**Poster discussion I**

**PDI/1 453**

**ABSORPTION OF 5-AMINOSALICYLIC ACID ENEMA IN PATIENTS WITH ILEORECTAL ANASTOMOSIS FOR ULCERATIVE COLITIS.**


Topical treatment with 5-aminosalicylic acid (5-ASA) is frequently given to patients with ileorectal anastomosis (IRA) after colectomy for ulcerative colitis (UC). 5-ASA absorption in this condition has not been investigated comprehensively. We therefore studied six UC patients with IRA in remission and six healthy volunteers: after at least 48 hours without treatment, half a Pentasa® enema (50 ml) was administered, and heparinized blood samples were taken immediately before administration, after 30 and 60 minutes and then hourly for 8 hours. Urine was collected after 4.5, 9 and 24 hours. Blood samples were centrifuged immediately. Plasma and urine were stored at -20°C and its major metabolite acetyl 5-ASA (ac5-ASA) was measured by HPLC. One patient retained the enema less than 5 hours, another 7 hours, and the others more than 9 hours. All the controls retained the enema at least 9 hours. Figure 1 shows the mean plasma concentrations of 5-ASA and ac5-ASA in patients and controls.

![Graph showing plasma concentrations of 5-ASA and ac5-ASA](image)

The mean total recovery of 5-ASA + ac5-ASA in urine was 12.2% in patients (range 7.1-20.6%) and 18.7% in controls (range 13.3-22.6%). Pentasa® enema is an acidic buffer suspension of 5-ASA (pH 4.8) and previous studies report that it is absorbed faster than a neutral solution of 5-ASA. The unexpectedly low absorption in IRA patients compared with controls (p<0.01, Kruskal-Wallis test) could be explained by a different luminal pH, or by mucosal differences.

In conclusion, absorption of 5-ASA enema in patients with IRA is low and thus this treatment can be considered safe.

**PDI/3 471**

**HUMAN COLONIC MUCUS: DEMONSTRATION OF A MEASURABLE ADHERENT GEL LAYER AND ITS RELATION TO INFLAMMATORY BOWEL DISEASE.**

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A continuous layer of adherent mucus gel covers the colonic mucosa. Its thickness may be relevant to mucosal protection and the pathophysiology of colonic disease but it has never been measured in man. We sought to identify and quantify this layer as part of ongoing work into the role of mucus in inflammatory bowel disease (IBD).

Thick mucosal sections taken from fresh colectomy specimens are viewed transversely on an inverse microscope under phase contrast illumination this allows direct measurement of the gel layer. The study was supported by the Cancer Research Campaign, 46 from carcinoma, 2 diverticular disease, 1 appendix mass, 1 lipoma, 17 ulcerative colitis (UC) and 15 Crohn’s disease (CD).

**Table: Adherent mucus thickness (SD) in μm**

<table>
<thead>
<tr>
<th>Site</th>
<th>Non-IBD</th>
<th>UC</th>
<th>CD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right</td>
<td>12</td>
<td>104 (88)**</td>
<td>125 (88)**</td>
</tr>
<tr>
<td>Left</td>
<td>17</td>
<td>134 (68)</td>
<td>43 (45)**</td>
</tr>
<tr>
<td>Rectum</td>
<td>21</td>
<td>155 (54)</td>
<td>60 (80)**</td>
</tr>
</tbody>
</table>

Adherent mucus thickness as mean (SD) in μm; N is number of specimens at site. Unpaired t-test for differences between non-IBD and UC or CD. (**P<0.05; ***P<0.01; **P>0.001)

**DISCUSSION:**

UC has a significantly thinner and more variable mucus layer with some sites denuded of mucus. This correlates directly with the severity of inflammation. In CD the layer is thicker than non-IBD. Our findings show that goblet cell depletion, which is a feature of UC as opposed to CD is reflected in the adherent mucus gel. Our hypothesis is that the adherent layer of mucus gel may be of importance in the pathophysiology of the different types of inflammatory bowel disease.

**PDI/2 485**

**QUANTIFICATION OF INFLAMMATORY BOWEL DISEASE (IBD) ACTIVITY USING TC99m HMPAO SINGLE PHOTON EMISSION COMPUTERISED TOMOGRAPHY (SPECT).**

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Tc HMPAO white cell scanning can be reliably used to assess extent of IBD on routine planar images. However assessment of disease severity by quantification of uptake in bowel is more difficult due to overlapping activity in bone marrow. SPECT is a technique already being applied to imaging of other organs e.g. heart and brain. It can provide trans-axial images of the abdomen in which uptake of Tc HMPAO in bowel is clearly separated from other structures, particularly the bone marrow. In order to assess the accuracy of this imaging technique in IBD, Tc HMPAO SPECT was performed in 20 patients with suspected colonic involvement. Uptake in each of 5 colonic segments (rectum, sigmoid; descending, transverse and ascending) was quantified on transaxial images and expressed as a ratio of marrow uptake.

Colonoscopy was then performed within 14 days and severity was assessed histologically in the same 5 segments and graded 0-3. Correlation of segment histology score vs. segment/marrow uptake ratio was r = 0.9 (p<0.001). In conclusion Tc HMPAO SPECT imaging can clearly localise actively affected bowel in IBD and allow accurate disease quantification. This non-invasive technique may be useful in objective evaluation of new therapies for IBD.

**PDI/4 452**

**NOVEL COLONIC FACTORS IN THE PATHOPHYSIOLOGY OF ULCERATIVE COLITIS.**


Tissue damage in the colon during acute exacerbation of ulcerative colitis (UC) is probably mediated by infiltrated and/or circulating polymorphonuclear (PMN) leukocytes. The source of infiltrating PMN, appear normal since (1) no systemic inflammation is present in UC patients, and (2) in vitro PMN respond normally to stimuli such as the bacterial protein formyl-methionyl-leucyl-proline (FMLP) or the phosphotidyl ether (PMA). Alternatively, we hypothesised that PMN functions, especially the ability to synthesize oxygen free radicals (OFR), may be triggered and/or exaggerated by exposure to colonic contents of the colonic lumen. To test this hypothesis, we evaluated the ability of PMN to produce OFR in response to stimuli after pre-exposure of PMN to rectal dialysates from 9 control patients or 9 with "active" UC. Dialysis bags containing dextran and having a 12kDa cutoff were used. Luminol (0.33mM) enhanced chemiluminescence was taken as a measure of OFR production by the PMN suspension. Each day freshly prepared PMN (0.5 x 107 cells in 1 ml) from 10 normal human subjects, was added to 1 ml PBS buffer. Stimuli were then added - either FMLP at maximal (100nM) or submaximal (1nM) concentrations, or phosphotidyl myristic acid (PMA) at 10ugassay tube. Chemiluminescence rates were measured for 15 min using a Thun/EMI single photon multiplier tube. Without dialysate, the mean stimulation of chemiluminescence by 100nM FMLP was 5.181 x 106 cpm (SEM=0.054 X 106; range=0.028 X 106 to 0.422 X 106) (baseline = 3 x 10 thousand). After pre-incubation with control rectal dialysate (CRD) at 16 fold dilution there was less stimulation by 100nM FMLP: 5.120 x 106 cpm (SEM=0.078, range=0.008 to 0.740), a 37% decrease. After pre-incubation with a similar diluted UC rectal dialysate (UCRD), there was more stimulation by 100nM FMLP: 5.441 x 106 cpm (SEM=0.024; range=0.022 to 1.927), a 131% increase. The difference between means for the CRD and UCRD groups was significant at the p<0.05 level (Wilcoxon test). At FMLP=100 nM, the ratio CRD mean/UCRD mean was 3.67/1; at FMLP=1 nM, the ratio was 9.47/1. At different dilutions of CRM (4, 16, 32 fold) the increase induced by 100nM FMLP was significantly attenuated to 50%, 63% and 20%, respectively. For PMA, the ratio of the mean of the UCRD/CRD ratios was 1.54/1 (p<0.05). These data indicate that there may be at least one factor in normal rectal dialysate which is capable of attenuating OFR production by PMN. The source of this factor could be bacteria in the colonic lumen or the colonic tissue itself. It further appears that rectal dialysates from UC patients, in contrast, have less of this factor and they have more of a second factor which stimulates OFR production by PMN. Characterization of these putative factors could lead to new drugs for IBD.
EFFECT OF BOWEL DECONTAMINATION ON THE INFLAMMATORY RESPONSE IN TRINITROBENZENESULFONIC ACID INDUCED COLITIS. M. Antoñelo, A. García-Lafuente, S. Videla, J. Vilaseca, E. Crespo, F. Guzmán, J-R Malagelada. Digestive System Research Unit, Hospital Vall d’Hebron, Barcelona, Spain.

The role of microorganisms in the development of inflammatory bowel disease has not been clarified. We studied the participation of colonic bacteria in the inflammatory response associated to colitis. Trinitrobenzenesulfonic acid (TNBS) treatment was given to control (CC) and antibiotic treated rats (Rifaximin + Vancomycin, I&V, 50+50 mg/kg/d). Antibiotics were started three days before TNB and continued up to day 7. A colonic segment of anesthetized CC and I&V rats was perfused at 0, 4, 12, 24, 48 and 72 h and days post TNB (n = 8-10 per point). Release of inflammatory mediators was measured in the colonic perfusate. PGE2, TXB2 and LTB4 concentration was assayed by specific RIA. Myeloperoxidase activity (MPO) was determined both in perfusates and in colonic homogenates. Stool samples from CC and I&V rats were cultured for aerobes and anaerobes. On day 7, lesions were scored (0 to 20) based on macroscopic and microscopic findings.

Release of PGE2 and TXB2 was elevated over baseline at 4 h after TNB, however LTb4 was only released in animals that had been infected with I&V than in CC (p < 0.05) but there were no differences in tissue MPO content. However, on day 7, eosinon and PGE2 release was reversed and became lower in I&V than in CC, without changes in MPO tissue content. On day 7, colonic lesions were scores were lower in I&V (8±1) than in control group (14±6). CC stool cultures showed both aerobic and anaerobic bacteria. I&V samples contained enterobacteria (E. coli, Proteus spp) and no anaerobes at 24 and 72 h, but on day 7 predominance of Candida spp was observed. Thus, as compared to mixed bacterial population, predominance of enterobacteria is associated to a high inflammatory response, and predominance of Candida spp to low inflammatory activity. We conclude that colonic inflammation and luminal mediators release is modulated by the composition of the fecal flora.

ANALYSIS OF DNA EXTRACTS OF NORMAL AND DISEASED INTESTINE USING PCR FOR THE 32Ka GENERAL MYCOBACTERIAL ANTIGEN. S. Bithel, D.S. Millar, N.J. Vaz, W.R. Ford, J.D. Sanders, S. Nemes. Department of Surgery, St. George’s Hospital Medical School, London, SW17 ORE, U.K.

IS900 is a multicopy DNA element insertion highly specific for the chronic enteric pathogen Mycobacterium avium (MA). PCR assays based on this element performed directly on DNA extracts of surgically resected human intestine from people in central and southern England have demonstrated this organism in 65% of Crohn’s disease (CD), 4.1% of ulcerative colitis (UC) and 12% of non-IBD (nIBD) controls. The increased proportion of M. para positive in CD was highly significant (Chi squared, p < 0.0001). Since mycobacteria in general are prevalent in the human intestine we have re-tested all the DNA extracts by PCR specific for the 32Ka general mycobacterial antigen using the primer 5’-CAGGCGTTGGCGCTGCGACG-3’ and 5’-ATGACACACGTGCACCGATGCA-3’. 56% of CD, 13% of UC and 50% of nIBD were positive. There was a significant difference between CD/nIBD comparison with UC (p = 0.0006) but not between CD and nIBD (p = 0.5681). These findings further support the conclusion that UC is not a mycobacterial disorder. If the presence of M. para in the substantial major tissue defects CD/UC and I&V (IS900 PCR) was a non-specific consequence of opportunistic invasion, we should reasonably expect similar results using the 32Ka/MA for mycobacteria in general absence of any significant difference in the presence of mycobacteria in general between CD and nIBD. Deletion of a specific causative relationship between the chronic enteric pathogen M. para, and chronic enteritis in humans.
ENHANCED MUCUS PHOSPHOLIPID SECRETION ASSOCIATED WITH THE GASTROPROTECTION BY EBROTIDINE. B.L. Sliomany, S. Sengupta, E. Piotrowski, V.L.N. Murty, and A. Sliomany. Research Center, UMDNJ, Newark, NJ, USA

Phospholipids of gastric mucus gel along with mucus play a major role in the inherent resistance of the stomach to a variety of luminal insults by maintaining the gel viscoelastic, permeselective and hydrophobic qualities. In this study, we present evidence that, in conjunction with demonstrated gastroprotective properties, ebrotidine, a new cytoprotective agent, stimulates mucous phospholipid secretion. Gastric mucosal cells were incubated for 3h in DMEM containing [3H]choline for phospholipid labeling, centrifuged, washed and transferred to a medium containing 0.15M NaHCO3. Following 30 min incubation, the cells were transferred to a fresh DMEM and incubated for various time periods up to 2h. The medium was centrifuged and the supernate used for the isolation of secreted [3H]phospholipids, while the pellet-containing cells were analyzed for cAMP and the content of cellular [3H]phospholipids. In the absence of drugs, the secretion of choline-containing phospholipids averaged about 4% of the total cellular phospholipids/h. Introduction of ebrotidine led to a dose-dependent increase in the rate of phospholipid secretion up to 1h followed thereafter by a decline. The maximal phospholipid secretory effect was attained at 1h, giving a 2.6-fold increase in phospholipid secretion. The phospholipid secretory response to ebrotidine was also accompanied by an increase in gastric mucosal cAMP content which reached a maximum value of 2.1-fold over that of controls at 1h. In contrast, ranitidine, a classical H2-blocker, neither evoked the increase in cAMP levels nor caused any discernible stimulation in gastric mucus phospholipid secretion. The results demonstrate that gastroprotective properties of ebrotidine are associated with the ability of the drug to elicit a transient stimulation in gastric mucous phospholipid secretion, and that ranitidine does not possess such property.


Growth factors such as EGF, TGF and BFGF are potent mitogens and play an important role in the healing processes but their gastroprotective activity has been little studied. The aim of this study was to compare TGF, EGF and BFGF in protection against acute gastric lesions and in expression of their receptors in the gastric mucosa. Gastric lesions were induced by an intragastric (i.g.) application of 1.5 ml of 100% ethanol, acetylated aspirin (ASA) (100 mg/kg) and water immersion and restraint stress (WRS) for 3.5 h. Parenteral infusion of EGF or TGF (12.5-100 ng/kg-h) i.v. -reduced dose-dependently gastric acid and protein secretion in chronic gastric fistula rats and prevented the formation of gastric damage in rats by ethanol, ASA and WRS, the dose inhibiting by 50% (ED50) the lesions being for EGF 77 or 33 μg/kg-h and for TGF 55, 60 or 25 μg/kg-h, respectively. This protection was accompanied by a significant rise of mucosal blood flow (MBF) measured by laser Doppler technique. Basic FGF (12.5-100 μg/kg) inhibited dose-dependently gastric damage induced by WRS (ED50 55 μg/kg) and increased MBF (by 28% but failed to affect the lesions caused by ethanol and ASA. Suppression of generation of prostaglandins (PG) by indomethacin (5 mg/kg i.p.) partly reversed the protection by EGF and TGF but not by ASA, and completely abolished the protective activity of growth factors against WRS ulcerogenesis. Ulcers generated by ethanol and ASA, respectively. (1) binding studies with 125I-labelled peptides showed a remarkable expression of EGF and TGF receptors, expression of BFGF receptors was detected to lesser extent. We conclude that 1) maintenance of MUCIN is an important protection in EGF and TGF against ethanol and ASA damage; 2) all three growth factors protect against WRS by enhancing the gastric mucosa, it is capable of expressing the receptors for all three growth factors.

INFLUENCE OF EPIDERMAL GROWTH FACTOR AND INSULIN ON THE WOUND REPAIR OF GASTRIC ULCER DISEASES... INVESTIGATION USING A NEW MODEL WITH CULTURED GASTRIC MUCOSAL CELLS. K. Hashiro, S. Watanabe, H. Hirose, A. Miyazaki, R. Okura, O. Kobayashi, Z. Murai, N. Sato. Dept. of Gastroenterology, Juntendo University School of Medicine, Tokyo, Japan

We assessed the influence of EGF and insulin on the wound repair process using a newly developed system for quantitative analysis of wound repair of gastric mucosal cells as a model for peptic ulcer diseases. [METHODS] Gastric mucosal cells prepared from rabbit were cultured in F-12 medium and formed complete monolayer cell sheet in 2 days. Artificial wounds (50% constant) were made all over cell denudation with rotating silicon tip. The process of wound repair was monitored by measuring cell free area every 12 h for 2 days. EGF(10ng/ml) and/or insulin(10-10 M) were added at wounding. Morphological investigation was performed by phase contrast- and electron microscopy. DNA synthesizing cells were detected by indirect immunocytochemistry using anti-BrdU antibody. [RESULTS] In controls, wound area was 2.0mm2 at 0 h, 0.9mm2 at 12 h, 0.36mm2 at 24 h, 0.22mm2 at 36 h and 0.07mm2 at 48 h. In EGF(10ng/ml) series, wound area was 0.8mm2 at 12h, 0.66mm2 at 24 h and 0.06 mm2 at 36 h. In EGF(10ng/ml) + insulin(10M) series, wound area was 0.5mm2 at 12 h and 0.3mm2 at 24 h. In control, BrdU positive cells were detected only around wounds. In EGF and/and insulin series, BrdU positive cells appeared in 12-24 h period in the same area. [CONCLUSIONS] In our newly developed model, the artificial wound made on the cell sheet was repaired with an initial migration and following proliferation stages. EGF and insulin significantly accelerated the gastric mucosal wound repair with the stimulation of cell migration and cell proliferation. These evidences suggested that EGF and insulin might contribute to the rapid healing of gastric ulcer diseases.

NITRIC OXIDE GENERATION MODULATES THE INHIBITION BY INTERLEUKIN-18 OF EPIDERMAL GROWTH FACTOR INDUCED ACID SECRETION IN THE RAT. M.D.Barrachina, S.Calatayud, L. Moreno, J.M. Piquéd, B.J.R. Whittle, L.Y. Espuñol, University of Valencia, Valencia, Spain; Hospital Clinic, Barcelona, Spain and Wellcome Research Laboratories, Beckenham, UK.

Cytokines induce the expression of a corticosteroid-sensitive nitric oxide (NO)-synthase in vascular and evaluated by involvement of NO in the inhibition by the cytokine interleukin-18 (IL-18) of pentagastrin-stimulated acid production. Wistar rats (190-250g) were anesthetized (urethane 1.5 kg/ml, i.p.), the stomachs continuously perfused with saline (0.9 ml/min), 5% pentagastrin-stimulated peristaltic and 10% inhibition, interfered with its action (5 kg/mg). In a previous study, we demonstrated that the NO synthase, the 3.5 h, with acid secretion being determined over the following 120 min. Results: Rats were treated (i.v.) 15 min before IL-18 administration with Nω-nitroarginine methyl ester (L-NNAME), L-arginine (100 mg/kg) or D-arginine (100 mg/kg). Results: Acid secretion stimulated by pentagastrin (34±3 μEq H+ 120min, n=4) was substantially inhibited (p>0.001) by IL-18 (18±2.7% inhibition, n=13). Prior administration of dexamethasone (5 mg/kg, i.c. 14h and 6h before study) did not modify the inhibitory effects of IL-18 (80±6.6% inhibition, n=4). Pretreatment with 5 (n=7) and 10 ng of L-NNAME significantly (p<0.01) restored the secretory response to pentagastrin in IL-18 rats (9.4±9% and 10±16% inhibition respectively). The actions of L-NNAME (5 mg/kg) were prevented (p<0.01) by the previous administration of L-NNAME, but not by its enantiomer D-arginine (p<0.01). The effects of IL-18 (5 mg/kg) were not significantly modified by L-NNAME (5 mg/kg), L-arginine or D-arginine did not significantly modify the secretory response to pentagastrin. IL-18 administration did not modify blood pressure and heart rate. L-NNAME (5 mg/kg) increased blood pressure, but not the mechanism by which interleukin-induced acid-inhibition was prevented, since simultaneous systemic pressor responses induced by phenylephrine (10 ng/kg i.v.) were equally potent. In contrast, 25 (8 mg/ml) did not significantly inhibit pentagastrin-stimulated (104 M) acid secretion in the rat isolated perfused stomach (n=5). These findings suggest a role of the acute release or action of NO in vivo in the gastric inhibitory response to IL-18, not involving the inducible NO-synthase.