Poster discussion I

PDI/1 453

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

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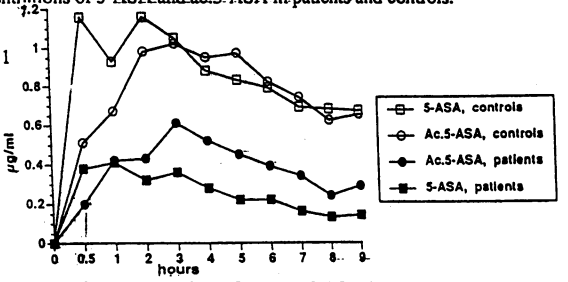
Topical treatment with 5-aminosalicylic acid (5-ASA) is frequently given to patients with ileorectal anastomosis (IRA) after colectomy for ulcerative colitis (UC). 5-ASA absorption in this condition has not been investigated. We therefore studied six UC patients with IRA in remission and six healthy volunteers: after at least 48 hours without treatment, half a Pentasa® 4% 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 -80°C. 5-ASA and its major metabolite acetyl 5-ASA (ac.5-ASA) were 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 ac.5-ASA in patients and controls.

FIG. 1



The mean total recovery of 5-ASA + ac.5-ASA in urine was 12.2% in patients (range 7.1-20.8%) 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 less 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 quantitate 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. 82 specimens were studied: 46 from carcinoma, 2 diverticular disease, 1 appendix mass, 1 lipoma, 17 ulcerative colitis (UC) and 15 Crohn's disease (CD).

Site	Non-IBD		UC		CD	
	N	Thickness	N	Thickness	N	Thickness
Right	12	107 (48)	14	97 (78)	12	195 (88)**
Left	17	134 (68)	14	43 (45)***	4	232 (40)**
Rectum	21	155 (54)	12	60 (86)**	5	294 (43)***

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

QUANTITATION 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 $r = 0.9$. ($p<0.001$). Total bowel uptake/marrow ratio correlated with Crohn's disease activity index $r=0.6$ $p=0.01$. In conclusion Tc HMPAO SPECT imaging can clearly localise actively affected bowel in IBD and allow accurate disease activity quantitation. 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.

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Tissue damage in the colon during acute exacerbation of ulcerative colitis (UC) is probably mediated by infiltrated neutrophils (PMN). However, circulating PMN, 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 stimulants such as the bacterial peptide formyl-methionyl-leucyl-proline (FMLP) or the phorbol ester (PMA). Alternatively, we hypothesized that PMN functions, especially the ability to synthesize oxygen free radicals (OFR), may be triggered and/or exaggerated by exposure of passing and/or infiltrating PMN to the 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 (0.333 mM) enhanced chemiluminescence was taken as a measure of OFR production by the PMN suspension. Each day freshly prepared PMN (0.5 to 2 X 10^6 cells in 1 mL) from 1 of 10 normal human subjects, was added to 1 mL PBS buffer. Stimuli were then added – either FMLP at maximal (100 nM) or submaximal (1 nM) concentrations, or phorbol myristic acetate (PMA) at 10 ug/assay tube. Chemiluminescence rates were measured for 15 min using a Thorn/EMI single-photon photomultiplier tube. Without dialysate, the mean stimulation of chemiluminescence by 100 nM FMLP was + 0.191 x 10^6 cpm (SEM = 0.054 X 10^6; range = 0.028 x 10^6 to 0.422 x 10^6) (baseline = 3 to 5 thousand). After pre-incubation with control rectal dialysate (CRD) at 16 fold dilution there was *less* stimulation by 100 nM FMLP: + 0.120 X 106 cpm (SEM=0.078, range=0.008 to 0.740), a 37% decrease. After preincubation with a similarly diluted UC rectal dialysate (UCRD), there was *more* stimulation by 100 nM FMLP: + 0.441 X 106 cpm (SEM=0.204; range= 0.024 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 CRD (4, 16, 32 fold) the increase induced by 100 nM FMLP was significantly attenuated to 50*, 43* and 20* %, respectively (*: $p < 0.05$). For PMA, 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 from 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/or 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.

PDI/5 1068

EFFECT OF BOWEL DECONTAMINATION ON THE INFLAMMATORY RESPONSE IN TRINITROBENZENSULFONIC ACID INDUCED COLITIS. M. Antolín, A.García-Lafuente, S. Videla, J. Vilaseca, E. Crespo, F. Guarner, 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. Trinitrobenzensulfonic acid (TNB) was given to control colitis (CC) and antibiotic treated rats (Imipenem + Vancomicin, 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 7 days post TNB (n= 8-10 per point). Release of inflammatory mediators was mesured in the colonic perfusated. PGE_2, TXB_2, and LTB_4 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 macro- and microscopic findings.

Release of PGE_2 and TXB_2 was elevated over baseline at 4 h after TNB, however LTB_4 and MPO release was not higher until 12 h in both groups. Inflammatory mediators were elevated until 7 days post TNB ($p<0.05$). The comparison of both groups showed that at 24 and 48 h the release rate of the mediators were at least two-fold higher in I&V than in CC ($p<0.05$) but there were no differences in tissue MPO content. However, on day 7, eicosanoid and MPO release was reversed and became lower in I&V than in CC, without changes in MPO tissue content. On day 7, colonic lesion scores were also lower in I+V (8 ± 1) than in control group (14 ± 1.6). CC stool cultures showed both aerobic and anaerobic bacteria. I&V samples contained enterobacterias (*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 enterobacterias is associated to a high inflammatory response, and predominance of *Candida spp* to low inflammatory activity. We conclude that mural colonic inflammation and luminal mediators release is modulated by the composition of the fecal flora.

PDI/7 144

CHRONIC NORMOVOLEMIC ANEMIA INCREASES GASTRIC MUCOSAL BLOOD FLOW AND PROTECTS AGAINST ETHANOL-INDUCED GASTRIC DAMAGE. N. Marroni, M. Casadevall, J. Panés, C. Piera, JM. Jou, JM. Piqué. Gastroenterology, Hematology and Nuclear Medicine Deptartments, Hospital Clínic, Barcelona University, Barcelona, Spain.

It has been demonstrated that gastric blood flow is increased under conditions of acute normovolemic anemia (Gastroenterology 1992;103:407), and this probably represents an intrinsic mechanism protecting the gastric mucosa against damage induced by reduced oxygen availability. The present study was aimed at investigating the effects of chronic normovolemic anemia on gastric mucosal blood flow (GMBF) and on ethanol-induced gastric mucosal damage in rats. **Methods:** A chronic model of normovolemic anemia was developed in rats by daily replacement of 1.5 ml of blood by a plasma expander through a catheter chronically implanted in the jugular vein. Control rats not submitted to blood exchange were also used. Hematocrit, blood volume and blood viscosity were measured in basal conditions and after four days blood exchange. At the fifth day, rats were anesthetized with ketamine (100 mg/kg, i.m.), a laparotomy was performed and the stomach was exposed. In basal conditions, GMBF was measured by hydrogen gas clearance technique and the hemoglobin (IHb) and the oxygen (ISO2) content of the gastric mucosa was estimated by reflectance spectrophotometry. After baseline measurements, 1 ml of 100% ethanol was administered intragastrically and GMBF, IHb and measurements were repeated after 60 minutes. Mean arterial blood pressure (BP) was monitored throughout the experiment. At the end of the experiments thats were sacrificed and the gastric damage was macroscopically assessed as the total area of the lesion in mm^2. **Results:** In comparison with basal values, anemic rats experienced a significant reduction in the hematocrit (from 49±3 to 34±4%; p<0.01) and a significant reduction in the blood viscosity (from 5.9+0.4 to 3.6 cP; p<0.05), without changes in the blood volume (from 5.7±1.8 to 6.1±2.8 ml/100g bw). BP was lower in anemic than in control rats (108±3 vs 120±4 mmKg; p<0.05). Compared with control animals, anemic rats had a higher GMBF (36±2 vs 31 ±2 ml/min/100g; p<0.05), a lower IHb (131±6 vs 102±5 arbitrary units; p<0.05), without changes in the ISO2 (35±2 vs 36±2 arbitrary units). A similar decrement in GMBF was observed after intragastric ethanol administration in anemic (30±5%) and in control rats (38±6%). However, the final GMBF after ethanol administration was still higher in anemic than in control rats (26±2 vs 19±2 ml/min/100g; p<0.05). A fewer ethanol-induced macroscopic gastric damage was observed in anemic than in control rats (10±3 vs 64±25 mm^2; p<0.05). **Conclusions:** 1)-Chronic normovolemic anemia induces a slight but significant increment in gastric mucosal blood flow, and a marked reduction in the gastric hemoglobin content without changes in the oxygen content. 2)-Rats submitted to chronic normovolemic anemia exhibit a protective mechanism against ethanol-induced gastric mucosal damage.

PDI/6 1273

ANALYSIS OF DNA EXTRACTS OF NORMAL AND DISEASED INTESTINE USING PCR FOR THE 32kDa GENERAL MYCOBACTERIAL ANTIGEN. S.Withey, D.S.Millar, M.L.V.Tizard, J.Ford, J.D.Sanderson, J.Hermon-Taylor. Department of Surgery, St.George's Hospital Medical School, London, SW17 ORE. U.K.

IS900 is a multicopy DNA insertion element highly specific for the chronic enteric pathogen Mycobacterium paratuberculosis (M.para). 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), 4.3% 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 32kDa general mycobacterial antigen using the primers 5'CGGCAGCTCGCTGGTCAG-3' and 5'ATCAACACCCCGGCGTTCGAG-3'. 56% of CD, 13% of UC and 50% of nIBD were positive. There was a significant difference between CD/nIBD compared 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 majority of CD tissues tested by IS900 PCR was a non-specific consequence of opportunistic invasion, we should reasonably expect similar results using the 32kDa PCR for mycobacteria in general. The absence of any significant difference in the presence of mycobacteria in general between CD and nIBD favours a specific causative relationship between the chronic enteric pathogen M.para, and chronic enteritis in humans.

PDI/8 831

DYNAMIC ASSESSMENT OF ENDOSCOPIC AND HISTOLOGIC CHANGES INDUCED BY INDOMETHACIN IN DIFFERENT PHASES OF EXPERIMENTAL GASTRIC ULCER HEALING

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Indomethacin (INDO) delays healing of experimental gastric ulcers, however, the dynamic assessment of indomethacin-induced delay in different phases of ulcer healing is less established. This was studied with a new videoendoscopic method which allows highly precise sequential monitoring of healing dynamics. **Methods:** 56 rats (8/group) with cryo-ulcers in the corpus were treated with either: (PLA) or INDO (2x0.5 mg/kg s.c.) during different phases of ulcer healing as shown in Table. Ulcer size was measured three times per week with a novel videoendoscopic method. On day 15, ulcer size was measured in standardized histologic mucosal specimens.

Results: Ulcer diameter on day 1 was 6.2 ± 0.1 mm ($\bar{x}$ ± SEM). INDO treatment (B) during 2 weeks predominantly delayed healing between days 8-10 and 10-15, respectively. INDO treatment during 2 weeks with 4 days pretreatment before ulcer induction (C) showed no difference to (B). INDO treatment in the first week for 7 days delayed ulcer healing predominantly between days 8-10, however, healing rates readapted between days 10-15 close to placebo healing rates. INDO treatment for 7 days in the second week (E) or for 4 days in the first week (F, G) showed no differences to placebo.

Treatment	Daily ulcer size reduction ‡			Ulcer size‡
	days 3-8 ($\bar{x}$ ±SEM)	days 8-10 ($\bar{x}$ ±SEM)	days 10-15 ($\bar{x}$ ±SEM)	day 15 ($\bar{x}$ ±SEM)
A: PLACEBO	7.1 ± 0.4	6.5 ± 0.4	3.9 ± 0.3	14 ± 2
B: INDO, days 1-15	6.1 ± 0.3	4.3 ± 0.4*	1.0 ± 0.2*	32 ± 2*
C: INDO, days (- 4)-15	6.3 ± 0.7	4.5 ± 0.3*	1.0 ± 0.2*	31 ± 4*
D: INDO, days 1-8	6.3 ± 0.3	3.9 ± 0.3 *	3.2 ± 0.3*†	24 ± 3*†
E: INDO, days 8-15	7.0 ± 0.2	6.4 ± 0.4†	3.6 ± 0.3†	15 ± 3†
F: INDO, days 1-4	7.0 ± 0.2	6.3 ± 0.4†	3.8 ± 0.3†	15 ± 3†
G: INDO, days 4-7	6.9 ± 0.2	6.4 ± 0.4†	3.6 ± 0.3†	15 ± 3†

‡ In % of initial ulcer size
* significant difference (p < 0.02, ANOVA) vs. placebo
† significant difference (p < 0.02, ANOVA) vs. group B (INDO days 1-15)

Conclusion: 1.) Ulcer healing is predominantly delayed by INDO in the second week. 2.) Only 2 days after discontinuation of indomethacin treatment, ulcer healing readapted close to placebo levels. 3.) Indomethacin administered either in the second week or only for 4 days shows no effect on ulcer healing dynamics.

PDI/9 119

ENHANCED MUCUS PHOSPHOLIPID SECRETION ASSOCIATED WITH THE GASTROPROTECTION BY EBROTIDINE. B.L.Slomiany, S. Sengupta, E. Piotrowski, V.L.N. Murty, and A. Slomiany. Research Center, UMDNJ, Newark, NJ, USA

Phospholipids of gastric mucus gel along with mucins play a major role in the inherent resistance of gastric mucosa to a variety of luminal insults by maintaining the gel viscoelastic, permselective and hydrophobic qualities. In this study, we present evidence that ebrotidine, a H_2-receptor antagonist with demonstrated gastroprotective properties, elicits a rapid stimulation of gastric mucus phospholipid secretion. Gastric mucosal cells were incubated for 3h in DMEM containing [^{3}H]choline for phospholipid labeling, centrifuged, washed and transferred to a medium containig 0-150μM of ebrotidine or ranitidine. 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 [^{3}H]phospholipids, while the pelleted cells were analyzed for cAMP and the content of cellular [^{3}H]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 120μM ebrotidine giving a 36% increase in phospholipid secretion. The phospholipid secretory response to ebrotidine was also accompanied by an increase in gastric mucosal cell cAMP content which reached a maximum value of 2.1-fold over that of controls at 1h. In contrast, ranitidine, a classical H_2-blocker, neither evoked the increase in cAMP level nor caused any discernible stimulation in gastric mucus phospholipid secretion. The results demonstrate that the gastroprotective properties of ebrotidine are associated with the ability of the drug to elicit a transient stimulation in gastric mucus phospholipid secretion, and that ranitidine does not possess such property.

PDI/10 026

MUCOSAL RECEPTOR EXPRESSION AND GASTROPROTECTIVE ACTIVITY OF TRANSFORMING GROWTH FACTOR ALPHA (TGF) EPIDERMAL GROWTH FACTOR (EGF) AND BASIC FIBROBLAST GROWTH FACTOR (bFGF). S.J. Konturek, T. Brzozowski, B. Slomiany, Drozdowicz, A. Garner, Inst. Physiol., Univ. Sch. Med., Krakow, Poland.

Growth factors such as EGF, TGF and bFGF are potent mitogenic and growth promoting agents essential for healing processes but their gastroprotective activity has been little studied. The aim of this study was to compared 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, acidified 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 μg/kg-h s.c.) reduced dose-dependently gastric acid and pepsin 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) these lesions being for EGF 45, 77 or 33 ug/kg-h and for TGF 35, 60 or 25 μg/kg-h, respectively. This protection was accompanied by a significant rise in mucosal blood flow (MBF) measured by laser Doppler technique. Basic FGF (12.5-100 μg/kg-h) inhibited dose-dependently gastric damage induced by WRS (ED50 55 μg/kg-h) 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 against ethanol and completely abolished the protective activity of growth factors against WRS ulcerogenesis. Isolated mucosal membranes binding studies with I^{125}-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 MBF is important in protection by EGF and TGF against ethanol and ASA damage; 2) all three growth factors protect against WRS by enhancing MBF, 3) gastric mucosa is capable of expressing the receptors for all three growth factors.

PDI/11 190

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. Maehiro, S. Watanabe, M. Hirose, A. Miyazaki, R. Ohkura, O. Kobayashi, T.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 wound with constant cell free area was made by 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^{-8}-10^{-9}M) were added at wounding. Morphological investigation was performed by phase contrast- and electron microscopy. DNA synthesizing cells were detected by indirect immunostaining using anti-BrdU antibody. [RESULTS] In controls, wounded area was 2.0mm^2 at 0 h, 0.9mm^2 at 12 h, 0.36mm^2 at 24 h, 0.02mm^2 at 36 h and 0mm^2 at 48 h. In EGF(10ng/ml) series, wounded area was 0.8mm^2 at 12h, 0.06mm^2 at 24 h and 0% at 36 h. In EGF(10ng/ml) + insulin(10^{-8}M) series, wound area was 0.5mm^2 at 12 h and 0mm^2 at 24 h. In control, BrdU positive cells were detected only around the wound in 24-36 h. In EGF and/or 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.

PDI/12 113

NITRIC OXIDE GENERATION MODULATES THE INHIBITION BY INTERLEUKIN-1ß OF PENTAGASTRIN-STIMULATED GASTRIC ACID SECRETION IN THE RAT. M.D.Barrachina, S.Calatayud, L. Moreno, J.M.Piqué, B.J.R.Whittle, I.V.Esplugues. 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 immune cells. We have now evaluated the involvement of NO in the inhibition by the cytokine interleukin-1ß (IL-1ß) of pentagastrin-stimulated acid production. Wistar rats (180-250g) were anaesthetized (urethane 1.5g kg^{-1}, i.p.), the stomachs continuously perfused with saline (0.9 ml min^{-1}) and acid output determined. Interleukin-1ß (2 μg kg^{-1} i.v., Genzyme Corp.) was administered once H^+ output stimulated by the i.v. infusion of pentagastrin (8 μg kg^{-1} h^{-1}) had remained constant for 120 min, with acid secretion being determined over the following 120 min. Rats were treated (i.v.) 15 min before IL-1ß administration with N^G-nitroarginine methyl ester (L-NAME), L-arginine (100 mg kg^{-1}) or D-arginine (100 mg kg^{-1}). Results: Acid secretion stimulated by pentagastrin (34±3 μEq H^+ 120 min^{-1}, n=42) was substantially inhibited ($p<0.001$) by IL-1ß (72±7% inhibition, n=13). Prior administration of dexamethasone (5 mg kg^{-1}, s.c. 16 and 4 h before study) did not modify the inhibitory effects of IL-1ß (80±6% inhibition, n=4). Pretreatment with 5 (n=7) and 10 mg kg^{-1} (n=9) of L-NAME significantly ($p<0.001$) restored the secretory response to pentagastrin in IL-1ß rats (9.4±8% and 10±16% inhibition respectively). The actions of L-NAME (5 mg kg^{-1}) were prevented ($p<0.01$) by the previous administration of L-arginine but not by its enantiomer D-arginine (n=6 and 14 respectively). In control animals pretreatment with L-NAME (5 mg kg^{-1}), L-arginine or D-arginine did not significantly modify the secretory response to pentagastrin. IL-1ß administration did not modify blood pressure. L-NAME (5 mg kg^{-1}) increased blood pressure but this was not the mechanism by which interleukin-induced acid-inhibition was prevented, since similar systemic pressor responses induced by phenylephrine (10 μg kg^{-1} min^{-1}, i.v.) had no such effect. In contrast, IL-1ß (8 ng ml^{-1}) did not significantly inhibit pentagastrin-stimulated (10^{-6} 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-1ß, not involving the inducible NO-synthase.