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MOTOR FUNCTION AND MUCOSAL INTEGRITY OF JEJUNAL AND ILEAL POUCHES USED AS RECTAL SUBSTITUTES AFTER PROCTOCOLECTOMY.

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Background: A jejunal pouch(JP) provides a lower resting pressure and a more distensible reservoir for stool than an ileal pouch(IP) (Takahashi *et al*, J Gast Surg 1998). We wondered whether a JP would maintain its mucosal integrity as well as an IP, in view of the stasis and bacterial overgrowth that occur in such pouches. Methods: Ten dogs underwent proctocolectomy; 5 had JP-distal rectal anastomosis(JP) and 5 IP-distal rectal anastomosis(IP). Five unoperated healthy dogs served as controls. Twelve weeks after operation pouch emptying was assessed scintigraphicaly after instillation per anus of 60 ml ^{99m}Tc-labeled egg white into the pouch. The number of bacteria per gram of stool was determined by culture. Pouch mucosal integrity was tested by documenting the ability of the mucosa to exclude ^{51}Cr EDTA. A 0.1mCi 51Cr EDTA bolus was instilled per anus, and the amount of isotope recovered in the urine over the ensueing 4hr was measured. Results: Pouch emptying was similar in both groups(JP=mean±SEM, 82±0.5 % of instillate emptied; IP=85±4 %,p=0.6), and both groups showed bacterial overgrowth in the feces(JP=aerobes,8 X 10^8±4 X 10^8 cfu/g stool;anaerobes, 18 X 10^8±5 X 10^8 cfu;IP=aerobes,9 X 10^8±5 X 10^8 cfu;anaerobes, 15 X 10^8±4 X 10^8 cfu;p>0.05). Mucosal integrity also was similar in both groups(JP=5.8±1.6% instilled isotope in urine; IP=5.8±1.3%, p>0.05) and differed little from that of the healthy rectum(4.1±2%, p>0.05). Gross and histologic exam of the pouches at autopsy 30 weeks after the operation showed no evidence of inflammation in either type of pouch. Conclusion: Jejunal pouches emptied as well as ileal pouches, when used as rectal substitutes in dogs. While bacterial overgrowth occured in both types of pouches, neither type showed loss of mucosal integrity or inflammation.

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ROLE OF GLUTATHIONE REDOX CYCLE IN PROTECTION OF CULTURED GASTRIC MUCOSAL CELLS AGAINST PEROXYNITRITE

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Background & Aims: *Helicobacter pylori (H. pylori)* infection is a known risk factor for peptic ulceration as well as for gastric cancer. It has beenshown that *H. pylori* infection leads to sustained production of the reactive nitrogen species nitric oxide and peroxynitrite ($ONOO^-$) as part of the host immune response and further to increased apoptosis of gastric epithelial cells in response to free radical-mediated DNA damage (Gut 1994;35:1394, Cancer Res 1996; 56:1279, Cancer Res (1996; 56:3238). Thus, it appears to be critical to determine possible protective mechanisms against $ONOO^-$ in the gastric mucosa. For this purpose, we have used 3-morpholinosydnonimine (SIN-1) as a continuous generator of $ONOO^-$, because SIN-1 generates both O_2^- and NO almost simultaneously in an aqueous solution (Nature 1993;364: 626). We also determined the cytotoxicity of SIN-1 and the effects of the glutathione (GSH) redox cycle on SIN-1-induced injury to gastric epithelial cells *in vitro,* since the GSH cycle is a potent antioxidant in the gastric mucosal cells (Am J Physiol 1991;260:G556, Gastroenterology 1994;106:1199). ***Methods:*** Primary monolayer cultures of rat gastric fundic mucosa were studied as a representative of normal gstric cells with functional integrity and cellular polarity (Methods Toxicol 1993;1A:182). These cells were exposed to SIN-1 or nascent $ONOO^-$, and cytotoxicity was assessed quantitatively by ^{51}Cr release from prelabeled cells. The effects of alterations of the GSH redox cycle were determined. ***Results:*** (1) SIN-1 caused a time-related and dose-dependent increase in ^{51}Cr release. (2) Pretreatment with extracelluar GSH, which accumulated intracellular GSH, prevented SIN-1-induced ^{51}Cr release dose-dependently. (3) In contrast, diethyl meleate (a depletor of intracellular GSH) increased SIN-1-induced ^{51}Cr release, corresponding inversely with the levels of intracellular GSH. (4) Preincubation with buthionine sulfoximine (an inhibitor of γ-glutamylcysteine synthetase), which inhibited intracellular GSH biosynthesis, caused a left shift of the dose response curve for SIN-1. (5) Moreover, pretreatment with 1,3-*bis*(chloroethyl)-1-nitrosourea rendered cells less resistant to SIN-1, corresponding with the inhibition of glutathione reductase activity. (6) Nascent $ONOO^-$ also caused a dose-dependent increase in ^{51}Cr release, which was similarly modulated by the alteration of the GSH cycle. ***Conclusions:*** We have found that $ONOO^-$ induces direct toxicity to cultured gastric epithelial cells. The GSH redox cycle provides significant protection of these cells against $ONOO^-$ *in vitro.* This system may provide a model which permits to determine the protection against mucosal injury and DNA damage in gastric inflammatory diseases (such as those induced by *H. pylori* infection) where generation of reactive nitrogen species and $ONOO^-$ is proposed to be pathophysiologically involved.

G3200

CAN *HELICOBACTER PYLORI* BE SUCCESSFULLY CULTURED FROM RAPID UREASE TESTS?

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Introduction: Culture of *H.pylori (Hp)* and the determination of antibiotic sensitivity is expensive if done routinely on every endoscopy patient but is of increasing importance with the rise in antibiotic resistance. **Aim**: To determine whether *Hp* can be isolated from biopsies used in rapid urease tests (CLOtests). **Method:** Antral biopsies from endoscopy patients were inserted into CLOtests to determine *Hp* status. If the CLOtest was red after the endoscopy session it was sent to the lab for culture. The biopsies were removed from the gel, macerated and plated out onto Blood Agar and Wilkens-Chalgren Agar with and without Dent selective supplement. Plates were incubated for 3–5 days at 37°C in 10% CO_2. **Results:** 63 positive CLOtests were studied at times between 1h and 6h post-endoscopy. Culture success was 88% in the 1st hour but decreased thereafter. **Conclusions:** *Hp* can be isolated if the biopsy is processed within 2h. As CLOtests contain a bacteriostatic agent, all bacterial growth is retarded, but *Hp* can be isolated if the time between endoscopy and plating out is minimized. If the CLOtest is positive, the biopsy can be used for culture and antibiotic sensitivity.

Hrs	Total	*Hp* Pos	*Hp* Neg	% Pos
1	8	7	1	88
2	16	12	4	75
3	20	6	14	30
4	12	3	9	25
5	5	0	5	0
6	2	0	2	0
Tot	63	28	35	

G3201

STRUCTURAL ANALYSIS OF A CD2 EPITOPE MEDIATING APOPTOSIS OF RAT CD4† LAMINA PROPRIA T LYMPHOCYTES

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Introduction: The homeostasis of the intestinal immune system depends on the tight regulation of proliferation and apoptosis. *In vitro* studies have previously shown that apoptosis of LPLs can be mediated via the adhesion receptor CD2. The purpose of the present study was therefore to examine whether CD2 ligation can induce apoptosis *in vivo* and to investigate the structural basis for this effect. Methods: Female Lewis rats (two animals per group) were treated with one of two cross-blocking anti-CD2 monoclonal antibodies (mAb) (OX34 [IgG2a] or OX53 (IgG1]) directed at the CD48 binding site of domain 1 (D1) or isotype control mAbs (2 mg each). Apoptosis was assessed by immunohistology (TUNEL, 3 sections per animal). Antibody affinity to wild type CD2 was measured by using surface plasmon resonance analysis (SPR). CD2 epitope differences were tested by site directed mutagenesis of rat CD2 followed by SPR. Results: OX34 but not OX53 nor the control mAb resulted in depletion of $CD4^+$ lamina propria T cells. This depletion was mediated by apoptosis as assessed by immunohistology (TUNEL positive cells/mm^2 lamina propria, median [range]): OX34: 5.5 [5.0–6.7]; OX53: 2 [1.8–2.4]. Similar results were seen in the white pulp of the spleen. The affinity of OX34 for wild type CD2 was 1000-fold higher than of OX53. Site directed mutagenesis demonstrated that OX34 and OX53 bind to the same site on CD2. However, the OX34 binding site was somewhat smaller and OX34 appeared to bind in a different orientation. Conclusions: These data demonstrate that the CD2 molecule is involved in apoptosis induction of $CD4^+$ lamina propria T cells *in vivo*. Apoptosis induction was accomplished by one of two antibodies that bind to the same region of CD2 molecule (ligand binding site). Our results suggest that the difference between these antibodies may be a consequence of them binding CD2 in a different orientation, or with different affinity of kinetic properties.