentable cellulose gum, caused no increase in EG. In contrast lactulose, which is non-viscous, unabsorbed and fermentable, caused a rise in plasma EG similar to that due to guar. Both mitosis and crypt length were increased in the distal ileum of rats fed guar gum, but not in those fed lactulose, despite their high level of EG. We conclude that EG is released by fermentation of non-absorbed carbohydrates. The probable source is crypt L cells rather than those of the small intestine. The absence of crypt cell hyperproliferation in rats fed lactulose indicates that high plasma EG does not necessarily cause increased mitosis in the small intestine.

This work was supported by the Ministry of Agriculture, Fisheries and Food.

H pylori USES UREA FOR AMINO ACID SYNTHESIS AT NEUTRAL pH.

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H pylori has the highest urease activity of all known bacteria. Its enzymatic production of ammonia protects the organism from acid damage by gastric juice. We have investigated the possibility that the urease activity may also allow the bacteria to utilise urea as a nitrogen source for the synthesis of amino acids.

An NCTC strain of H pylori (11038) was incubated for 5 min at 37°C with 50 mM urea enriched with 5 atom % 15N urea in the presence of either 0.9% NaCl pH 6.5 or 0.2 M citrate pH 6. The suspensions were then centrifuged and washed twice to remove any 15N absorbed to the surface of the bacteria. E coli NCTC 9001 was used as a urease negative control. 15N enrichment of the washed organism was detected by isotope ratio mass spectrometry. H pylori showed intracellular incorporation of 15N in the presence of pH 6 citrate buffer. There was no significant incorporation of 15N by H pylori in unbuffered saline or by E Coli in either pH 6 citrate buffer or unbuffered saline.

Further studies were undertaken to determine the intracellular fate of the urea - nitrogen by means of gas chromatography/mass spectrometry analysis. These were performed following incubation with 15N enriched 5mM urea in the presence of either 0.2 M citrate buffer pH 6 or 0.2 M acetate buffer pH 5. Following 5 min incubation with either buffer there was 15N enrichment of glutamate, glutamine, phenylalanine, aspartate and alanine.

Conclusions At pH and urea concentrations typical of the gastric mucosal surface H pylori utilises exogenous urea as a nitrogen source for amino acid synthesis. The ammonia produced by H pylori urease activity thus facilitates the organism’s nitrogen metabolism at neutral pH as well as protecting it from acid damage at low pH.

COLONIC ELECTROGENIC K+ HYPERSECRETION INDUCED BY STARVATION: PUTATIVE INVOLVEMENT OF BASOLATERAL K+ CONDUCTANCE. A Brunsden & R.J. Levin, Department of Biomedical Science, University of Sheffield, Sheffield S10 2TN.

Starvation in humans is associated with diarrhoea of unknown aetiology. In distal colon removed from starved rats cholinergic stimulation activates in vitro Cl secretion compared to fed controls (Nzegwu & Levin, 1990, Proc. Nutr. Soc. 49, 178A). As secretagogue-induced basolateral K+ conductance may be involved in maintenance of Cl secretion we examined possible changes. Colon segments were removed from anaesthetised fed and 3 day-starved (at) rats and incubated as muscle-stripped sheets in Ussing-type chambers. Electrogenic Cl- secretion activated by 1 mM serosal betahexanol (Beth), a cholinergic agonist, was monitored as the short-circuit current (Isc in μamps/cm2) using bicarbonate saline. The K+ conductance of the basolateral membranes was assessed as the Isc generated across the colons by an imposed K+ concentration difference (mucosal fluid 149 mM K+, serosal 5.9 mM K+, all Cl-replaced with gluconate) after pre-treatment with mucosal amphotericin (40μM). No significant difference was observed in the basal Isc in bicarbonate saline between fed and starved colons. Beth caused a significant (p<0.05) hypersecretion in bicarbonate saline but only in the starved distal colon (Iscmax, fed, 82 ± 15 (6) v starved, 146 ± 10 (6), Mean ± S.E., (no. of rats)). The ‘basal’ K+ conductances of the starved proximal and distal colons were not significantly different from the fed but Beth caused larger significant (p<0.01) maximal increases of the K+ Isc in the starved distal colons (st, 25 ± 3 (5) v fed, 10 ± 2 (8)) not observed in the starved proximal colons (st, 59 ± 7 (7) v fed, 72 ± 11 (7)). The duration of Beth’s action was significantly less in starved proximal (fed, 22 ± 4 min v st, 9 ± 1 min, p<0.01) but not in the distal colons (fed, 4 ± 1 min v st, 6 ± 2 min). These basolateral K+ changes may be significant factors involved in the Cl hypersecretion of the distal starved colon.

EFFECT OF HELICOBACTER PYLORI ON GASTRIC MUCOSAL AMMONIUM CONCENTRATIONS.

S J Middleton, J Calam, S F Moss, M Shorthouse, J O Hunter, Department of Gastroenterology, Addenbrooke’s Hospital, Cambridge CB2 2QQ, and Hamersham Hospital, London W12.

H pylori (Hp) gastritis is associated with hypergastrinaemia. This may be explained by elevated juxta-mucosal pH and reduced somatostatin brake on G cell activity. The ammonium concentration in the gastric lumen from patients with Hp is elevated which may be a consequence of Hp urease activity. However, to cause an alteration in G or D cell activity, mucosal ammonium concentrations should also be increased. The aim of this study was to measure mucosal ammonium and urea concentrations.

Snap-frozen gastric antral biopsies from 12 patients with duodenal ulceration were taken before and after eradication of Hp infection and thus healing of duodenal ulceration. Biopsies were homogenised and cytosolic supernatants obtained. The concentrations of urea ammonium and 34 aminoacids were measured by Pharmacia Cambridge UK using an aminoacid analyser (LKB Biochem 20). Urea and ammonium concentrations were expressed as a percentage of the total aminoacid concentration.

Mucosal urea and ammonium concentrations were respectively 0.7 ± 0.5% and 10.6 ± 3.2% before, and 1.2 ± 0.4% and 7.7 ± 2.3% after eradication of Hp.

The rise in urea and fall in ammonium concentrations on eradication of Hp was significant in both cases. (p <0.05, Wilcoxon Signed Rank test). The only amino-acid whose concentration changed was aspartate, which fell after eradication.

This study demonstrates that central mucosal ammonium concentration is increased by Hp infection and supports the role of ammonium in the associated tissue damage and hormonal changes.
IS GASTRIC MUCOSAL NITRIC OXIDE SYNTHASE ACTIVITY INCREASED IN PATIENTS WITH H. PYLORI ASSOCIATED DUODENAL ULCERATION? S.J. Middlxgen, S.F. Mouw, M. Shorthouse, J. Calan, J. O. Hunter. Department of Gastroenterology, Addenbrooke's Hospital, Cambridge CB2 2QQ, and Department of Gastroenterology, Hammeon Medical, London W12

The mechanism by which H. pylori causes duodenal ulceration is uncertain, but it has been suggested that nitric oxide (NO) has a pathogenic role. Nitric oxide synthase (NOS) catalyses the conversion of arginine to equimolar quantities of NO and citrulline. Gastric mucosal NOS activity was investigated in patients with H. pylori-associated gastritis and duodenal ulceration. Antral mucosal biopsies were taken from 12 patients before and after successful eradication of H. pylori and thus healing of duodenal ulceration. Total NOS activity in cytocytic supernatants of biopsies was estimated from the inhibitory effect of monomethyl-L-arginine (L-NMMA) on the conversion of ^4C-arginine to ^4C-citrulline in the presence of cofactors NADPH and tetrahydroporphyrin. In the absence of calcium this procedure gave an estimate of the inducible enzyme activity. Measurement of ^4C-citrulline was initially performed with Dowex AG 50W-X8 columns. However, analysis of the column eluate by thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) demonstrated the presence of several other substances carrying the ^4C label including ornithine and arginine succinate. Subsequent experiments were therefore carried out using TLC. In no in citrulline production is detectable using this method, and further experiments in which mucosal citrulline concentrations were measured using an amino acid analyser (LKB Biochem 20, by Pharmacia, Cambridge, UK) also failed to demonstrate significant alteration in concentrations before and after eradication of H. pylori (mean ± SD 0.12 ± 0.08% and 0.08 ± 0.08% of total amino acids respectively). Both these methods have demonstrated increased NOS activity in ulcerative colitis. Antral mucosal NOS activity is not significantly increased in H. pylori-associated gastritis.

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Recent in vitro and laboratory animal studies have suggested that copper and zinc have an anti-inflammatory effect while iron has a pro-inflammatory effect. The local effect of H. pylori on these trace metals has not been studied probably because of major limitations of traditional techniques. We aim at assessing gastric levels of copper, zinc, and iron in H. pylori gastritis using a novel method capable of detecting atomic and cellular levels of the above trace metals. Patients and methods: Eight well-nourished patients, median age 52 years, were studied. Gastric antral biopsies were taken at base-line and 6 weeks after completing a 2 week course of triple therapy. EELS relies on the following principle: the amount of electronic energy lost (AE) from a standard beam directed at the electron shells of an atom is specific to each element. Ultrathin sections (10/biopsy) were examined using a Zeiss TEM 902 energy filtering electron microscope set up for elemental EELS of nitrogen (AE=405 eV), iron (AE=808 eV) and copper (AE=931 eV), and zinc (AE=1020 eV). Spectra were used to determine these elements' ratios to nitrogen within the mucous layer and the cytoplasm of the epithelial and endothelial cells. Results: the median copper/nitrogen ratio within the epithelial cells rose from 0.01 in the presence of H. pylori to 0.15 after the eradication of H. pylori (p<0.07 within the endothelial cells (p<0.001). However, the same ratio fell from 0.12 to 0 within the mucous layer (p<0.001). The changes in iron or zinc were not significant. Conclusions: H. pylori gastritis is associated with a relative deficiency in local gastric epithelial and endothelial copper which probably results from loss of this metal into the adjacent mucus layer where H. pylori lives. These changes are relevant to our understanding of H. pylori pathogenesis and are correctable by its eradication.

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Background: Gastric mucosal hydrophobicity protects against acid/peptic attack. It is reduced in peptic ulcer or gastritis associated with H. pylori and reverts to normal with eradication of the infection. Increased antral infection density is associated with development of duodenal ulceration. Our hypothesis is that this is because H. pylori (which produces phospholipase A2) degrades phospholipid in the mucus layer, thus reducing hydrophobicity. Aim: To investigate the relationship between mucosal hydrophobicity, mucosal phospholipids and infection density of H. pylori.

Methods: 12 H. pylori positive (mean age: 43yr, range 27-70, m: 5.5) and 12 H. pylori negative (mean age: 48yr, range 25-64, m: 5.7) dyspeptic patients attending for endoscopy were recruited. At endoscopy 5 antral biopsies were taken; 1 for ClO test, 2 for a plate counting assay using electronic microscopy; 1 for histological assessment of H. pylori infection density (graded 0-3). Antral surface mucous was obtained using a cytology brush and phospholipids were extracted ( Folch) after homogenisation; separated on TLC and measured. Bicarbonate ratio of phospholipothiololylcholine/phospholipidoline (pm/pc) was taken as an index of phospholipolysis. Results: H. pylori positive patients had a mean (SE) contact angle of 48°.5 (± 2.5) compared with that of H. pylori negative patients of 60°.3 (± 2.5, p<0.005). In H. pylori positive patients there was a strong negative correlation between contact angle and H. pylori infection density in the surface mucus layer (R=0.72, p<0.02) but not with crypt infection density (R=0.26, NS). There was a trend for lower contact angles to correlate with higher lipicpc ratio but this was not statistically significant. Conclusions: Gastric mucosal hydrophobicity is confirmed as being reduced in patients who have H. pylori infection. The degree of reduction is related to the density of infection and tends also to be related to the degree of phospholipolysis in the mucus layer.

References:
4.Khulusi S et al Gastroenterology 1994;106(4);A106
W41

FREQUENCY OF ANTRAL INTESTINAL METAPLASIA AFTER H. PYLORI ERADICATION IN DUODENAL ULCER PATIENTS.

J L Wyatt, Histopathology Dept, St. James's Hospital, Leeds, LS9 7TF.

Eradication of H. pylori results in improvement of gastrointestinal gastritis, but it is unclear whether intestinal metaplasia is reversible in these circumstances.

301 patients with duodenal ulcer who were enrolled into two trials of Omeprazole 20mg qid (4 weeks) and clarithromycin 500mg tid (2 weeks) vs. Omeprazole 20mg qid (4 weeks) and clarithromycin placebo (2 weeks) had repeat endoscopy with biopsy 4-6 weeks post treatment; 227 were re-biopsied at 6 months post treatment and 64 at 12 months. H. pylori status was assessed by urea breath test, histology and bacteriology. The gastritis in paired antral biopsies from each of these endoscopies was graded by the Sydney System.

Intestinal metaplasia (IM) occurred usually as small isolated foci in 100/602 biopsies from 73/301 (24%) patients at initial endoscopy; its detection was very dependant on sampling error. The number of biopsies showing IM at follow-up is shown in the table.

<table>
<thead>
<tr>
<th>Time Post Treatment</th>
<th>6 Weeks</th>
<th>12 Weeks</th>
</tr>
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<tbody>
<tr>
<td>IM No IM</td>
<td>6/120</td>
<td>2/64</td>
</tr>
<tr>
<td>IM No IM</td>
<td>48/120</td>
<td>16/64</td>
</tr>
<tr>
<td>IM No IM</td>
<td>92/120</td>
<td>36/64</td>
</tr>
<tr>
<td>P&lt;0.001</td>
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</table>

There was no trend for the grade of IM to decrease in patients rendered Hp negative.

We conclude that focal IM is relatively common in the antrum of DU patients; this large study showed a trend towards reduction in IM post treatment, but this was not statistically significant.

W42

RELATIONSHIP BETWEEN H. PYLORI INFECTION, INFLAMMATION AND DUODENAL GASTRIC METAPLASIA.

S. Khalili, S Badve, P Patel, AG Lim, R Lloyd, MA Mandal.

*Histopathology, St George's Hospital Medical School, London, UK.

Introduction: Gastric metaplasia (GM) is present in the duodenal bulb of up to two thirds of dyspeptic subjects. Its prevalence is not influenced by H. pylori status, but its extent is greater in those who are positive suggesting that H. pylori may be responsible for extending pre-existing GM. The extent of GM in the duodenum and the density of H. pylori infection in the gastric antrum are greater in duodenal ulcer (DU) than non-DU subjects. We aimed to study, in DU and non-DU subjects, the relationship between extent of GM and a) H. pylori density and b) the severity of duodenitis and of gastritis.

Method: 127 H. pylori positive subjects, 97 with DU and 30 with non-ulcer dyspepsia, were studied. Three duodenal bulb biopsies were obtained per patient. Biopsy sections were stained with diastase PAS/Alcan blue and the extent of gastric type mucosa in each biopsy section was determined as a percentage of the entire sections epithelial lining. The severity of duodenal inflammation was assessed in the same sections, and graded on a scale of 0-3. Two gastric antral biopsies were assessed for the severity of inflammation as well as the density of H. pylori colonisation, and also graded 0-3. For all histological parameters, the mean score of the biopsies obtained from the same area in the same subject was used.

Results: In DU compared to non-DU subjects, there was a significantly greater mean extent of GM (38.3 vs 6.6, p<0.001), as well as a greater mean score of antral H. pylori density (8.9 x 0.9, p<0.001). Severity of antral (1.7 vs 0.7, p<0.001) and of duodenal inflammation (1.8 vs 0.5, p<0.001) were also greater in DU. The extent of GM in the duodenal bulb was also associated with the severity of both duodenitis (R2=0.84, p<0.001) and gastritis (R2=0.54, p<0.001) as well as the density of H. pylori colonisation in the gastric antrum (R2=0.58, p<0.001).

Conclusion: This study shows that duodenal and gastric inflammation as well as the density of H. pylori colonisation in the gastric antrum are related to the extent of GM in the duodenal bulb, providing further evidence that H. pylori plays a part in the pathogenesis of GM, possibly through greater colonisation of the duodenum and more severe duodenitis.

W43

THE EFFECT OF HELICOBACTER PYLORI ERADICATION ON GASTRIC METAPLASIA IN THE DUODENAL BULB.


Parkside Helicobacter Study Group, Central Middlesex and St Mary's Hospitals, London, UK.

Duodenal gastric metaplasia (DGM) is induced in at least part by gastric acid, other factors are ill-defined. DGM has been reported as more frequent and extensive in the presence of H. pylori infection. This study investigated the effect of Hp eradication on DGM is unclear. Previous studies may be inaccurate due to sampling and observer variations as biopsies were taken from a single location in the duodenal bulb and only semi-quantitative methods used to assess DGM. We took multiple duodenal bulb biopsies and used a quantitative method to examine the effect of Hp eradication on DGM.

We studied 10 Hp-ve healthy controls (4 men, mean age 33, range 24-40) and 10 Hp-ve DU (7 men, mean age 37, range 22-58). We restudied 8 of them (6 men, mean age 39, range 24-58) six months after Hp eradication. Hp status was determined by histology, antral, and duodenal bulb biopsies routinely processed and stained with H & E and Gimenez, microscobic culture of antral and duodenal biopsies in selective and non-selective media at 37°C for up to 10 days, and by the 14C-urea breath test (European Standard Protocol, positive if excess 14CO2 excretion > 5 per ml). The quadrants of the duodenal bulb were biopsied >1cm from the edge of any ulcer. The biopsies were stained with PAS to assess DGM. Presence and extent of DGM was determined by a single blinded observer using a computer-enhanced image intensifier (Searson Imaging, Cambridge). Extent of DGM was calculated as a percentage of the duodenal epithelial surface of each biopsy.

Prevalence of DGM was significantly higher (p<0.05, Mann Whitney test) in Hp-ve patients with DU (9/10) than in the Hp-ve controls (5/10). The prevalence of DGM (68%) was not altered significantly by Hp eradication. The extent of the DGM was significantly greater (p<0.05) in Hp-ve patients with DU (median 38, range 0-54) than in the Hp-ve controls (median 1%, range 0-33). Six months after Hp eradication the extent of DGM (median 20%, range 0-74) had not changed significantly (p>0.05, Wilcoxon signed rank test).

DGM is indeed more common and greater in extent in Hp-ve patients with DU than Hp-ve controls, but neither its prevalence nor its extent changed significantly six months after Hp eradication. Factors determining the continued presence of DGM need to be elucidated.

AWH is supported by a grant from Loderle Laboratories, UK.

W44

H PYLORI-INDUCED DISTURBANCE OF GASTRIC ACID SECRETION IS UNRELATED TO BACTERIAL CAGA STATUS.


University Department of Medicine & Therapeutics, Western Inflammatory, Glasgow, Division of Medicine, University of Leeds, Leeds, IRCIS, Siena, Italy.

Subjects with H. pylori who develop duodenal ulceration (DU) have a markedly increased acid secretory response to gastric releasing peptide (GRP) which results from eradication of the infection. Subjects with H. pylori who develop DU are also more likely to have bacterial strains which are CagA positive and consequently show a serological response to the bacterial protein. We have investigated whether the disturbance in gastric secretory function is related to the bacterial strain.

Patients and Methods: Sixty seven subjects with non-ulcer dyspepsia (NUD) were examined. They had suffered from dyspepsia for > 6 months and no microscopic lesion had been found despite at least two upper GI investigations. All were positive for H. pylori by 14C-ura breath test, and histology and CLO test of endoscopic antral biopsies. Any medication was discontinued at least 2 weeks prior to the study. Twenty five age and sex matched controls without H. pylori infection were also examined. In all subjects acid output was measured in response to GRP (40 mmol/kg/h) administered i.v. for 60 mins. In those with H. pylori, CagA status was determined indirectly by assaying serum CagA IgG antibodies by ELISA using a purified recombinant fragment of CagA.

Results: The median acid output to GRP in the 67 NUD subjects with H. pylori was 18 mmol/h (range 0-54) which was higher than that in the pylori negative subjects (8.1 mmol/h) of the 67 H pylori positive NUD subjects were CagA seropositive and their GRP stimulated acid output (median 19, range 54) was similar to that in those who were CagA seronegative (17.5, 1-32).

Conclusions: These results indicate that the disturbance of gastric acid secretion associated with H. pylori is independent of bacterial CagA status. It must be related to host or other bacterial factors.
**Helicobacter pylori (clinical) W45-W51**

**EFFECT OF H PYLORI ERADICATION ON THE SEVERITY OF DYSPEPTIC SYMPTOMS IN DU PATIENTS: A ONE YEAR FOLLOW UP STUDY.**

E. B. Omar, A. Wizk, K. E. L. McColl, University Department of Medicine and Therapeutics, Western Infirmary, Glasgow, Scotland.

Eradication of H pylori markedly reduces the DU relapse rate. However, very little data is available regarding the effect of eradication on the severity of dyspeptic symptoms in DU patients. We have recently developed and validated a new tool for the global measurement of dyspepsia (The Glasgow Dyspepsia Severity Score). This tool scores the severity of dyspeptic symptoms on the basis of: frequency and severity of symptoms, frequency of medical consultation and investigations, time off work, and usage of prescribable and off-the-counter medication. Each category if scored on a sliding scale giving a combined maximum possible score of 33. We used this tool to study the symptomatic response in DU patients receiving eradication therapy.

**Subjects and Methods:** Thirty one endoscopically proven DU patients with H pylori had their dyspeptic symptoms scored before and at one year following eradication therapy consisting of two weeks treatment with Triprostatin (120 mg t.i.d.), Metronidazole 400 mg t.i.d. and Amoxicillin 500 mg t.i.d. The DU dyspeptic scores were compared with those from eighty age and sex matched healthy subjects selected randomly from the same catchment area. None of the healthy subjects had sought medical advice for dyspepsia though 28 (35%) were positive for H pylori infection. H pylori status of all subjects was determined by the 

**Results:** The mean dyspeptic score in the 31 DU patients before eradication therapy was 12.7 (range: 7-17) compared to 1.7 (range: 0-8) in the 80 healthy subjects. In 28 DU patients the infection was successfully eradicated and their mean dyspeptic score fell to 1.8 (range: 0-9) which was equivalent to that in the healthy subjects. In the three DU subjects in whom the infection was not eradicated their dyspeptic scores were similar before (9,11,11) and one year after receiving the therapy (10,11,10 respectively).

**Conclusions:** Eradication of H pylori markedly reduces dyspeptic symptoms in DU patients, restoring their severity and frequency to that of the general population.

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**IS ABSENCE OF DYSPEPTIC SYMPTOMS A USEFUL TEST TO ASSESS H. PYLORI ERADICATION?**

**D. Halliday, AB Price, MR Jacyns, Dept. of Gastroenterology, Histopathology & Nutrition Research, Northwick Park Hospital, Harrow, England.**

One of the issues limiting the more widespread use of H. pylori eradication therapy is wether all patients should have a test performed to confirm successful eradication. Many of the tests are expensive and therefore invasive, time-consuming and expensive. Widespread use of non-invasive tests such as urea breath tests is limited by availability and/or expense. The aim of this study was to assess the usefulness of the absence of dyspeptic symptoms as a test for H. pylori eradication.

**Methods:** Data on all H. pylori positive patients with duodenal ulcer disease who were treated with eradication therapy at this institution between 1991-1994 was reviewed. H. pylori status was confirmed by histology and/or 14C-urea breath test (UBT) prior to treatment. Patients were reviewed at 1 and 6 months after completion of the treatment course when UBT was performed and data collected on dyspeptic symptoms using the Gastrointestinal Symptom Rating Scale (GSRS).

**Results:** 112 patients had undergone eradication therapy. 80(71.4%) successfully eradicated H. pylori as defined by a negative UBT.

**DYSPEPTIC SYMPTOMS ERADICATED NOT ERADICATED **n=80 n=32

<table>
<thead>
<tr>
<th></th>
<th>Absent</th>
<th>Present</th>
<th>Absent</th>
<th>Present</th>
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</thead>
<tbody>
<tr>
<td>1 month:</td>
<td>70 (87.5%)</td>
<td>14 (43.7%)</td>
<td>10 (12.5%)</td>
<td>18 (56.3%)</td>
</tr>
<tr>
<td>6 months:</td>
<td>78 (97.5%)</td>
<td>3 (9.4%)</td>
<td>2 (2.5%)</td>
<td>29 (90.6%)</td>
</tr>
</tbody>
</table>

Using the absence of dyspeptic symptoms as a test for confirming successful H. pylori eradication, the sensitivity was 87.5% at 1 month & 97.5% at 6 months, with the corresponding figures for the specificity being 56.3% & 90.6%.

**Conclusion:** In duodenal ulcer patients the absence of dyspeptic symptoms may be a useful and cheap alternative to conventional tests for assessing H. pylori eradication.

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**CHILDHOOD DOMESTIC HYGIENE, HELICOBACTER PYLORI INFECTION AND INFLAMMATORY BOWEL DISEASE: IS THERE AN ASSOCIATION?**

**A.E. DAGNIS, I. USMANI, K.R. NEAL, R.F.A. LOGAN, University Dept. of Public Health & Epidemiology and Division of Gastroenterology, University Hospital, Nottingham, NG2 2UH, UK.**

It has been suggested that improvements in domestic hygiene in childhood with resulting delayed exposure to enteric infection could account for the emergence of IBD in developed countries. Gent et al. (Lancet 1994;343:766-7) found that Cronin's disease (CD) was more common in subjects whose first home had a hot water tap and a separate bathroom. Helicobacter pylori (Hp) infection has also been related to childhood hygiene and could be a marker of childhood hygiene. We have therefore enquired about childhood domestic conditions and Hp status in a case-control study of 213 clinic attended with UC, 110 with CD and 334 age and sex frequency matched controls having elective 'repair' surgery. Data was collected using a self-completed questionnaire and Hp status was assessed by an ELISA for Anti-H pylori IgG (Helico-G,Porton, Cambridge).

74% of CD patients reported always having had hot water at home before age 11 compared with 63% UC and 61% of controls (Odds ratio (OR) for hot water always v.less often for CD 1.81 (95% confidence limit 1.1-3.0) for UC OR 1.1 (0.7-1.5). This relationship was unaffected by controlling for age, sex, and other domestic conditions using unconditional logistic regression. There was no association with having an Ulcerative colitis (UC) history in the subject or their parents. There was a significant difference in Hp seropositivity between either IBD group and controls (35% UC, 33% CD, 36% controls seropositive for Hp). However logistic regression showed frequent sharing of a bed before age 11 was associated with being Hp seropositive while living in a house with a bath was protective. Sulphasalazine usage was not associated with lower Hp positivity.

The greater availability of hot water in childhood in CD confirms the earlier report and supports the hypothesis that improvements in domestic hygiene in childhood are involved in the development of CD.

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**Association of Helicobacter pylori infection with coronary heart disease (CHD) in the CHILDHOOD DOMESTIC HYGIENE and HELICOBACTER IS THERE AN ASSOCIATION?**

**K.R. NEAL, R.F.A. LOGAN, University Dept. of Public Health & Epidemiology and Division of Gastroenterology, University Hospital, Nottingham, NG2 2UH, UK.**

Background: A preliminary case-control study has suggested that H. pylori infection may be associated with risk of CHD in adults, but the results may have been biased due to case ascertainment and confounding from socioeconomic factors. We postulated that a possible mechanism linking H. pylori infection with CHD could be through the effects of increased inflammatory factors (fibrogen, white cell count and sialic acid) related to CHD. Aim: to investigate the relationship between seropositivity to H. pylori and (1) objective evidence of CHD; (2) conventional cardiovascular risk factors and risk factors related to inflammation; and (3) to assess whether these relationships are independent of confounding factors. **Methods:** A population based random sample of 388 white caucasian men, aged 50-69, were recruited into a cross-sectional study. The presence of CHD was assessed by electrocardiogram (ECC). Serum levels of antibodies to H. pylori were measured, levels of risk factors determined and a questionnaire administered. **Results:** 47 subjects had evidence of CHD. Odds ratio for H. pylori seropositivity and abnormal ECC was 3.82 (95% CI 1.60-9.10) p<0.002, after adjustment for a range of socioeconomic indicators (father's and own social class, own housing tenure) and risk factors for both H. pylori (age, childhood smoking and Helicobacter pylori infection) and CHD having smoking, history of hypertension, diabetes and hyperlipidaemia. Conventional cardiovascular risk factors, cholesterol, triglycerides and lipoprotein (a), was related to H. pylori seropositivity. The adjusted mean differences (± 95% CI) in fibrinogen and log. white cell count between H. pylori positive and negative patients were 0.18 (±0.14) g/l, p=0.05, and 0.12 (±0.08) log(10), p<0.01, respectively. **Conclusion:** H pylori infection is associated with CHD independently of a wide range of confounding factors examined in this study. A possible mechanism may include increases in risk factor levels due to chronic active inflammation in the stomach.
IS HELICOBACTER PYLORI INFECTION OF ANY SIGNIFICANCE IN THE AETIOLOGY OF PRIMARY GASTRIC LYMPHOMA? - A PROSPECTIVE STUDY.

Karat D, O' Hanlon DM, Raines S, Scott D*, Griffin SM. Depts of Surgical Gastroenterology and Pathology*, Newcastle General Hospital, Newcastle-upon-Tyne.

Helicobacter pylori (HP) is implicated in the aetiology of peptic ulcer disease and has been suggested as a risk factor for gastric adenocarcinoma. Recent studies have proposed a role for HP in the aetiology of primary gastric lymphoma (PGL). This study prospectively assessed all patients presenting over a 30 month period (from Jan 1992) with PGL to determine the presence of HP in patients with PGL.

Specimens were assessed for HP using 3 different techniques, the CLO test (urease test) on antral biopsy specimens, serology (ELISA technique) and histological analysis of antral biopsy and tumour biopsy specimens. Twelve cases of PGL were diagnosed (6% of all gastric malignancies), 8 were male and the mean(SEM) age of patients was 61.7(3.6) years. Six patients had no evidence of HP on any investigation, 4 were HP positive using all 3 techniques, 1 patient had 1 test positive and 1 patient had 2 tests positive. Results as number (%); see table.

<table>
<thead>
<tr>
<th>HP negative</th>
<th>HP positive</th>
<th>Significance</th>
</tr>
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<tbody>
<tr>
<td>Number</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low grade</td>
<td>3 (50%)</td>
<td>5 (83%)</td>
</tr>
<tr>
<td>Invasion of serosa</td>
<td>2 (33%)</td>
<td>2 (33%)</td>
</tr>
<tr>
<td>LN positive</td>
<td>2 (33%)</td>
<td>3 (50%)</td>
</tr>
<tr>
<td>MALT</td>
<td>1 (17%)</td>
<td>3 (50%)</td>
</tr>
</tbody>
</table>

Statistics: Mann-Whitney U, Fisher Exact test, Significance at P < 0.05.

Of note only 6 (50%) patients were HP positive (5 (40%) if histology alone is considered) which is similar to levels found in age-sex matched patients with peptic disease. HP was associated with MALT lesions and lower grade tumours but no significant differences were observed.

In conclusion this study fails to support a primary aetiological role for helicobacter pylori in the genesis of primary gastric lymphoma, although an adenovate role in MALT and low grade tumours can not be excluded.

CagA EXPRESSION IS RELATED TO THE SEVERITY OF PREMALIGNANT HISTOLOGICAL CHANGE IN THE STOMACH. SE Paxton, PH Katerzlis, L Lin, M Wilks, ZW Zhang, P Domizio, S Tabachchali, MGY Farthing. Digestive Diseases Research Centre, Depts of Microbiology and Histopathology, Medical College of St Bartholomew’s Hospital, London, UK.

Approximately 60-70% of Helicobacter pylori (H. pylori) strains express the CagA antigen and infection with CagA positive strains has been shown to be important in the development of peptic ulcer disease. H. pylori infection is also causally related to the development of gastric adenocarcinoma and recent data suggest that strains expressing the CagA antigen may further enhance this cancer risk. The aim of this study was to examine the relationship between expression of the CagA antigen detected serologically and the presence and severity of premalignant histological changes in the stomach.

94 consecutive patients (mean age 54, range 23-78), attending for upper gastro-intestinal endoscopy were studied. 4 antral biopsies were obtained from each patient for culture, gram stain and histological examination. All histology was examined ‘blind’ by a single pathologist and activity, severity, atrophy and intestinal metaplasia were graded according to the Sydney system. CagA was detected serologically both by ELISA using a recombinant CagA fusion protein as antigen and by Western blot analysis. Overall, 62 patients (71%) were found to be CagA positive. 19 patients had completely normal antral histology, 38 had chronic active gastritis alone, 42 had gastritis with atrophy, and 16 showed evidence of intestinal metaplasia. CagA seropositivity rates were 26%, 52%, 90.4% and 100% respectively. Antibodies to CagA were not detected in only four patients with atrophy and in each case this was graded as mild. All 16 patients with intestinal metaplasia were positive for CagA.

H. pylori strains expressing the CagA antigen are closely associated with more severe premalignant histological changes in the stomach. These findings support epidemiological evidence relating CagA positivity to the development of gastric neoplasia.

THE INCIDENCE AND SEVERITY OF FOLLICULAR GASTRITIS IN CASES OF GASTRIC CARCINOMA.


Helicobacter pylori infection is the only known cause of lymphoid follicles in the basal gastric mucosa. The follicles have been reported to be present in between 27 and 100% of cases of H. pylori infection.

In a retrospective review of 77 cases of gastric carcinoma operated on between 1990 and 1994, we examined the non-malignant mucosa in uninvolved resection margins, and assessed the incidence, frequency and size of follicles. As a control group, we examined 12 gastric resection margins from pancreatocoduodenectomy specimens over the same period.

Of those with follicular gastritis, the mean number of follicles/mm in all the carcinoma cases was 0.32 compared to 0.23 in the pancreatic carcinoma group. With data from the operation notes and pathology reports it was possible, in 69 cases, to divide the gastric carcinomas into two groups, depending on the site of origin of the tumour. The cases with carcinoma arising in the cardia and gastro-oesophageal junction had a mean 0.25 follicles/mm compared to 0.32 follicles/mm in the cases arising in the body and antrum (significant at p<0.05 by pooled t-test). A similar relationship was found between the body/antrum group and the pancreatocoduodenectomy group (p<0.05). The relationships between the groups was similar when the size of the follicles was considered.

Our data has shown that in cases of carcinoma arising in the proximal stomach, the incidence and severity of the follicular gastritis is similar to a group with no gastric carcinoma. In contrast, cases with carcinoma in the body and antrum of the stomach, have a higher incidence of follicular gastritis of significantly greater intensity, suggesting that H. pylori infection may play a greater role in the development of carcinoma in the distal stomach.

Oesophagus W52–W60

MALLORY-WEISS REVISITED. AFRAZ J Masson PN Bramley G McKnight NAG Mowat. GI Unit, Aberdeen Royal Infirmary.

In western literature over the past 10 years, little has been written on the incidence and natural history of Mallory-Weiss tear.

Over a two year period there were 1098 admissions with confirmed upper GI bleeding, to a Bleeding Unit serving all of Grampian. One hundred and thirteen(10.3%) had an endoscopically proven diagnosis of Mallory-Weiss tear (12.9 per 100 000 per annum). The male/female ratio was 2.23/1, and 39.8% occurred during the 3rd and 4th decades. According to standard criteria, 19(16.8%) had significant bleeds requiring transfusion of up to 9 units of blood. One patient received injection therapy, 1 rebled and 1 man with severe coagulopathy due to alcoholic liver disease, died.

Fifty-five of 113 (48.7%) bleeds had an associated potential bleeding pathology at the time of endoscopy. These included; oesophagitis/oesophageal ulcer 23(20.4%), oesophageal varices 1(1.8%), gastritis erosions 16(13.2%), gastric ulcer 2(1.8%), duodenitis 4(3.5%), duodenal ulcer 6(5.3%). The Mallory-Weiss tear was of secondary importance with regard to the source of bleeding, in 14(12.4%) cases.

Of all patients with Mallory-Weiss tear, 37.8% had been drinking alcohol in the previous 24 hours, compared with 22.3% of all upper GI bleeds from other causes (p<0.002) and 53.6% were smokers compared with 35.1% of other bleeds (p<0.0003). There was no correlation between bleeding from Mallory-Weiss tear and aspirin/NSAID usage.

Liver function tests were deranged in 51.2% of bleeds due to Mallory-Weiss tear, compared with 36.1% of upper GI bleeds from other causes (p<0.04). Symptoms of gastro-oesophageal reflux were present in 59.5% of trivial and 62.5% severe bleeds due to MW tear.

This community based data suggests that Mallory-Weiss tear is an important cause of upper GI bleeding, particularly in the younger age group. It normally runs a benign course, although an important sub-group have significant bleeds. There appears to be an association with alcohol ingestion, smoking and deranged liver function.

We intend to perform a prospective study of possible aetiological factors in the more severe bleeds.
ANzelcHlK - THE FINAL Nail?
C Maxwell-Armstrong, RJc Steele, S Amar, C Robertson, D Morris, D Evans, JD Hardcastle. Dept of Surgery, University Hospital, Nottingham NG7 2UH

Despite a high complication rate, the use of the Angelchik prosthesis for gastro-oesophageal reflux is still a subject for debate. In our unit, 46 prostheses were inserted between 1982 and 1989 providing an unusually long follow up period of 7 to 14 years. During this time, 11 (24%) of the prostheses were removed, usually for dysphagia, at times varying from 1 to 109 months postoperatively (mean: 47 months). Seven of these patients had a fundoplication at the time of removal, and four had no other procedure.

In 1994, 36 of the patients were interviewed, and underwent a barium/marshmallow swallow. All had had a swallow in the first postoperative year. Of 26 of these patients who still had an Angelchik prosthesis, 1 had severe symptomatic reflux, and 4 required anti-reflux medication. Dysphagia was reported by 20 (77%), and this necessitated dietary restriction in 18 (69%). The marshmallow swallow time in the patients was significantly prolonged when compared with an age and sex matched control group (p<0.001), but paired analysis revealed no significant deterioration with time in the individual patients.

Insertion of an Angelchik prosthesis is associated with an unacceptable removal rate, and despite lack of objective evidence of worsening of dysphagia, this rate appears to be cumulative with time.

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IS LAPAROSCOPIC FUNDOPLICATION A MORE ATTRACTIVE OPTION THAN LONG-TERM OMEPRAZOLE IN GASTRO-OESOPHAGEAL REFLUX DISEASE?
A Watson, N Peck, N Callander
Department of Surgery, Royal North Shore Hospital, Sydney and The Wellington Hospital, London

Referral for anti-reflux surgery occurs in a small proportion of patients with GORD because of the perceived success of long-term Omeprazole, balanced against the invasiveness of surgery, hospitalisation, time off work and concern about its results. The emergence of laparoscopic fundoplication may influence that balance. Forty patients referred for laparoscopic fundoplication underwent symptomatic assessment, endoscopy, oesophageal manometry and 24 hr pH monitoring. Post-operative assessment was performed at a median of 5 months, and NHS cost compared with that of long-term Omeprazole 20mgs daily.

Sixteen patients (40%) were deemed not to have responded to Omeprazole and 13 (33%) expressed concern about prolonged medication. Fundoplication was completed laparoscopically in 36(90%). Median hospital stay was 3 days, and time off work 2 weeks. 34(94%) were symptom-free and all 14 patients undergoing repeat objective studies to date were restored to a physiological pH profile. The cost of fundoplication was £1700, compared to an annual cost of Omeprazole £436.

Failure to respond to Omeprazole and concern about long-term medication are not uncommon. Early assessment of laparoscopic fundoplication shows that it is safe and highly effective, and associated with a relatively short hospital stay and time off work. Its cost is equivalent to approximately 4 years of Omeprazole therapy. Providing durability is confirmed, laparoscopic fundoplication has several advantages over long-term Omeprazole.

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RANDOMISED CONTROLLED TRIAL OF NISSEN VERSUS LIND FUNDOPLICATION: RESULTS AT TEN YEAR FOLLOW-UP. ST Baxter, SJ Walker, R Sutton. Department of Surgery, University of Liverpool, L69 3BX.

Studies have demonstrated that Nissen (NF) and Lind (LF) fundoplication are equally effective when assessed in the early postoperative period. Long term results were assessed in this study. Twenty one patients (NF: n=11, LF: n=10), of an original total of 53, were available for follow-up at a median of 10 years (range 8.5-12.5) and were assessed by symptom score, pH monitoring and oesophageal manometry. (Statistics: Wilcoxon signed rank test).

At early assessment (median 13 months, range 5-39) both operations produced significant improvements in symptoms (LF and NF: p<0.01) and pH results (LF and NF: p<0.01). At late follow-up, symptoms (LF and NF: p<0.01), and pH results (LF: p<0.01) were still better than before surgery but the improvement in pH results in the NF group was no longer significant. Between early and late assessments symptoms deteriorated slightly in both groups (p>0.05), pH results also deteriorated but this was only significant in the NF group (p<0.025).

<table>
<thead>
<tr>
<th>Total % time pH&lt;4.0:</th>
<th>Visick heartburn score</th>
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<tbody>
<tr>
<td>median &amp; (range)</td>
<td>median, (mean) &amp; (range)</td>
</tr>
<tr>
<td>PRE</td>
<td>EARLY</td>
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<td>15.8</td>
<td>0.9</td>
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<tr>
<td>(5-50)</td>
<td>(0-46)</td>
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<td>NF</td>
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<td>12.6</td>
<td>0.25</td>
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<td>(8-31)</td>
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This suggests that both operations produce continued relief of symptoms at ten years, but there is a trend towards deterioration with both. Although there is a significant deterioration in pH results in the NF group, there is no firm evidence for the superiority of either procedure.

Causes Of Failure To Respond To Omeprazole In 44 Patients With Gastro-Oesophageal Reflux Symptoms.
SD Singh, J Wang, A Anggianah, WA Owen, AR Jones, JW Owen. Department of Surgery, Guy’s Hospital, London.

Omeprazole is a strong inhibitor of gastric acid secretion and has been used in the treatment of gastro-oesophageal reflux disease. However, some patients continue to have symptoms in spite of omeprazole therapy.

Forty-four patients (17 male, 27 female) with symptoms of gastro-oesophageal reflux which did not respond to omeprazole therapy, with persistent heartburn, regurgitation, chest and epigastric pain, dysphagia and globus, were studied. Age group 27-73 years (mean 39 years), duration of symptoms 1-13 years (mean 3 years), period of treatment 1-12 months (mean 5.3 months), daily doses 20-80 mg of omeprazole (20mg 17/44, 40 mg 21/44, 80mg 6/44).

All patients underwent 24 hour simultaneous oesophageal and gastric pH monitoring. Oesophageal pH was recorded at 5 cm above the lower oesophageal sphincter as determined manometrically. Gastric pH was monitored at 15 cm below the proximal pH sensor.

Twenty-one (47.7%) patients had pathological acid reflux, 14 (31.8%) patients had an alkaline shift in the stomach (as defined by gastric pH>4 and >10% in supine position), 9 (20.5%) patients were normal. Of the 21 pathological refluxers, 10 (47.6%) patients had hiatus hernia and 8(38.1%) patients had oesophagitis. Six (15.6%) patients had both pathological acid reflux in the oesophagus and alkaline shift in the stomach. Forty (90.9%) patients recorded normal oesophageal motility where as 4 (9.1%) patients had motility disorders. Thirty-three (75%) patients had low lower oesophageal sphincter pressure (<8mm Hg), where as 1 (2.2%) patient had high lower oesophageal sphincter pressure (>15mm Hg).

Failure of omeprazole therapy to relieve symptoms of gastro-oesophageal reflux may be due to 1. inadequate acid suppression as 48% of patients had proven pathological acid reflux. 2. Alkaline gastric shift. 3. Lower oesophageal sphincter pressure. 4. Symptoms in absence of reflux suggests a psychological cause.
THREE YEAR FOLLOW-UP OF OESOPHAGITIS WITH ENDOSCOPY AND OESOPHAGEAL PH MONITORING. 
NL McDermott, SW McKelvey, CL Bevan, AJG Love. 
Dept. of Medicine, Queen’s University Belfast, Royal Victoria Hospital, Belfast, and Ulster Hospital Dundenald, Northern Ireland.

Aims: Data on long term natural history of reflux oesophagitis (RO) are sparse. This study was designed to determine the changes in grade of oesophagitis and ambulatory oesophageal pH monitoring (amb. pH) 3 to 4 years after initial diagnosis of RO.

Method: Twenty-five patients (20 male) with mean age 53 yrs (range 24-78) in whom grade II-III oesophagitis was diagnosed and treated in one centre were followed-up after a mean of 38 months (range 34-46) with repeat upper GI endoscopy, amb. pH (off acid suppression) and symptomatic questionnaire.

Results:Twenty-three patients had repeat investigations; 3 asymptomatic patients refused follow-up. Repeat endoscopy (18 patients) showed that 3 patients (12% of original 25) had developed Barrett’s oesophagus in spite of maintenance acid suppression therapy (AST). Five patients still had grade II RO (none of whom were on a proton pump inhibitor (PPI)) and 10 patients had a normal OGD (7 of whom were on a PPI, 1 on an H-2 receptor antagonist (H2RA)).

Initial amb. pH 3 years ago was abnormal in 21 (84%) patients and normal in four. Nineteen patients had repeat amb. pH, the mean score of % time pH<4 +/-SEM improving from 15.6+/-2.48% three years ago to 12.1+/-1.95%. In spite of an apparent trend for % time pH<4 to improve, the difference was not significant using Wilcoxon matched pairs signed rank test. Two patients changed from normal to abnormal amb. pH, 3 changed from abnormal to normal and the remainder (14) were abnormal on both occasions.

Before repeat investigation, 15 (60%) patients were on AST. After repeat investigation 4 changed from an H2RA to a PPI due to endoscopy findings, and a further 5 (20%) required commencement of AST for RO.

Conclusions: The majority of oesophagitis patients still have abnormal amb. pH 3 yrs after initial diagnosis and 80% still require AST (mostly PPI). Repeat endoscopy showed Barrett’s mucosa had developed in at least 12%.

OESOPHAGEAL WALL TENSION DETERMINES SENSITIVITY IN PRIMARY OESOPHAGEAL MOTILITY DISORDERS

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University of Manchester Department of Surgery, Hope Hospital, Salford, U.K.

Introduction: The natural history of primary oesophageal motility disorders is unknown.

Aim: To assess which oesophageal function tests identify the clinical progress of patients.

Method: 80 patients underwent conventional oesophageal manometry. 63 also had intraoesophageal balloon distension (IOBD) and 45 had traction force (TF) studies with a balloon and force transducer. Clinical assessment two years later with questionnaire.

Results: 8 achalasia, 10 diffuse oesophageal spasm, 5 nutcracker oesophagus, 22 nonspecific motility disorders and 35 normal. Only patients with achalasia were significantly better following treatment. Patients presenting with dysphagia fared worse than those without dysphagia. Patients with a mean amplitude of oesophageal contractions in the distal oesophagus (MDA) less than 30mmHg or a traction force to IOBD or wet swallows less than 15g were significantly worse 2 years later. Manometric responses to IOBD did not predict progress nor did any other feature on conventional manometry.

Conclusion: Apart from achalasia the manometric diagnosis or response to IOBD does not predict prognosis. However, poor oesophageal contractility is associated with progressively severe symptoms despite treatment.
LONG TERM RESULTS OF TOTAL PELVIC FLOOR REPAIR FOR NEUROTOPHIC FAECAL INCONTINENCE
S. Korzen, K. Deen, M.R.B. Keighley, University Department of Surgery, Queen Elizabeth Hospital, Birmingham

The results of surgery for neuropathic faecal incontinence are variable. From 1988 to 1993 we performed a combination of postanal repair, anterior levatorplasty and anterior sphincter plication (total pelvic floor repair, TPFNR) on 75 women with neuropathic faecal incontinence excluding sphincter repairs. 6 required either a colostomy or a graciloplasty for persistent incontinence. All were invited for anorectal physiology and were sent a questionnaire assessing their continence and satisfaction with the procedure. 57 of 69 returned the questionnaire (83%) and 39 (57%) identified bowel control and anorectal physiology. Analysis is based on the 57 returned questionnaires.

Preoperatively, 55 women had at least weekly incontinence. Although 43 were improved postoperatively (75%), 37% still had weekly incontinence, 49% occasional accidents and 14% were continent.

None of the standard anorectal parameters changed postoperatively apart from a slight fall in resting and squeeze pressures, and none had predictive value regarding outcome. Obesity and straining were associated with poorer outcome, but perineal descent or difficult deliveries were not. Despite these results, 42 (74%) reported satisfaction with the procedure. Although TPFNR can offer improvement in 75% of women, it restores complete continence in only a minority of cases. Apart from obesity and a habit of straining we were unable to identify predictive factors regarding outcome.

Magnetic Resonance Imaging of the Anal Sphincter using an Endoanal coil
NM de Sousa*, WA Kmin*, R Puni*, CI Bartram**, GM Bydder*
From: *The Robert Steiner Magnetic Resonance Unit and the Departments of **Radiology and *Surgery, Royal Postgraduate Medical School, Hammersmuth Hospital, Du Cane Road, London W12 0HS.

The magnetic resonance imaging (MRI) appearances of the anal sphincter were demonstrated using a receiver coil within the anal canal and these data were compared with clinical and surgical findings where available.

Six normal volunteers, (3 male and 3 female ages 31 to 77 years, mean 53.3 years) and 14 patients (6 male and 8 female ages 26 to 72 years, mean 46 years) with a variety of sphincter pathology were imaged. Four had idiopathic fistula-in-ano, 3 had perianal Crohn’s disease, 3 had idiopathic faecal incontinence, 3 had previous obstetric trauma and 1 had previous surgical trauma and anal stenosis. A cylindrical receiver coil 50mm long and 9mm in diameter was placed internally across the sphincter. T1 and T2 weighted axial and T1 weighted coronal images were obtained. In addition, Gadolinium-DTPA 0.1ml/kg was administered in 7 patients.

The external anal sphincter was identified from its annular subcutaneous portion to the levator ani. In females, the superficial component of the external sphincter appeared deficient anteriorly between 11 and 1 o’clock and this gap was bridged by the transverse perineal muscle. The crescentic internal anal sphincter and the longitudinal muscle of the rectum were easily distinguished. In 3 patients with suspected perianal infection, abscesses were correctly identified and confirmed at surgery. One patient with anal stenosis showed marked compensatory hypertrophy of the internal sphincter. Two patients with previous obstetric trauma had external sphincter tears identified prior to surgical repair. Two patients with idiopathic faecal incontinence had marked external sphincter atrophy while a third had a small anterior rectocele. MRI using a dedicated anal coil provides high resolution detailed images of sphincter pathology and has considerable diagnostic potential.

SEX DIFFERENCES IN THE HEALTH LOCUS OF CONTROL AND LEARNED ILLNESS BEHAVIOUR IN PATIENTS WITH THE IRITABLE BOWEL SYNDROME (IBS) AND INFLAMMATORY BOWEL DISEASE (IBD).

Irritable bowel syndrome is associated with psychiatric illness, neuroticism and environmental stress, but causality remains unclear.

Aim:
To examine for predisposing psychosocial factors in patients with IBS and IBD.

Method:
42 outpatients with IBS and 44 with IBD were studied by questionnaire. Comparisons were made between self-report measures of psychological distress and health locus of control, illness behaviour and vicarious learning and operant conditioning of illness behaviour.

Results:
1. IBS was associated with greater psychological disturbance than IBD, particularly in men (p<0.01).
2. As expected, women with IBS viewed their health as controlled by external chance factors to a greater extent than women with IBD, whilst interestingly, for men, the opposite effect was observed (p<0.01).
3. IBS patients reported greater severity of gastrointestinal symptoms (p<0.001) and more medical consultations in the previous six months (p<0.05) than patients with IBD. On a third measure of illness behaviour, men with IBS reported markedly greater perceived impact of gastrointestinal symptoms than did men with IBD, whereas for women the trend was in the opposite direction (p<0.01).
4. Surprisingly, the IBD group reported greater parental reinforcement of illness behaviour associated with gastrointestinal disturbance than the IBS group (p<0.05), but on measures of modelling of illness behaviour or reinforcement of non-gastrointestinal ill health, there were no group differences.

Conclusion:
Sex differences, especially viewed health control by external chance factors and perceived impact of gastrointestinal symptoms, may play a significant role in the symptomatology of IBS and IBD.

PATHOPHYSIOLOGICAL ASPECTS AND CLINICAL OUTCOME OF INTRA-ANAL APPLICATION OF ISOSORBIDE-DINITRATE IN PATIENTS WITH CHRONIC ANAL FISSURE W.R. Schouten, J.W. Briel, M.O. Boerma and J.J.A. Auwerda, University Hospital Dijkzigt, Rotterdam, The Netherlands.

Recently we have demonstrated that local ischemia, due to increased activity of the internal anal sphincter (IAS), is a major contributing factor in the pathogenesis of anal fissure. Relaxation of the IAS can be achieved by local application of exogenous nitric oxide donors, such as isosorbide-dinitrate (ISDN). Aim of this study was to assess, if the local application of 1% ISDN-ointment on anal pressure, anodermal bloodflow and fissure healing. Sixteen consecutive patients (male/female: 10/6; median age: 35; range: 18-51) with a chronic anal fissure were studied. Before treatment and at 3 and 6 weeks all patients underwent conventional manometry and laser Doppler flowmetry of the anoderm. All patients experienced mild, transient headache during the first 2 days. Within 10 days the fissure related pain was resolved in all patients. At 6, 9 and 12 weeks anal fissure was completely healed in respectively 9, 11 and 15 patients. Prolonged manometry, started directly after ISDN application, showed that the maximal anal resting pressure (MAP) decreased within 5 minutes (median pressure drop: 50%; median duration: 39 min). This pressure reduction was the short-term effect of ISDN itself. At 3 and 6 weeks manometry was performed at least one hour after the last ISDN application. These recordings revealed a reduction of MARP (mean values: pre: 116±36 mm Hg; 3 weeks: 87±18; 6 weeks: 97±30, p<0.03, paired t-test). This pressure reduction represents the long-term effect of ISDN, caused by its active metabolite isosorbide-mono-nitrate. At the same time anodermal bloodflow showed a significant increase (0.59±0.17 V; 3 weeks: 0.88±0.17 V; p<0.001). Conclusion: local application of ISDN reduces anal pressure and improves anodermal bloodflow. This dual effect results in a healing rate of 94% at 12 weeks.
PREGNANCY IS ASSOCIATED WITH SUBSEQUENT REDUCED RECTAL MAXIMAL THRESHOLD PRESSURE. R. Farouk and G.S. Duthie: Academic Surgical Unit, Castle Hill Hospital, Hull HU16 1NU.

Pregnancy has previously been shown to affect pelvic floor function and anal sphincter activity. Little is known about rectal function after childbirth however. Rectal function in 11 pririparous women (age 26-37 years) prior to delivery, in the immediate postpartum period, and six months after birth using the proctometergram.

The proctometergram was measured by placing a pressure transducer in the mid-rectum and inflating a latex balloon with degassed water (temperature 37°C; rate 1 ml./second). The volumes required to produce first rectal sensation (ml.), maximum tolerated volume (ml.), and maximum tolerated rectal pressure (cm.H2O) were recorded.

The median volume to produce first rectal sensation was not significantly affected by pregnancy (ANTEPARTUM = 32.5 ml.; POSTPARTUM (1 month) = 28 ml.; POSTPARTUM (6 months) = 26 ml.). The maximum tolerated volume was also similarly unaffected (ANTEPARTUM = 104 ml.; POSTPARTUM (3 months) = 146 ml.; POSTPARTUM (6 months) = 151 ml.). The maximum tolerated rectal pressure was reduced however after delivery (ANTEPARTUM = 41 cm.H2O; POSTPARTUM (1 month) = 35 cm.H2O (p < 0.05); POSTPARTUM (6 months) = 29 cm.H2O (p < 0.03).

Normal vaginal delivery is associated with a significantly higher intra-rectal pressure at the maximum tolerated volume. This may contribute to the feeling of faecal urgency experienced in up to 25% of such women.


While anal endosonography has been shown to reliably identify anal sphincter defects, its direct comparison with conventional needle EMG is rare but was the aim of the present study. Methods: In 23 consecutive patients (47.2 ± 25.6 years, 20 females) with defaecatory problems referred for needle EMG, anal endosonography by means of a 7.5 MHz 355° rectal scanner (Kretz, Gelsenkirchen, Germany) was performed prior to EMG, if sphincter defects were found they were described in terms of location and extend (0 to 12 h in knee-elbow-position) but not given to the neurologist performing EMG (HLJVG). Conventional concentric needle EMG of the external anal sphincter was done circumferentially in 1-hour-steps to locate muscle parts exhibiting signs of denervation/reenervation.

They were described in analog to sonography. Results: Of 23 patients referred for EMG, 8 had no EMG abnormalities; in none of these cases, sonography showed any defect. In the remaining 15 cases, EMG (n. Fig 1 forground circles) and sonography agreed in all but one case (No.9).

Thus, specificity was 100% and sensitivity was 94%. Correlation between EMG and sonographic locations of defects was r = 0.92, p < 0.001. In some cases, however, either technique may show a larger area affected. Conclusion: While EMG is the current gold standard for sphincter mapping, anal endosonography may become the technique of choice in the future. (Supported by DFG grant EN 59/10).

OTHER POSSIBLE ROLE OF CELL ADHESION MOLECULES IN TUMOUR GROWTH AND METASTASIS. Atta M Abbasi, Ian C Talbot*, Alastair Forbes. St Mark's Hospital, and *ICRF Colorectal Cancer Unit, City Road, London, EC1V 2PS.

Abnormal cell adhesion has been proposed as a critical event in neoplasia, but most investigation has been of experimental systems. Cell adhesion molecules (CAMs) representative of the 3 principal surfaces of the normal colorectum have therefore been studied in human colorectal carcinoma. CEA is normally expressed at the apical (luminal) surface, E-cadherin at the lateral surface (adherens junction), and CD44 at the cell base.

Immuno-fluorescence and western blotting were performed on frozen sections from 3 polyps, 29 carcinomas (25 well to moderately, 4 poorly-differentiated), biopsies from normal colon, and on 2 colonic cell lines: LIM 1863 (which forms glandular structures in vitro) and HT 29 (which grows as a monolayer).

There was apical staining for CEA in normal colon, in the polyps, in 20 of the differentiated carcinomas, and in LIM 1863 cells. Basal staining was observed in the other 5 differentiated tumours. Uniform cytoplasmic staining was observed in the poorly differentiated tumours, and in HT29 cells. E-cadherin was expressed at lateral cell borders in normal colon, polyps, differentiated tumours and LIM 1863 cells, and was not detected in poorly differentiated tumours or HT29 cells. There was strong cytoplasmic staining for CD44 in normal colon, polyps, tumours, and cell lines, corresponding to areas of cell proliferation as supported by staining for Ki-67 (a well recognised marker for proliferation) in adjacent sections.

The present results, combined with prior evidence of the importance of down regulation of E-cadherin in emigration of cells from tumours, of CD44 and CEA in protection of cells in circulation, and of CEA in implantation, promote a hypothesis for colorectal metastasis in which the 3 CAMs have a crucial role.

FREQUENCY AND TYPE OF TRANSMISSION OF HEREDITARY NON POLYPOSIS COLORECTAL CANCER. P. Benatti, C. Scapoli, L. Roncucci, A. Percepepe, M. Ponz de Leon. Dept. of Internal Medicine, University of Modena, Via del Pozzo, 71, 41100 Modena, Italy.

Recent clinical and biomolecular studies estimated that the susceptibility gene for HNPCC, one of the most common cancer predisposing syndromes, is carried by as many as one in 200 individuals in Western countries. Affected individuals develop tumors of the colon (mainly proximal), endometrium, stomach, ovary and other organs, often before 50 years of age. Previous studies carried out by means of segregation analysis also support the hypothesis of a major gene predisposing to colorectal cancer, inherited in an autosomal dominant fashion. In 1984 a Colorectal Cancer Registry was instituted in our District with the aim to evaluate hereditary factors contributing to the pathogenesis of colorectal cancer. In the present study, our purpose was twofold: 1) to establish the frequency of HNPCC through a population-based approach, 2) to assess the genetic pattern of transmission by complex segregation analyses.

RESULTS: Using clinical criteria, each suggestive of an increased susceptibility to hereditary cancer, we identified 32 unrelated families affected by HNPCC, for an overall frequency of 3.9% of all cases. Segregation analysis, by means of POINTER programme - testing whether the distribution of a complex phenotype is the consequence of a major locus or multifactorial inheritance - suggested the presence of a major gene, with an estimated frequency of 0.0044 and a lifetime penetrance between 72 and 77%, transmitted autosomally with intermediate dominance (additive transmission). This type of transmission is further supported by the results of the COMDS analysis programme, fitting an oligogenic model from which the presence of at least a second gene with modifying effect is indicated. CONCLUSIONS: 1) the frequency of the disease is in the order of 4% of all colorectal malignancies, 2) the results of segregation analyses are in accordance with a two-loci model, suggesting that at least two genes might be involved in the pathogenesis of HNPCC.
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INHIBITION OF GROWTH OF HUMAN COLORECTAL CANCER BY GASTRIN RECEPTOR ANTAGONISM AND THE SIGNIFICANCE OF GASTRIN RECEPTOR EXPRESSION. R.J.C. Steele, T. Clifford, E. Robinson, S. Watson, Departments of Surgery and Pathology, University of Nottingham, UK

Previous studies have shown that gastrin is a potent growth factor for colorectal cancers and high affinity gastrin receptors have been measured on the plasma membranes of tumour cells derived from human colorectal adenocarcinomas. The aim of this study was to determine the effect of the gastrin receptor antagonist, CR2093 on basal and gastrin-stimulated growth of short term in vitro cultures of primary human colorectal adenocarcinomas and to relate this to gastrin receptor expression.

Tumour cells were derived from surgical specimens by enzymatic dissociation and grown on matrices of type 1 collagen and irradiated fibroblasts. Cell proliferation was assessed by [3H]-thymidine uptake. Gastrin receptor expression was determined by an immunocytochemical technique using the mouse mononclonal antibody directed against the gastrin receptor, 2C1. Staining was detected with an avidin-biotin method.

Increased growth in the presence of gastrin-17 (1x10^-10 and 5x10^-9M) was shown in 16/54 (47%) tumours. The gastrin receptor antagonist significantly reversed this stimulated growth (p<0.05) in 13/16 (81%) of the gastrin-sensitive tumours. In addition, CR2093 inhibited the basal growth of 11/34 (32.4%) tumours and this inhibition could be reversed by gastrin-17, in the majority. All tumours which responded to gastrin expressed the gastrin receptor, but there was no correlation between the intensity of expression and the degree of response. In addition, tumours which did not show an in vitro response to the hormone were also receptor positive. However, there was a significant correlation between intensity of receptor expression and inhibition of basal growth by CR2093 (p<0.05, r=0.54).

Gastrin receptor expression in colorectal adenocarcinomas is widespread yet does not predict the sensitivity of tumours to the proliferative effects of gastrin. However, expression does predict sensitivity to the inhibitory effects of a gastrin receptor antagonist on basal growth. Gastrin receptor expression may be related to endogenous gastrin production by colorectal tumour cells.

W70

'FAILED' LATERAL INTERNAL ANAL SPHINCTEROTOMY FOR CHRONIC ANAL FISSURE: A STUDY USING EDDANOAL ULTRASOUND. R. Fazook, G. S. Duthie, Academic Surgical Unit, University of Hull, Castle Hill Hospital, Hull HU6 5JQ.

Lateral internal sphincterotomy has become the operation of choice for chronic anal fissure secondary to internal anal sphincter hyperkinesia in the number of patients, however, the fissure persists. We have assessed 7 patients 16 male; median age 43 (range 23-60)years with symptomatic fissures following previous attempted lateral internal sphincterotomy.

A 7 MHz. , 360° rotating ultrasound transducer was used to assess the anal sphincters of each patient. A recording was made of the examination using a thermal image printer. Each assessment was carried out with the patient in the left lateral position. No bowel preparation was required.

In two patients, a defect was recorded in the central third of the internal anal sphincter. All of the remaining patients did not have any evidence of a defect in the internal sphincter. In no case, there were defects in the external sphincter in the left lateral position which were consistent with the position of previous surgery.

Failed lateral internal sphincterotomy for chronic anal fissure is unusual. In such patients however, endoanal ultrasound has shown the amount of sphincterotomy performed to be inadequate, or for the external anal sphincter to have been divided instead.

W71

ANTINEUTROPHIL CYTOPLASMIC ANTIBODY TITRE BUT NOT IgG SUBCLASS DISTINGUISHES BETWEEN PRIMARY SCLEROSING CHOLANGITIS AND AUTOIMMUNE HEPATITIS. D.S. Bansi, RW Chapman, K.A. Fleming, Department of Gastroenterology and Nuffield Department of Pathology and Bacteriology, Oxford Radcliffe Hospital, Oxford, UK.

We determined the prevalence and specificity of antineutrophil cytoplasmic antibodies (ANCA) and their IgG subclass in chronic liver diseases. METHODS: 63 primary sclerosing cholangitis (PSC), 28 autoimmune hepatitis (AIH), 34 primary biliary cirrhosis, 12 alcoholic liver disease, 5 large duct obstruction, 4 haemachromatosis and 36 normals were tested for ANCA using the alkaline phosphatase technique. IgG subclass distribution of 33 PSC and 11 AIH was determined using monoclonal antibodies, HP 6001 for IgG1, HP 6002 for IgG2, HP 6050 for IgG3 and SK 44 for IgG4 (Sigma Immunochemicals).

RESULTS

<table>
<thead>
<tr>
<th>PSC(n=33)</th>
<th>AIH(n=28)</th>
<th>PBC(n=34)</th>
</tr>
</thead>
<tbody>
<tr>
<td>%ANCA at 1:5</td>
<td>65</td>
<td>50</td>
</tr>
<tr>
<td>%ANCA at 1:50</td>
<td>11</td>
<td>10</td>
</tr>
</tbody>
</table>

All other controls were ANCA negative at 1:5. The IgG subclass distribution was:

<table>
<thead>
<tr>
<th>IgG1</th>
<th>IgG2</th>
<th>IgG3</th>
<th>IgG4</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANCA(n=11)</td>
<td>82%</td>
<td>9%</td>
<td>27%</td>
</tr>
</tbody>
</table>

CONCLUSION: ANCA is specific to the autoimmune liver diseases. Titres were highest in PSC with a sensitivity of 98% and specificity of 91% at 1:50. The similar IgG subclass distribution of PSC and AIH ANCA may reflect similar mechanisms of immune regulation and an identical antigenic species.

W72

UPTAKE OF CHOLYL-LEUYL-FLUORESCENE BY ISOLATED HUMAN HEPATOCYTES. VA SARASWATI, CO MILLSA, KEEGH, ELIAS, LIVER RESEARCH LAB, QUEEN ELIZABETH HOSPITAL, BIRMINGHAM.

Cholyl-leucyl fluorescent (CLF) is the prototype of a family of fluorescent bile salts that retain many of the physiochemical properties of the parent bile acids. They have been used to study the uptake, transport, distribution and secretion of bile acids in isolated hepatocytes. We have used CLF in a number of patient livers and in the intact rat. CLF is a stable entity that accumulates in the liver by active, sodium-dependent process mediated by sodium cotransport proteins that have been characterized in detail. Hepatocytes isolated from tissue left after trimming donor livers used in 'reduced-size' transplantation in children were incubated with 5 to 200uM concentrations of CLF or C14 GC for 15sec to 30 min. at 37°C in Krebs-Henseleit buffer and uptake of bile acid measured by a fluorimeter or liquid scintillation counter. Parameters were calculated from duplicate readings using isolates from 4 donor organs. Viability of hepatocyte preparations ranged from 95 and 99% by Trypan blue exclusion. Uptake for both CLF and C14 GC plateaued by 30 sec with little change over 30 min. Peak uptake was seen at 20uM concentration with little further increase at higher concentrations up to 200uM. Hepatocyte affinity for both CLF and C14 GC was similar (Km 37.13 x 10^-5 M at 10^-5 M respectively) as was the maximal transport velocity (Vmax = 11.8 x 10^-5 mol/min/cell/ml resp. respectively). Coincubation of CLF with C14 GC at 20uM concentration resulted in 87% inhibition of uptake. We conclude that the uptake kinetics of CLF are very similar to the handling of C14 GC by normal human hepatocytes and the two molecules may possibly share the same transporter. CLF can be used to explore bile acid metabolism in health and in diverse liver diseases.
**W73**

**EXPRESSION OF ADHESION MOLECULES IN CHRONIC LIVER ALLOGRAFT REJECTION**

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1 University Department of Surgery, Liver Transplantation Unit and 2 Department of Histopathology, Royal Free Hospital School of Medicine, Pond Street, London NW3 2QG.

Chronic rejection following liver transplantation is a process characterised by a progressive loss of interlobular bile ducts and arteriopathy. Progressive bile duct loss has been referred to as the vanishing bile duct syndrome (VBDS) or ductopenia. The end result is graft failure, requiring retransplantation.

Adhesion molecules are involved in leukocyte-endothelial cell interaction. Expression of adhesion molecules in histologically proven VBDS may give further clues as to the mechanism of graft injury. Frozen sections from liver biopsies and explanted liver (total 6) were immunohistochemically analysed for expression of intercellular adhesion molecule-1 (ICAM-1), platelet and endothelial cell adhesion molecule (PECAM), E-Selectin and vascular cell adhesion molecule (VCAM). Sections were analysed for intensity of stain and distribution compared to stable grafts and normal liver.

ICAM-1 staining was strongly expressed on sinusoidal endothelium and was also associated with chronic inflammatory cells. Surviving bile ducts appeared to stain positively for ICAM-1. PECAM was present on all endothelial cells, as in normal liver. Expression of VCAM and PECAM on sinusoidal endothelium was increased markedly compared to stable graft and normal controls. E-selectin was only expressed on large vessel endothelial cells.

Expression of cytokine-inducible adhesion molecules may play an important role in the pathogenesis of chronic rejection. The accumulation of chronic inflammatory cells resulting in bile duct destruction may, in part, be determined by the presence of an adhesive sinusoidal bed, and persistence of expression of adhesion molecules, despite immunosuppression, may be detrimental to the graft and recipient.

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**W74**

**INDUCTION OF NITRIC OXIDE SYNTHASE IN THE HEPATIC ARTERY DURING HEPATIC FAILURE IN MAN**

NM Robinson, REA Smith, JR McPeake, JF Martin, R Williams, S Mondon. Dept of Medicine and Institute of Liver Studies, King's College School of Medicine and Dentistry, London SE5 9SR, UK.

Hepatic failure is characterised by arterial vasodilatation and reduced sensitivity to vasoconstrictors. Nitric Oxide (NO) synthesised in large quantities by inducible NO Synthase (iNOS) may be an important cause of the pathophysiological vasodilatation seen in high output cardiac states such as hepatic failure. This contrasts with the normal control of physiological vascular tone by a low output of NO from endothelial NOS (eNOS). Human hepatic artery was obtained from donor (n=7) and recipient (n=10) patients at the time of liver transplantation for hepatic failure associated with a low systemic vascular resistance. Arterial rings were removed and frozen at -70°C for molecular analysis. We detected mRNA iNOS and mRNA eNOS using the Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR). We designed novel oligonucleotide primers based on the published sequence of human iNOS and eNOS. DNA products from the RT-PCR were subsequently sequenced to confirm their identity and to exclude contamination. Messenger RNA iNOS was detected in recipient arteries only whereas mRNA eNOS was found in all arteries. We have previously shown a pharmacological resistance to phenylephrine in similar hepatic rings. This resistance was blocked by inhibitors of NOS. In conclusion, we have detected inducible NOS gene transcription in human systemic arteries from patients with hepatic failure. This expression of iNOS may account for the resistance to vasoconstriction seen in similar clinical syndromes in man.

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**W75**

**CYCLOSPORIN-A (CSA) DOES NOT INHIBIT TRANSCYTOTIC VESICULAR TRANSPORT: A MORPHOMETRIC ANALYSIS**

L. Lora, E. Mazza, C. Carletto, C. Milanesi, R. Niccazzo, D. Martines

Gastroenterology Department and Biology Department of Padova University - Italy.

Inhibition of hepatocytic vesicular transport, induced by CSA, has been observed in a bile-fantsia rat model. The aim of this study was to verify if the influence of CSA (1 mg) on transcytotic vesicular pathways in perfused rat liver. This was achieved measuring the biliary excretion of horseradish peroxidase (HRP) and examining HRP labeled vesicles in the perisinusoidal (PS) and pericanalicular (PC) areas, using ultrastructural morphometric analysis. Male Sprague Dawley rats were perfused with Krebs Henseleit buffer (albumin 1%, RIC 20% and aminoacid mixture). Taurodeoxycholate (1 μmol/ml) was infused into portal vein. 1 μmol of CSA, dissolved in Cremophor EL (CSA livers) or the vehicle alone (CEL livers) was added to the medium. Two perfusion protocols were used in the study. First, the pattern of HRP biliary excretion, HRP (25 mg) was given as a 1 hour pulse, under single pass conditions, after 30 min of recirculating perfusion. Bile samples were collected to measure the 24 hour output of HRP by HRP activity in bile duct bile. A second protocol was used, with bile duct bile collected to measure the pattern of HRP and HRP labeled vesicles, a 1 hour pulse of high dose of HRP (500 and 200 mg, respectively) was given. Two and 18 min after a single-pass perfusion the livers were fixed with 2.5% glutaraldehyde-0.8% paraformaldehyde in 0.1 M cacodylate buffer. The total pericanalicular area, the area and the number of HRP-containing structures were quantitated morphometrically in liver samples by analysing semi-quantitative electron micrographs obtained by a Zeiss 108 transmission electron microscope at 80 KV. RESULTS: The appearance of the second biliary HRP peak (transcytotic vesicular pathway) was observed at 15 min in CSA and also in CEL. Livers. The area under the second peak of biliary HRP excretion curve was similar in CSA and CEL livers (39.9±12.8 vs 38.6±15.9 mm expressed as Mean±SE of 5-6 observations). Morphometric analysis confirmed that CSA perfusion did not affect neither percent area (Tab) nor density (data not shown) of HRP labeled vesicles, both in pericanalicular (PC) and perisinusoidal (PS) area at 2 min (rapid pathway) as well as at 18 min (late pathway).

Transcytotic vesicular pathways:

- 2 min after 500-μg HRP
- 18 min after 200-μg HRP

<table>
<thead>
<tr>
<th>n = 3</th>
<th>PC area %</th>
<th>PS area %</th>
<th>PC area %</th>
<th>PS area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEL Livers</td>
<td>0.47 ± 0.35</td>
<td>0.59 ± 0.03</td>
<td>1.39 ± 0.21</td>
<td>0.09 ± 0.05</td>
</tr>
<tr>
<td>CSA Livers</td>
<td>0.34 ± 0.09</td>
<td>0.63 ± 0.16</td>
<td>1.27 ± 0.44</td>
<td>0.10 ± 0.05</td>
</tr>
</tbody>
</table>

CONCLUSIONS: These results indicate that CSA does not inhibit transcytotic vesicular pathways and therefore cholesterol is not related to transcellular vesicular transport alterations.

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**W76**

**PRODUCTION OF INTERFERON-γ BY LIVER AND PERIPHERAL BLOOD T-LYMPHOCYTES IN PRIMARY SCLEROSING CHOLANGITIS**

EB Martina, RW Chapman, KA Fleming. Dept. of Gastroenterology and Nuffield Dept. of Pathology, John Radcliffe Hospital, Oxford, OX3 9DU UK.

The genesis of primary sclerosing cholangitis (PSC) is unknown, but autoimmunity has been suggested. So far little is known about the cytokine profile in PSC, in particular the role of interferon-γ (IFN-γ), a cytokine of the Th1 type, which is involved in cell mediated immunity. Aim: To investigate the cytokine profile of liver derived and peripheral blood lymphocytes in PSC.

Materials and methods: 10 PSC were studied. Liver derived lymphocytes (LDL) were obtained by collagenase digestion of fresh liver biopsies. Peripheral blood lymphocytes (PBL) were obtained at the same time by gradient centrifugation. Cytokine production from individual cell level was assessed by the reverse haemolytic plaque assay. Cell suspensions were incubated on a monolayer of protein-A conjugated sheep erythrocytes with the appropriate polyclonal anti-cytokine serum (IFN-γ, TNF, IL-2 and IL-4). The reaction was developed using complement, with a ring of haemolysis formed around the cytokine secreting cells (CSC) whose phenotype was subsequently determined by immunocytochemistry. The area of haemolysis was measured to quantify the cytokine production.

Results: 6/10 PSC had detectable IFN-γ secretion by LDL, and 3 of these had detectable IFN-γ secretion by PBL. IFN-γ was secreted by PBL but not LDL in 1 patient. LDL secreted TNF-α in 1 patient and IL-4 in another, both also positive for IFN-γ. The cytokine production (assessed by haemolysis area) was higher in LDL than PBL (p=0.005). On immunocytochemistry the IFN-γ secreting cells were activated αβ T-lymphocytes (CD3+, Tcrb+, HLA-DR+, CD8+). Conclusion: In PSC, the secretion of the pro-inflammatory cytokine IFN-γ by activated liver and peripheral blood T-cells is up-regulated, suggesting a Th1 profile in this condition. Moreover the production of IFN-γ is higher by LDL than by PBL. These results confirm an important role of the LDL in the pathogenesis of PSC which may be in part mediated by IFN-γ.
ELEVATION OF IgA AND IgM RHEUMATOID FACTORS IN CHRONIC LIVER DISEASES

AC Douds, AG Lim, *D Rees, and JD Maxwell
Departments of Biochemical Medicine and *Rheumatology, St. George's Hospital Medical School, London SW17 ORE, England

Background A non-deforming polyarthropathy is known to occur in patients with primary biliary cirrhosis (PBC), autoimmune chronic active hepatitis (AIH), and chronic viral hepatitis B/C. Increased circulating immune complexes are a feature of these chronic liver diseases and it has been postulated that arthropathy may arise as a result of joint deposition of excess circulating immune complexes. In rheumatoid arthritis, immune complex joint deposition is enhanced in the presence of rheumatoid factor (RF), an antibody to the Fc portion of IgG. The aim of this study was to determine if RFs were present in chronic liver diseases and to determine their disease specificity.

Methods 44 patients with rheumatoid arthritis (RA), 19 with alcoholic liver disease (ALD), 9 with PBC, 12 with ‘other liver diseases’ (AIH, n=5, chronic viral hepatitis C, n=7) and 37 normals (age matched) were assayed for IgA and IgM RFs by an ELISA.

Results IgA and IgM RF were significantly elevated in all disease groups, p<0.05, compared to normal controls. Comparison of levels of IgA and IgM RF between disease groups showed significant elevation of IgM RF, p<0.05, ‘in other liver diseases’ versus ALD.

Conclusions IgA and IgM RF are elevated in a range of chronic liver diseases at levels comparable to those found in rheumatoid arthritis. IgA RF appears to be a non-specific marker of chronic liver disease whereas IgM RF elevation is more characteristic of liver diseases associated with arthropathy.

IMUNOGLYCOLIN G GLYOSYLATION PROFILE IN CHRONIC LIVER DISEASES

AC Douds, AG Lim, *A Bond, *G Hayes, JD Maxwell, and +FC Hay
Departments of Gastroenterology and +Immunology, St. George’s Hospital Medical School, London SW17 ORE, England

Background The carbohydrate moieties of glycoproteins are thought to play a fundamental role in cellular and protein function. When disease is present changes in glycan composition of glycoproteins may occur. Galactosylation abnormalities of immunoglobulin G (IgG), measured by lectin binding ratios, have been documented in rheumatoid arthritis and malignancy but not in liver disease. As the liver is an important site of immunoglobulin catabolism, we hypothesised that damage to Kupffer cells in chronic liver disease may produce defective clearance of IgG with a net change in circulating IgG glycosylation profile. The aim of this study was to determine the glycosylation of IgG in chronic liver diseases.

Methods Purified IgG was obtained from sera of 14 alcoholic liver disease (ALD), 10 primary biliary cirrhosis (PBC), 14 other liver disease patients (hepatitis C, autoimmune chronic active hepatitis, drug induced and cryptogenic) and 12 normals (age matched). Terminal N-acetylgalactosamine and galactose residues of IgG were detected by lectin binding of Banderaeae simplicifolia II (BSII) and ricin communis agglutinin (RCA) respectively.

Results Lectin ratios of BSII to RCA, and BSII levels were significantly elevated in all liver disease groups, p<0.05, compared to normals, with no differences between disease groups by analysis of variance.

Conclusions The glycosylation profile of serum IgG is significantly altered in chronic liver disease conditions and not specific to any one liver disease.

THE ROLE OF DUPLEX AND COLOUR FLOW DOPPLER ULTRASOUNDOGRAPHY IN THE ASSESSMENT OF TIPS SHUNT FUNCTION


Introduction: Shunt dysfunction following TIPS occurs in 20-60% of patients and is the major cause of variceal rebleeding. The aim of follow up is to identify patients with dysfunction prior to complications. The “gold standard” for identifying shunt dysfunction is direct portography and measurements of the portal pressure gradient (PPG). The aim of this prospective study was to assess the role of Doppler examination as a screening test for shunt dysfunction following TIPS.

Materials and Methods: Twenty three patients with TIPS followed for at least 3 months with no variceal rebleeding were the subjects of this study. Portography with measurement of PPG and Doppler examination were performed within 24 hours of each other at 3 months by 2 separate observers. Peak velocity (PV), time averaged velocity, blood flow, and resistive index were measured in the portal vein and the shunt. Results were expressed as mean and standard error and correlations between parameters calculated using linear regression.

Results: The shunt was not visualised due to the body habitus in 4 patients (17%). All patients with a PV in the portal vein of greater than 90 cm/sec (40%) had a PPG of <12mmHg. A PV of less than this was not specific. No correlation was observed between any of the other measured parameters and PPG. A TIPS: portal vein blood flow of >1 indicating intrahepatic vascular steal, was found in the only 2 patients who developed encephalopathy.

Conclusions: The results of this study suggests that portography remains the “gold standard” for accurate follow up and is required for the majority of the patients. However, if the Doppler criteria of PV in the portal vein of >90 cm/sec are satisfied then portography may not be necessary. Ratio of TIPS to portal vein blood flow of >1 appears predictive of subsequent encephalopathy.

OSTEOPENIA AFTER LIVER TRANSPLANTATION: AN OVERESTIMATED RISK?

SH Hussain, SP Stewart, F Roman, B Oldroyd, P Brandle, M Simpson, MA Smith, J O’Grady, MS Losowsky, Departments of Medicine and Liver Unit, St James’ University Hospital, Leeds and Centre for Bone and Body Composition Research, University of Leeds, Leeds UK.

Background: Prospective longitudinal studies, after orthotopic liver transplantation (OLT), report an initial reduction in bone mass which later returns to normal. Aims: To further examine the extent of osteopenia after OLT. Methods: Therefore, we performed sequential measurements of bone mineral density using dual energy x-ray absorptiometry in 26 patients who underwent OLT for liver failure due to: (i) cholestatic (n=15) and (ii) non-cholestatic (n=11) liver disease. Total body (TB), lumbar spine (LS) and femoral neck (FN), bone mineral densities (BMDs) were assessed before OLT (n=26), at 1 month (n=18) and at 6 months (n=13). Age matched Z scores were calculated from the BMDs to correct for factors causing osteopenia in the general population (<2 indicating osteopenia). Results: In all patients before OLT, the mean Z scores were within the normal range (TB Z score -0.58±0.3, LS Z score -0.71±0.2, FN Z score -0.19±0.3). Patients with cholestatic liver disease compared to those with non-cholestatic liver disease had a similar: (i) TB BMD (mean±SEM g/cm2) 1.07±0.05 vs 1.11±0.02, (ii) LS BMD (1.01±0.07 vs 1.05±0.02) and (iii) FN BMD (0.94±0.07 vs 0.92±0.02). The sequential studies following OLT, at one month and six months showed no significant change in TB BMD (1.10±0.03, 1.13±0.04, 1.11±0.05 respectively) or in LS BMD (1.04±0.04, 1.02±0.04, 1.02±0.04). The FN BMD before OLT (0.94±0.05) remained unchanged (0.95±0.06) one month after OLT, but by six months had fallen slightly but significantly to 0.90±0.06 (p<0.003). Discussion: Both bone mineral density in cholestatic and non-cholestatic liver disease was similar and usually normal before OLT. In contrast to a number of studies which suggest a fall in bone mineral density post OLT, the data after OLT from the present study show only a marginal fall in femoral neck bone mineral density. These results suggest that the risk of worsening osteopenia post-OLT, as a result of immunosuppressive treatment, may be over-estimated.
Clinical trials  W81–W90

A RANDOMISED TRIAL OF 3 ERADICATION REGIMES FOR HELICOBACTER PYLORI

Gibbons AH, Beales JP, Boulton RA, Mulla RJ*, Want SV* and Calam J. Dept. of Gastroenterology and Microbiology*, Hammarsmith Hospital, London.

Our inner-city area contains many immigrants who are likely to have metronidazole-resistant strains of H. pylori (MRHP). This drug is a frequent cause of side effects and poor compliance in eradication regimes. Therefore we asked: (i) What is the local prevalence of MRHP? (ii) Is it worth adding metronidazole to omeprazole and amoxycillin in eradication therapy in our area? (iii) Can thecourse of metronidazole be shortened?

Method: We treated 60 patients with omeprazole 40mg OD and amoxycillin 500mg QDS for 2 weeks, but randomised them to receive either no metronidazole (OA), metronidazole 400mg TDS throughout (OAM) or metronidazole at this dose for the first 5 days only (OAM2). Patients found to be infected at endoscopy were recruited. All had a positive 14C-urea breath test and were retested by this method at least 4 weeks after completing therapy. Metronidazole resistance was determined using disc sensitivity testing.

Results: 65% of the isolates were metronidazole resistant. The eradication rate in patients who received OAM (89%) was significantly higher than in those who received OA (58%) (p<0.03). In the OAM2 group it was slightly lower than OA (82%). Side effects led to the withdrawal of 1 patient from groups OA and OAM. Mild side effects occurred in 4 patients on OA, compared to 9 on OAM and 8 on OAM2.

Conclusion: Our population has a high rate of MRHP, as has been reported in other inner-city areas. Despite this it is worth adding metronidazole therapy to OA in our patients. Shortening the duration of metronidazole therapy to 5 days only slightly reduced the eradication rate.

EFFECT OF OMEPRAZOLE THERAPY ON HELICOBACTER PYLORI, UREASE ACTIVITY AND ANTRAL GASTRIC HISTOLOGY IN PATIENTS WITH DDU UNLCE

M Mukahabadu, Q B Zhang, M Kokkashi, C Gemelli, F D Lee, R I Russell. Department of Gastroenterology, Department of Pathology, & Department of Bacteriology, Royal Infirmary, Glasgow G31 2ER.

Helicobacter pylori (HP) is associated with chronic active gastritis and peptic ulceration (PU). Omeprazole is a proton pump inhibitor which is effective in healing PU and gastritis. Omeprazole has been shown to have some activity against HP both in vitro and in vivo as well as inhibiting urease activity in vitro. AIM: to evaluate the effect of Omeprazole on urease activity in vivo, HP colonization of the gastric mucosa, associated gastritis and CLO-test in patients with duodenal ulcer (DU).

PATIENTS AND METHODS: 12 patients (7 men and 5 women, age 22-68 yr) with DU > 3 mm in diameter and a positive CLO test were studied. Omeprazole 20 mg b.d. was given to each patient. Patients were re-endoscoped 8 weeks later to check for healing of DU and repeat biopsies from the gastric antrum for histology, CLO test culture and urease activity.

RESULTS: All patients had complete healing of the DU, significant reduction in the urease activity (95% CI of the difference between means 14.1 to 32.7, p<0.001), HP density (95% CI of the difference between means 590.5 to 1466.1, p<0.001) and gastritis activity. HP microorganisms were reduced on the histological sections of the gastric antrum after Omeprazole therapy and disappeared in three cases. Corresponded to negative CLO-tests results after 24 hours reading. CONCLUSION: Omeprazole at a dose of 20 mg b.d is capable of reducing HP density, urease activity in vivo and antral gastritis activity in patients with DU.

EFFECT OF OMEPRAZOLE ON THE DISTRIBUTION OF ANTIBIOTICS IN GASTRIC JUICE

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Omeprazole potentiates the anti-Helicobacter effect of amoxycillin and clarithromycin. We tested the hypothesis that this is partly due to enhanced gastric drug delivery.

METHODS: Amoxycillin 750 mg, clarithromycin 500 mg, or metronidazole 400 mg was given in randomised order as single doses either i.v. or p.o. to 24 healthy male subjects whilst taking placebo or omeprazole 40 mg b.d. in a cross-over design i.e. 8 subjects per antibiotic. Saliva and plasma samples were taken for 4 hours and 8 hours respectively. In the i.v. experiments gastric juice was aspirated continuously for 4 hours and 15 minute aliquots were quickly neutralised and frozen. Pyloric losses were measured using a phenol red marker. Antibiotic concentrations were measured in all samples by HPLC and Bioassay.

The maximum concentration (CMax) and area under concentration curve (AUC) were calculated for each antibiotic.

RESULTS: Estimated means and 95% Cl. CMax in mg/l.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Placebo</th>
<th>Omeprazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxycillin</td>
<td>0.18(0.07-0.48)</td>
<td>13.1(8.2-21)</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>10.6(6.7-17)</td>
<td>8.0(6.3-10)*</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>80(60-100)</td>
<td>62(48-78)</td>
</tr>
<tr>
<td>Placebo</td>
<td>91(72-109)</td>
<td></td>
</tr>
<tr>
<td>Omeprazole</td>
<td>69(54-83)</td>
<td>102(84-121)</td>
</tr>
</tbody>
</table>

*p<0.06, p<0.0001 (Difference omeprazole vs placebo)

CONCLUSIONS: 1. Amoxycillin can be detected in gastric juice following i.v. dosing, and is found in higher concentrations during omeprazole treatment. 2. Metronidazole, but not clarithromycin, secretion into gastric juice is reduced by omeprazole, probably because of differing pKa values. 3. Omeprazole treatment does not alter the absolute plasma bioavailability of these antibiotics.

A NEW SHORT, HIGHLY EFFECTIVE HELICOBACTER PYLORI ERADICATION REGIME


The efficacy, compliance and side effect profile of a new anti-Helicobacter pylori (HP) regimen was investigated on 62 patients with dyspeptic symptoms. A combination of positive urea breath test and either positive histology, urease or culture was necessary for inclusion in the HP positive group. 50 patients were HP positive. A one week course of clarithromycin 500mg t.d.s., omeprazole 20mg bd and De-Nol two tabs bd was used. 29 patients with active duodenal ulceration who were positive on histology and had 21 days of omeprazole at a dose of 20 mg nocte. 25 (86%) of these patients with ulcers had their HP eradicated and 96% of patients with ulcers who reattended were healed. Four weeks after completion of the one week course of HP eradication therapy, assessment was repeated in all cases. Diary cards were used to measure compliance and to record side effects. An overall eradication rate of 92% was achieved on an intention to treat basis. Compliance was excellent (93%). The most common side effect was taste disturbance in 34% due to clarithromycin. In vitro sensitivity to clarithromycin, amoxycillin and metronidazole was high in 29 randomly selected cases. However, metronidazole resistance was universal. This new drug regimen is very efficacious, well tolerated and has a high compliance rate. Seven days of treatment with clarithromycin, De-Nol and omeprazole eradicates HP in 92%.
GASTRIC ULCER (GU) RECURRENT FOLLOWING TREATMENT WITH OMEPRAZOLE (OM) AND AMOXICILLIN (AMOX) OR OMEPRAZOLE ALONE: A MULTICENTRE STUDY IN THE UK AND REPUBLIC OF IRELAND.


1The General Infirmary, (for the Study Group) and 2Astra Clinical Research Unit, Edinburgh.

We have investigated the effect of a Helicobacter pylori (Hp) eradication regimen on eradication rate and ulcer recurrence in patients with gastric ulcer.

METHODS: Consecutive patients with endoscopically confirmed gastric ulcers were entered into the study. Hp infection was assessed at entry and subsequent visits by histology and microbiology. All patients received omeprazole 40 mg od for 6 weeks. Thereafter they were randomised to receive in addition either amoxicillin 750 mg bid or placebo for 2 weeks in the ratio 2:1. Patients with healed ulcers then entered a 12 month untreated follow-up. Hp eradication was assessed one month after stopping treatment. Unhealed patients were regarded as having zero relapse days in follow-up. An all patients treated analysis was done.

RESULTS: 126 patients with Hp-associated benign gastric ulcers entered the study and 107 (72 OM-AMOX and 25 OM) were eligible for the analysis. 97% and 88% of OM-AMOX and OM treated patients respectively had healed ulcers after 8 weeks treatment. 24 patients relapsed or were lost to follow-up before the 1 month follow-up and are included as Hp positive. Hp was eradicated in 42/72 (58%) and 2/35 (6%) in the OM-AMOX and OM groups respectively ($X^2$ test, p < 0.001). In the 12 month follow-up 9/72 (13%) of the OM-AMOX and 13/35 (37%) of the OM groups had relapsed. The patterns of recurrence were highly significantly different between the two groups (Logrank test, P < 0.001). Regardless of treatment, recurrence was less likely in patients with successful Hp eradication (3/44 eradicated vs 17/39 non-eradicated; Logrank test, p < 0.001).

CONCLUSION: Treatment with OM-AMOX was effective in eradicating Hp infection in patients with Hp-associated gastric ulcers. This regimen was successful in reducing relapse in the following 12 month period.


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Mucosal colonic protease activity is elevated in Ulcerative Colitis (UC) and is associated with a thinner and discontinuous colonic mucous barrier. The polycrylic acid Carbopol 934P can interact with mucin and has faecal protease inhibiting activity. Here we describe effects of Carbopol 934P on the clinical course of UC and faecal protease activity.

100ml 1% (w/v) carbomer enema was given twice daily and 400mg orally 3 times daily to 8 patients with active distal UC for 4 weeks. Stool samples were collected weekly, histology, sigmoidoscopic appearance and clinical grade were assessed at weeks 0, 2 and 4. Stool protease activity was estimated as nmols new peptide N-terminals mmol-1 dry weight faeces-1 (U). 7 patients completed the study (6 fully, 1 minus week 4 stool sample), 1 was withdrawn for failing to adhere to the protocol.

The results are shown below, all values=mean SEM (n).

<table>
<thead>
<tr>
<th>Sigmoidoscopic Grade</th>
<th>Clinical Grade</th>
<th>Histological Grade</th>
<th>Faecal Protease Activity (U)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wk 0</td>
<td>3.14±0.40</td>
<td>1.85±0.14</td>
<td>2.72±0.57</td>
</tr>
<tr>
<td>(n=7)</td>
<td>(n=7)</td>
<td>(n=7)</td>
<td>(n=6)</td>
</tr>
<tr>
<td>Wk 2</td>
<td>2.29±0.52</td>
<td>1.71±0.28</td>
<td>1.43±0.53</td>
</tr>
<tr>
<td>(n=5)</td>
<td>(n=5)</td>
<td>(n=5)</td>
<td>(n=5)</td>
</tr>
<tr>
<td>Wk 4</td>
<td>1.57±0.65</td>
<td>1.14±0.26</td>
<td>1.57±0.43</td>
</tr>
<tr>
<td>(p&lt;0.05)</td>
<td>(n=7)</td>
<td>(n=7)</td>
<td>(n=6)</td>
</tr>
</tbody>
</table>

The study shows in all cases a marked improvement in mean sigmoidoscopic, clinical and histological grading of active UC in patients after 4 weeks of Carbopol 934P therapy compared with mean values prior to treatment. This was associated with a decrease in humoral colonic protease activity in 5 out of 6 patients. The results suggest a possible therapeutic potential for polyacrylic acids which previously have been shown to bind mucin and inhibit mucolysis of the protective barrier.

A RANDOMISED TRIAL OF 1.5g CALCIUM IN PATIENTS WITH COLORECTAL ADENOMATA.


Department of Surgery, University Hospital, Nottingham.

An increased dietary intake of calcium has been associated with a low incidence of colorectal cancer (CRC). In vitro studies and non randomised trials suggest an anti-neoplastic role for calcium in CRC. Eighty individuals (mean age 61.4 years (sd 9.4) m:f 49:31) with colorectal adenomata removed were randomised to receive 1.5g Calcium or placebo daily for two years. Seventy patients completed the study and had a final colonoscopy.

END POINTS: a) Rectal mucosal proliferation as measured by crypt cell production rate (CCPR). b) Adenoma occurrence.

RESULTS: There was no significant difference in CCPR between calcium and placebo groups at two years.

<table>
<thead>
<tr>
<th>RESULTS AT TWO YEARS</th>
<th>Patients with polyps</th>
<th>Patients with adenomata</th>
<th>Patients with metaplasic polyps</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(No. of polyps)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All n=70</td>
<td>29 (65)</td>
<td>8 (19)</td>
<td>21 (44)</td>
</tr>
<tr>
<td>Placebo n=37</td>
<td>16 (37)</td>
<td>4 (12)</td>
<td>12 (25)</td>
</tr>
<tr>
<td>Calcium n=33</td>
<td>13 (26)</td>
<td>4 (7)</td>
<td>9 (19)</td>
</tr>
</tbody>
</table>

CONCLUSION: Calcium supplementation appears to have no direct effect upon rectal mucosal proliferation. The percentage of patients who developed polyps was 39% and 43% respectively for the calcium and placebo groups. However, the adenoma occurrence rate was only 11% for both groups which is lower than other published series. The anti-neoplastic effect of calcium supplementation in patients with adenomata has not been supported by this study.
LANSOPRAZOLE PROVIDES GREATER SYMPTOM RELIEF THAN OMEPRAZOLE IN REFUX OESOPHAGITIS. A S Mee, J L Rowley and the Lansoprazole Study Group, Royal Berkshire & Battle Hospitals NHS Trust, Reading, RG1 5AN, UK.

Lansoprazole, a second generation proton pump inhibitor, provides effective symptom relief and healing in reflux oesophagitis. Its greater bioavailability compared to omeprazole results in superior initial acid suppression. This study was designed to compare symptom relief and healing rates in patients with reflux oesophagitis.

Methods 604 patients with endoscopically proven reflux oesophagitis, were randomly assigned to receive lansoprazole 30mg or omeprazole 20mg daily for up to 8 weeks. Daily assessment of symptoms was made by the patient using a Visual Analogue Scale. Clinical symptoms were evaluated at weeks 1, 4 and 8. Endoscopic assessment of healing, defined by normalization of the oesophageal mucosal appearance, was made at weeks 4 and 8.

Results 282 patients in the lansoprazole group and 283 patients in the omeprazole group were eligible for inclusion in the protocol analysis. At 3 days, there was a significant improvement in daytime symptoms of heartburn for patients in the lansoprazole group, compared to the omeprazole group (p=0.05). A similar but non significant trend was seen at 7 days. Clinical assessment at 7 days demonstrated significant improvement in daytime epigastric pain in the lansoprazole group, compared to the omeprazole group (p=0.03), with a similar but non significant trend in night-time epigastric pain. Oesophagitis was healed in 69% of patients in the lansoprazole group and 63% of patients in the omeprazole group at 4 weeks. This increased to 87% and 82% respectively at 8 weeks. Both treatments were well tolerated.

Conclusion Lansoprazole provides greater symptom relief compared to omeprazole during the first week of treatment, with a greater number of patients healed after both 4 and 8 weeks treatment.


Aim: As faecal tumour necrosis factor-alpha (TNF-α) is elevated in most HIV-positive patients with microsporidiosis, we used thalidomide, an anti-TNF-α agent, as a novel treatment.

Patients and methods: 12 HIV-positive subjects (mean CD4 count 28 cells/mm³) with chronic diarrhoea of twelve months duration (range 2-24, sd 9) due to microsporidiosis were recruited. All had received albendazole with no effect on their symptoms. FTNF-α was measured using a standard sandwich ELISA. Subjects were prescribed a four week course of thalidomide, 100mg nocte. At the end of the study period a repeat stool sample for microbiology and TNF-α was provided and weight, symptoms and anti diarrhoeal use prior to and during treatment were recorded.

Results: Mean bowel frequency fell from 6/day (range 2-10, sd 2.4) to 1.8/day (range 1-4, sd 0.9) (p<0.0001). Mean weight increase was 1.4 kg (range 0-3, sd 2). Mean anti diarrhoeal use fell from 6 tablets a day (range 0-15, sd 5) to 2.8 a day (range 0-15, sd 5.1) (p<0.005). FTNF-α fell from 19.4 u/ml (range 7.8-72.9, sd 26) to 8.3 u/ml (range 7.8-11, sd 1.3) (p=0.3). Microsporidial spores were still identified in the stools post therapy.

Three patients experienced a generalised rash and one was sedated. A further patient had a relapse of diarrhoea at one week.

Conclusion: Thalidomide is a novel and effective treatment for diarrhoea due to microsporidiosis.


COLORECTAL CANCER SCREENING: THE EFFECT OF COMBINING FLEXIBLE SIGMOIDOSCOPY WITH A FARCIAL OCCULUT BLOOD TEST. D H Bennett, M R Robinson, F Preece, V Moshakis, K D Vellacott, J Desbeas, J Kewenter, O Kronborg, B Monrad & J J Chamberlain & J D Hardcastle; Department of Surgery, University Hospital, Nottingham NG7 2UH.

The role of endoscopy in colorectal cancer screening is currently under discussion. A prospective, randomised European study is in progress assessing the benefit of combining flexible sigmoidoscopy (S) with Haemocult (H) in screening asymptomatic persons between 50-74 years. Subjects were randomised to screening by both S+H (Group 1) or H alone (Group 2).

<table>
<thead>
<tr>
<th>Number Randomised</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>14,538</td>
<td></td>
</tr>
<tr>
<td>Group 2</td>
<td>14,479</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number Screened</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 (N+S)</td>
<td>4,645</td>
<td></td>
</tr>
<tr>
<td>Group 2</td>
<td>8,809</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Adenomas &gt;1cm Detected</th>
<th>Group 1</th>
<th>H +ve</th>
<th>Group 2</th>
<th>H +ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>31.8 / 1000 screened</td>
<td>3.9 / 1000 screened</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 2</td>
<td>3.9 / 1000 screened</td>
<td>0.7 / 1000 screened</td>
<td></td>
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</tbody>
</table>

Combining S with H resulted in a significantly greater yield of both adenomas >1cm (p<0.001; Chi-squared test) and cancers (p<0.01, Chi-squared test), even though the compliance with screening by H was poor (34.7% for both tests in Group 1 compared to 66.1% in Group 2). Economic evaluation is in progress to calculate the cost-effectiveness of this regime.


Aim: As faecal tumour necrosis factor-alpha (TNF-α) is elevated in most HIV-positive patients with microsporidiosis, we used thalidomide, an anti-TNF-α agent, as a novel treatment.

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