83 USE OF A WATER-SOLUBLE CROSSLINKING AGENT TO STUDY EPIDERMAL GROWTH FACTOR (EGF) RECEPTOR ON THE SURFACE OF RAT INTESTINAL EPITHELIAL CELLS

Warren P. Bishop Univ. of IA, Iowa City, IA, USA

The use of conventional ligand binding methods to study EGF receptor in intestinal epithelia is difficult due to high non-specific binding. We have developed a method of crosslinking ^{125}I-EGF to cell surface receptors on isolated intact enterocytes. This enables direct visualization of specifically-labeled receptor after SDS-PAGE. Villus and crypt cells were isolated from adult (200-225g) rat jejunum or ileum by gently shaking everted segments in ice cold 200mM NaCl/2.5mM EDTA/20mM NaPO4, pH 7.4. 6 fractions were collected over 30 minutes. Pelleted cells were resuspended in PBS at 2-10 mg protein/ml. $6$100 ul of cell suspension was incubated with 5 ul (10 ng, 2x10^{6}cpm) ^{125}I-EGF, +/-12.5 ul 100 ug/ml cold EGF, for 2 hr. at 0° C. $_{3}$Cells were pelleted and resuspended in 100 ul of 1.3 mg/ml BS^{3} (Pierce, Rockford, IL) and incubated for 4 hr. 50 ul of 3x Laemmli sample buffer was added, followed by heating to 100°C x 3 min. Samples were then analyzed on 8% SDS-PAGE gels and autoradiographed for 2 days at -70°C. RESULTS: A 170kD specifically-labeled band was observed in both villus and crypt cell fractions of jejunum and ileum. In jejunum only, relatively more p170 was observed in the crypts. CONCLUSION: Use of the water-soluble crosslinker, BS^{3}, enables direct visualization of cell surface EGF receptor on isolated enterocytes. Preliminary results suggest that this molecule is relatively more abundant in the crypts of jejunum, but not ileum. EGF receptor present on non-proliferating villus cells may inhibit excessive mucosal growth by sequestering luminal EGF.

84 LONG TERM PARENTERAL NUTRITION IN CHILDREN (PN): NON ALUMINUM DEPENDENT OSTEOPATHY.

Moukarzel A, Ament ME, Vargas J, McDiarmid S, Reyen L, Najm I, Guss W. University of California, Los Angeles CA.

We previously reported a bone disease with PN related aluminum toxicity. In a prospective study, we assessed the trabecular bone mineral content of 14 asymptomatic patients, age 9.1 ± 3.9 years, on long term parenteral nutrition (PN). The PN was started in the first month of life in 11 pts. and between 3 and 5 years of age in 3 pts. In all children PTH, calcitonin, Vit D,25-OH, Vit D,1,25-(OH)$_2$ and serum aluminum level were within normal range. Computerized tomography was performed in the lumbar region. The mean trabecular bone mineral content measurement of L_1, L_2, L_3 and L_4 was compared to the mineral content of a normal population of the same age and sex. 7/14 pts. had trabecular bone mineral content below -2 SD (108.1 ± 21.6 mg/cc; 62.9% ± 13.9% of the normal mineral content), 5/14 pts were below -1 SD (142.4 ± 12.8 mg/cc; 83.9% ± 6.25%) and 2/14 had normal mineral content. There is a significant correlation (r=0.56, p<0.05) between the duration of PN and the decrease in mineral content. In 50% of the pts., there is a loss of more than 35% of the trabecular bone mineral content, even if they are asymptomatic. Children on long term PN who have never been aluminum toxic still may have a PN dependent osteopathy. The basis for this is unknown.

85 EARLY RESPONSE OF INTESTINAL PROGLUCAGON AND ODC mRNAs DURING ADAPTATION TO JEJUNECTOMY.

M.H.Ulshen, D.B.Rountree, S.E.Selub, R.Fuller, P.K.Lund. Depts of Pediatrics, Medicine and Physiology, Core Center for Diarrheal Diseases, University of North Carolina School of Medicine, Chapel Hill, NC, USA.

Locally produced peptide hormones may mediate adaptive growth of ileum after jejunectomy. Previously, we found that proglucagon (P) mRNA is elevated in adapting ileum at 1 and 5 wk after jejunectomy. A transient rise in mucosal ornithine decarboxylase (ODC) activity identifies early adaptation. In this study, we contrasted patterns of abundance of P and ODC mRNAs in remnant ileum early after 80% resection of proximal small bowel. Controls had transection and anastomosis. Animals were fasted for 24 h after surgery and then pair-fed. Ileum was removed at 4,12, 24,28,36,48 and 96 h after surgery; poly A+ RNAs were analysed by quantitative Northern blot hybridizations using rat P cDNA and synthetic ODC oligomer probes. Abundance of P and ODC mRNAs in ileum of resected animals tripled that of controls by 12 h after surgery; P mRNA remained elevated up to 96 h after surgery while ODC mRNA fell to control levels by 36-48 h. Conclusion: After jejunectomy, there are rapid increases in P and ODC mRNAs, independent of enteral nutrient. Whereas the rise in ODC mRNA is transient and brief, the elevation in P mRNA persists for weeks. This pattern of abundance of P mRNA is consistent with a role of one or more P mRNA products as a mediator of adaptive growth of bowel.

86 EFFECTS OF GASTROINTESTINAL (GI) PRIMING PRIOR TO FULL ENTERAL NUTRITION IN VERY LOW BIRTH WEIGHT (VLBW) INFANTS.

J. Neu, W. Meetze, C. Valentine, N. Sacks, J. McGuigan, M. Conlon. Dept. of Pediatrics and Clinical Research Ctr., University of Florida

In order to determine whether GI priming of small volume enteral feeds (beginning at 2 and increasing to 18 Kcal/Kg/day) improves subsequent growth and tolerance of feedings, and increases trophic hormone levels, VLBW (<1250g) infants were randomized to NPO and GI Priming (PO) groups. Feedings were given in three phases: I - Priming phase (days 3-14) the NPO group received TPN and the PO group received TPN plus GI priming; II - Accelerated phase (days 15-20) both groups increased enteral feedings daily to a maximum of 120 kcal/Kg/Day; III - Maintenance phase (days 21-30) both groups received full enteral feedings. Intakes were controlled to be equal during the first two phases.

	Group	Phase I	Phase II	Phase III
Wt Gain gm	PO(5)	47.0 ± 34.6	180 ± 80.9*	324 ± 148*
Cumulative	NPO(7)	40.0 ± 93.5	101 ± 80.5	275 ± 128
Gastric residuals	PO(5)	0	1.0 ± 1.4	1.6 ± 2.3*
> 3 cc	NPO(7)	0.43 ± 0.73	1.6 ± 1.9	4.2 ± 4.1
Days feedings	PO(5)	1.2 ± 1.5	0.3 ± 0.6*	0.7 ± 1.1*
withheld	NPO(7)	(per protocol)	1.1 ± 1.2	2.5 ± 2.9

Results stated mean ± SD. * p < 0.10

Serum Gastrin concentration (Pg/ml) measured by Radioimmunoassay:

	Day 3(prefeeding)	Day 10	Day 17	Day 24
PO (2)	27.5 ± 9.2	236 ± 65*	111 ± 35	183 ± 61
NPO (2)	34 ± 2.8	40 ± 17	234 ± 128	339 ± 114

Results stated mean ± SD. * p < 0.05

Results suggest that GI priming will improve weight gain and tolerance to subsequent accelerated enteral intake. Even very small enteral intake will stimulate gastrin release, which is known to be trophic to small intestinal development.

87 6 MERCAPTOPURINE (6MP) REDUCES FORMYL-METHIONYL-LEUCYL-PHENYLALANINE (fMLP) INDUCED MACROMOLECULAR UPTAKE IN RAT ILEUM.

V.Khoshoo, F.Daum, P.Karl, J.Markowitz, S.Fisher, M.Silverberg. Dept. of Peds and Research. North Shore University Hospital-Cornell University Medical College, Manhasset, NY.

Increased ileal permeability may play a role in the pathogenesis of Crohn's disease (CD). fMLP, a common bacterial peptide, initiates inflammation and increased permeability in the rat ileum via neutrophil activation and chemotaxis. We investigated the effect of 6MP often used to treat CD, upon fMLP-induced changes in ileal macromolecular uptake. METHODS: Using in situ ileal perfusion, permeability to 0.5% horseradish peroxidase (HRP), ± simultaneous luminal perfusion of 10 $^{-8}$M fMLP, was measured by blood levels over 90 min, in each of 3 groups of male rats: Group I = daily 48 mg 6MP m^{-2} for 14 days; Group II = vehicle-injected, pair fed controls; Group III = ad libitum controls. RESULTS: In the absence of fMLP, HRP uptake was low in all Groups (90 min blood HRP <37 ± 5 units)(mean ± SE). With fMLP, HRP uptake was markedly elevated over basal in rats not receiving 6MP: Group II = 210 ± 12; Group III = 263 ± 24 units, ($p<0.01$). Conversely, 6MP treatment (Group I) ameliorated the fMLP response (106 ± 8 units, $p<0.01$). 6MP therapy decreased the circulating neutrophil count, but did not alter mucosal myeloperoxidase, a neutrophil marker. CONCLUSION: 6MP therapy decreases macromolecular uptake induced by fMLP. SPECULATION: In CD, 6MP may be therapeutic through diminished neutrophil inflammatory response and reduced antigen uptake.

88 CONTRIBUTION OF COLONIC ACETATE (Ac) TO CIRCULATING ACETATE POOL IN AN INFANT PIG MODEL.

KL Freeman, DG Burrin, and Carlos H. Lifschitz. USDA/ARS Child Nutr Res Ctr, Baylor Coll Med, Dept Pediatr, Houston, TX.

Ac results from colonic fermentation of carbohydrate and fatty acid metabolism, and can be an energy source for infants. The contribution of colonic Ac to the circulating Ac pool was studied in 4 weaned, fasted, anesthetized neonatal pigs (age 5-11 d, wt 1.7-2 kg). Baseline Ac entry rate was determined from the plateau enrichment of ^{3}H-Ac in the portal vein during a 1-h infusion. Subsequently, ^{14}C-Ac (1 mmol/kg, 185-243 dpm/nmol) was infused intracecally for 1 h. ^{3}H-Ac dilution in the portal vein was used to calculate the Ac entry rate. The dilution of ^{14}C-Ac provided a measure of endogenous Ac in the circulating Ac pool. Colonic infusion increased portal Ac by 321% over baseline (% DOB). In 2 animals, no dilution of ^{14}C-Ac indicated that all circulating Ac was derived from the colonic infusion. In 1 animal, dilution of ^{14}C suggested that endogenous production of Ac was not suppressed. The table shows values before (Baseline) and after 1 h of intracecal infusion (End and Portal Ac).

Pig	Ac Entry Rate (^{3}H)			^{14}C-Ac Specific Activity		
	Baseline	End	% DOB	Infused	Portal	Entry %
	μmol/kg/h			dpm/nmol		
1	80	333	316	191	182	95
2	141	613	335	200	205	103
3	86	382	344	243	208	86
4	175	682	290	185	125	68

Colonic Ac may make a major contribution to the circulating Ac pool and thereby affect endogenous production.