149 mRNAExpression of Three Different PLA2-Types in Human Gastrointestinal Mucosa
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Phospholipase A2 (PLA2; EC 3.1.1.4) is a key enzyme in inflammation and is thought to play an important part in inflammatory diseases of the gastrointestinal tract. Three genetically, biochemically and functionally different forms of PLA2 have been identified in human tissues, two low molecular weight (14 kDa) forms (PLA2-1 and PLA2-II) and one high molecular weight (85 kDa) form (cPLA2). It is still unknown which type of PLA2 that can be found in the human gastrointestinal tract. Here, the presence of PLA2-1, PLA2-2 and cPLA2 mRNA was investigated in the human stomach, ileum and colon.
Methods: RNA was isolated from the tissues, mRNA was identified and functionally measured by using the reverse transcriptase-polymerase chain reaction (RT-PCR).
Results: (i) mRNA for PLA2-1, PLA2-II and cPLA2 was detected in the stomach, ileum and colon. (ii) In the ileum and the colon, the mRNA expression of PLA2-II markedly exceeded that of PLA2-1 and cPLA2. (iii) PLA2-1 mRNA was markedly increased in the ileum of patients with Crohn’s disease, whereas PLA2-1 and cPLA2 mRNA were unaffected. Conclusion. These results indicate that human gastrointestinal mucosa contains mRNA for three different forms of PLA2. Moreover, the results suggest that PLA2-II might be of importance in Crohn’s disease.

150 Secretion Causes H+ Secretion from Pancreatic Ductules by Vacular Type H+-ATPase
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Secretin stimulates pancreatic ductules to secrete HCO3- into pancreatic juice and H+ to interstitial fluid. The present study was undertaken to examine whether the H+ ion secretion is inhibited by micromolar concentrations of bafilomycin A1, at which concentration the bafilomycin A1 blocks vacuolar H+-ATPase by specific action. Thus, the Aim of the present study was to determine whether the secretin-induced H+ secretion is due to activation of a vacuolar type H+-ATPase.
Methods: Net H+ secretion was estimated from the rate of intracellular pH (pHi) recovery after acid loading (24 mM NH4Cl) of microdissected pancreatic ductules from pig, mounted in a flow-through perfusion chamber on the stage of a fluorescent microscope. pHi was measured from an estimated average of 10–15 cells using the fluorescent pH indicator BCECF and dual-wavelength excitation of fluorescence. The ducts were superfused with HCO3-free HEPES buffers. We used the inhibitor bafilomycin A1 to test whether the secretin-induced H+ secretion was due to vacuolar type H+-ATPase. To identify Na+-H+ exchange, we used the inhibitor amiloride or removal of extracellular Na+ by substitution with choline.
Results: Secretin-induced net H+ secretion of 2.11 ± 0.2 μmol·ml cell volume·min−1 in NH4Cl pulsed, amiloride-treated bile ductules. The net H+ secretion was blocked by bafilomycin A1 (10−6 M), but not by Na+ removal (JH+ 2.02 ± 0.4 μmol·ml cell volume·min−1). The inhibitory effect of bafilomycin A1 was not due to general disruption of cell pHi homeostasis, since normal pHi recovery to NH4Cl pulsed pancreatic ductules was restored by withdrawing amiloride from the superfusion fluid (JH+ 1.98 ± 0.2 μmol·ml cell volume·min−1). Bafilomycin A1 did not block Na+-H+ exchange in pancreatic ductules.
Conclusion: Secretion causes H+HCO3- secretion from pancreatic ductules by a mechanism involving primary active H+ secretion to interstitial fluid by vacuolar type H+-ATPase.

151 Effects of E-64, U-PA and T-PA on Tumor Cell Invasion In Vitro
In order to invade normal tissue and metastasize, cancer cells have to degrade different barriers like basement membranes. As proteolytic enzymes are believed to be necessary for this process, we have studied the effects of the serine protease inhibitor E-64 and the serine protease tissue plasminogen activator (t-PA) and urokinase plasminogen activator (u-PA) on tumor cell invasion in vitro.
Materials and methods Human malignant melanoma cells (LOX) were studied in an in vitro system using transwell chambers containing membranes of 8 μm pore size. Each membrane was coated with the solubilized tissue basement membrane Matrigel. To the upper transwell chambers the LOX cell suspension was added. The transwells were incubated for 72 hours and cells on the lower part of the membrane were counted. For studying possible effects on invasion, nontoxic concentrations of E-64, U-PA and t-PA were added to the upper chambers just prior to adding the cell suspension.
Results: E-64 inhibited the invasion of LOX cells through Matrigel in a dose-dependent manner. When E-64 was added to a final concentration of 400 μM, only 25% of the cells passed the membrane compared with controls. E-64 did not significantly reduce the motility of the tumor cells and did not alter cellular attachment to Matrigel coated dishes. There was no complete inhibition of invasion. U-PA and t-PA increased the invasion through Matrigel. The highest concentration of the serine proteases (2500 ng/ml) gave a 50% increase in invasion through the membrane.
Conclusion: Our results indicate that serine and cysteine proteases are involved in the degradation of basement membranes and thus contribute to the invasion of malignant melanoma cells.

152 Effect of Octreotide Acetate (OA) on Refractory Diarrhea of Patients with AIDS. Randomized Controlled Trial
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OA is a somatostatin analogue. We have previously observed its beneficial effect in severe diarrhea. The aim of this study is to compare the effect of OA to antidiarrheal drugs plus placebo on refractory diarrhoea of AIDS. Methods: 20 male patients with AIDS and refractory diarrhoea were included. Patients were randomly assigned to receive during 10 days either octreotide acetate (mean dose of 600 mcg/kg SC) or high doses of loperamide and diphenoxylate plus placebo (glucose solution). Fecal weight and frequency of bowel movements were daily recorded before and after treatment.
Results:

<table>
<thead>
<tr>
<th>Control</th>
<th>OA</th>
</tr>
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<tbody>
<tr>
<td>P</td>
<td></td>
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<tr>
<td>Age, y, ± sd</td>
<td>35 ± 9</td>
</tr>
<tr>
<td>Death of AIDS, months</td>
<td>23 ± 9</td>
</tr>
<tr>
<td>Diarrhea, months</td>
<td>7 ± 6</td>
</tr>
<tr>
<td>Before treatment</td>
<td>2753 ± 840</td>
</tr>
<tr>
<td>Fecal weight, g/mol</td>
<td>94 ± 2.8</td>
</tr>
<tr>
<td>Response 10 Days after treatment</td>
<td>485 ± 480</td>
</tr>
<tr>
<td>Fecal weight</td>
<td>2 ± 1.6</td>
</tr>
<tr>
<td>Response</td>
<td>1 ± 0.5</td>
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</tbody>
</table>

There was a complete response (fcal weight < 300 g/day) in 2 patients with OA and no control: good partial response (decrease >50% in fecal weight) in 5 with OA and 1 control: partial response (decrease <50%) in 2 with OA and 4 controls and no response in 1 with OA and 4 controls (P < 0.05). Side effects of OA were muscular pain and ileus in 2 patients respectively. Conclusions: OA was superior to antidiarrheal drugs plus placebo for refractory diarrhea in AIDS since complete and good partial response were observed in 70% vs 20% from controls.

153 Fluconazole vs Fluycytosine in the Treatment of Esophageal Candidiasis in AIDS Patients: A Double-Blind, Placebo-Controlled Study
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Objective: Candida albicans is the most frequent cause of esophagitis resulting in dysphagia and odynophagia in AIDS patients. Contrasting opinions exist about the pharmacological treatment of esophageal candidiasis in HIV-positive patients. Aim of this study has been to value the role and the therapeutic efficacy of fluconazole and fluycytosine, compared with placebo, in the treatment of endoscopically-diagnosed esophageal candidiasis in AIDS patients.
Methods: The study has considered 60 HIV-positive patients (36 males and 22 females, mean age 22 ± 2) at first episode of esophageal candidiasis diagnosed by endoscopy. No other opportunistic infection of the esophagus was detected. The patients selected for the study did not follow therapy with AZT and/or with any other antiretroviral drug; in these patients, the mean value of T cells subset CD4+ was 75 ± 20/mm3. The patients have been double-blindly randomized in 3 groups of 20 patients each in relation to pharmacological therapy: (a) the patients of 1st group received fluconazole (F) [3 mg/kg daily
per os (1 tablet 100 mg/twice/daily); (b) the patients of 2nd group received fluoxetine (FCT) [150 mg/kg/daily per os (1 tablet 500 mg/twice/daily); (c) the patients of 3rd group received placebo (P) (1 tablet/twice/daily).

In order to evaluate the efficacy of pharmacological therapy, clinical examination was performed every week up to the end of follow-up (3 months); endoscopic examination was performed at the end of pharmacological treatment (5 weeks). All the patients selected for the study provided informed consent.

Results. After 5 weeks of treatment, complete remission of endoscopic lesions was observed in 14 patients of F-group and in 4 patients of FCT-group (p < 0.05); partial remission of endoscopic lesions was observed in 6 patients of F-group and in 10 patients of FCT-group (p = n.s.), whereas 2 patients of P-group presented partial remission of esophageal lesions. No response was observed in 6 patients of FCT-group and in 18 patients of P-group, with a difference statistically significant in comparison with F-group (p = 0.01 vs FCT-group and p < 0.001 vs P-group).

As regards clinical symptomatology, complete remission was observed in 2 patients of FCT-group and in 14 patients of P-group (p < 0.001). Partial clinical remission was observed in 4 patients of F-group in 6 patients of FCT-group (p = n.s. vs F-group) and in 6 patients of P-group. No clinical response was observed in 2 patients of FCT-group and in 14 patients of P-group (p < 0.001). No remarkable side-effect has been observed in the patients of both groups of treatment.

Conclusions. The results of this study have demonstrated that both F and FCT are efficacious in the treatment of esophageal candidiasis in AIDS patients with a difference statistically significant in comparison with P. F demonstrated a greater therapeutic efficacy than FCT, with a difference statistically significant, as regards both endoscopic and clinical response. Nevertheless, further controlled investigations are needed to improve our knowledge about the therapeutic action of antifungal drugs in the treatment of Candida esophagitis in HIV disease.

154 The Role of Colonoscopy in the Differential Diagnosis of Acute, Severe Haemorrhagic Colitis


The diagnostic value of colonoscopy was assessed in 88 consecutive patients presenting with first attack of severe haemorrhagic colitis [shcH: 2.6 bloody bowel motions daily, fever and abdominal pain]. Blood tests and cultures, stool microscopy, parasitology and culture, abdominal films and sigmoidoscopy were routinely performed on admission. Colonoscopy was performed within 24 hours of admission (no later than 4 days after symptom onset) by a blinded endoscopist and biopsies were taken. Exclusion criteria were age over 65 years, regular use of NSAI/s and acute abdomen. The definite diagnosis of shcH was based on results of histology and stool culture. Core self limiting colitis (ASLC) was diagnosed in 37, infectious colitis (IC) in 31 (salmonella 20, shigella 3, campylobacter 5, pseudomembranous colitis), in 31 patients 4F and UC in 42 patients. 6 patients were correctly classified as IC/ASLC or IB by colonoscopy. Prominent endoscopic features in IC/ASLC were erythema, oedema, telangiectasia, erosions, spontaneous bleeding and small aphthoid ulcers surrounded by a red halo. These lesions were patchily distributed; the sigmoid colon was always severely involved, followed by the rectum and the splenic flexure. The caecum was involved in only 31 IC/ASLC patients. Distal migration of lesions was seen in 15 IC/ASLC patients, who had rectal sparing on admission but subsequently showed severe rectal inflammation on colonoscopy. There were no complications. Repeat colonoscopies and biopsies every 6 months for 18 months were considered that no IC/ASLC case had been initially mis-diagnosed. Thus, colonoscopy is invaluable in the early diagnosis and appropriate treatment of shcH.

155 Helicobacter Pylori Associated Gastritis in HIV Infection: Endoscopic and Histological Features in 32 Pts

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Helicobacter Pylori (HP) is the major etiologic agent associated with acute or chronic gastritis in immunocompetent patients. Moreover HP presence is demonstrated in 40-70% of biopsies of unselected patients undergoing upper endoscopy. Several recent studies have demonstrated low frequency of HP infection in HIV pts [1]. To verify the HP frequency in Italian HIV infected pts we have performed in the last three years, 220 symptomatic pts e.g. duodenal endoscopy with multiple antral biopsies (mean 6). In 32 pts (14.5%) the Gastritis was positive for HP. Endoscopic and histological features are showed in the table.

<table>
<thead>
<tr>
<th>Gastric Location</th>
<th>Endoscopic Features</th>
<th>Histologic Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastric Ulcer</td>
<td>1(3%)</td>
<td>Antral gastritis 15(47%) Duodenitis 8(25%) Normal 17(53%)</td>
</tr>
<tr>
<td>Superficial gastritis</td>
<td>3(9%)</td>
<td>HIV 18 pts AIDS 14 pts</td>
</tr>
<tr>
<td>Chronic gastritis</td>
<td>6(33%)</td>
<td>2(11.5%)</td>
</tr>
<tr>
<td>Chronic active gastritis</td>
<td>6(33%)</td>
<td>1(7%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Histologic Features</th>
<th>Total (32 pts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV</td>
<td>18 pts</td>
</tr>
<tr>
<td>AIDS</td>
<td>14 pts</td>
</tr>
</tbody>
</table>

Our data confirm the low frequency of HP infection in HIV infected pts even if symptomatic for epigastric pain, dyspepsia and vomiting. In all pts we observed various degrees of histologic gastritis even if endoscopic features were macroscopically normal. Not significant difference was registered in frequency and type of gastritis between HIV and AIDS pts. This low frequency of HP infection in HIV pts can be explained by hypochlorhydria typical of all pts. No remarkable side effect was observed even if this suggestion requires subsequent studies to be confirmed [3].


156 The Enteropathogenic E. coli Strain RDEC-1 produces a new enterotoxin active on rabbit ileum in vitro

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RDEC-1 is known to possess biological features similar to human EPEC strains and to be diarrheogenic in the rabbit. We have found that RDEC-1 elaborates an enterotoxin inducing intestinal secretion in the small bowel in vitro.

Rabbit distal ileum and colon, stripped of the serosal and muscular layers were mounted in Ussing chambers where they were bathed by a Ringer solution at 37°C and gassed with 95% O2/5% CO2. In distal ileum both the whole ileum and the segment from RDEC-1 induced a significant increase in potential difference (Delta PD) and short-circuit current (Delta ISc) that was abolished by substituting the chloride ion with sulphate in the bathing solution, by treatment with proteinase K and by heating the supernatant at 90°C for 15 minutes. After fractionating the supernatant, the activity could be demonstrated in the 30-100 kDa fraction.

Curing RDEC-1 of a 42 MDa conjugate plasmid of previously unknown function (which we named pSR) eliminated the enterotoxic activity. Transforming pSR into E. coli HB101 conferred enterotoxic activity to native strain. This strongly suggests that the gene encoding for the toxin is located on pSR. Preliminary attempts to establish the second messenger mediating the action of this new toxin showed no additive effect with maximal stimulation by theophylline, 8BrcGMP and calcium ionophore A23187.

In conclusion, our data suggest that RDEC-1 elaborates a new plasmid-encoded enterotoxin that we named RET for RDEC Enterotoxin.

157 Development of Retinoic Acid as a New Therapeutic Strategy for the Treatment of Pancreatic Cancer


The goal of this study was to evaluate the potential role of retinoid acid (RA) in the treatment of human pancreatic adenocarcinoma. Six different human pancreatic adenocarcinoma cell lines were characterized by cytokeratin phenotyping and found to express cytokeratins 8, 18 and 19 that are typically expressed in ductal pancreatic carcinomas. RA resulted in a time- and dose-dependent decrease of anchorage-dependent and -independent growth of all cell lines. In addition to growth inhibition, all cell lines demonstrated increased cellular differentiation as demonstrated by increased protein synthesis, decreased saturation density, decreased expression of protein kinase C, increased expression of ductal specific marker genes as well as morphological criteria. All-trans, 9-cis and 13-cis retinoic acid were found to be the most potent retinoids regarding growth inhibition and induction of differentiation. To evaluate the molecular basis of the RA action we used a combined approach of Western and Northern Blotting, immunoprecipitation and RT-PCR. In all cell lines tested we found a homogenous expression pattern of the intracellular RA effector molecules. All cell lines expressed the retinoic acid receptors (RAR) alpha, beta and gamma. RAR gamma is usually expressed in epithelial cells, the most sensitive target organ of retinoids. In contrast, retinoic acid receptor isofom (RARX) beta could not be detected. Of the cellular retinoid binding proteins we demonstrated the expression of CRABP and CRBP I but not CRBP II. To verify the expression of RAR in human pancreatic cancer we performed in situ hybridization of 25 human pancreatic carcin-