● G1758

LISOFYLLINE AMELIORATES ILEAL ISCHEMIA/REPERFUSION-INDUCED MUCOSAL BARRIER DYSFUNCTION IN RATS. S. Wattanasirichaigoon, M.J. Menconi, R.L. Delude, M.P. Fink. Department of Surgery, Beth Israel Deaconess Medical Center, Boston, MA.

Lisofylline (LSF) blocks synthesis of phosphatidic acid, which is a second messenger for several pro-inflammatory stimuli. We hypothesized that intestinal mucosal ischemia/reperfusion injury might be mediated *via* phosphatidic acid signaling and that might be ameliorated by treatment with LSF. Ischemia was induced in the distal ileum of Sprague-Dawley rats (250-350 grams) by occluding the regional mesenteric vessels for 60 min, followed by 60 min of reperfusion. Animals were randomized to receive *i.v.* LSF (15 mg/kg bolus + 10 mg/kg/hr), or an equivalent volume of Ringer's lactate solution (control). An everted gut sac technique was used to assess ileal mucosal permeability to fluorescently-labeled dextran (M.W.=4,000 Daltons), expressed as an apparent clearance(AC). Levels of reduced glutathione (GSH) were determined as a measure of oxidant stress. GSH was measured spectrophotometrically after oxidation with 5-5'-dithiobis (2-nitrobenzoic acid). No significant difference in maximal blood flow of reperfused guts was observed between the LSF-treated and control groups.

Study	Ileum	AC (x10-3µl/min/cm²)		GSH (µg/mg prot)	
		Control (n=8)	LSF (n=8)	Control (n=8)	LSF (n=8)
1	Baseline	6.0±1.1	4.0±0.6	11.8±2.4†	11.6±2.5†
	Post-ischemia	64.0±7.1*	34.4±6.1*	5.3±1.3†	5.7±1.4†
	Post-reperfusion	43.0±8.8	48.3±7.4	5.9±1.1	6.3±2.0
2	Baseline	6.4±0.7	6.9±1.0	7.7±1.0†	11.0±1.6†
	Post-ischemia	42.8±6.7	56.3±10.3	3.3±0.3†	4.3±0.7†
	Post-reperfusion	40.2±4.5*	23.1±3.8*	2.9±0.4	3.0±0.6

Note: Study 1=drug given 5 min prior to ischemia, Study 2=drug given 1 min prior to reperfusion, * unpaired t-test (control *vs* LSF); $p < 0.05$, † paired t-test (baseline *vs* post-ischemia); $p < 0.05$

Our results suggest that LSF is effective in reducing ischemia/reperfusion-induced gut barrier dysfunction, however the mechanism of action remains unknown.

This research was partially supported by a grant from Cell Therapeutics, Inc., Seattle, WA.

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ENHANCED EXPRESSION OF MCP-3 IN CHRONIC INFLAMMATORY BOWEL DISEASE (IBD). J. Wedemeyer, A. Lorentz, M. Göke, P. Flemming*, M.P. Manns, S.C. Bischoff. Dept. Gastroenterology & Hepatology, *Dept. Pathology, Medical School Hannover, Germany

Monocyte chemotactic protein 3 (MCP-3) belongs to the family of C-C-chemokines and has chemoattractant and activating capabilities in monocytes, lymphocytes, eosinophils and basophils. The role of MCP-3 in IBD has not been studied yet. We examined biopsies from patients with IBD (ulcerative colitis n=10, Crohn's disease n=8) derived from inflamed (n=16) and non-inflamed (n=10) mucosal areas, and healthy controls (n=19). Tissue sections were stained immunohistochemically using an anti human MCP-3 mAb. In addition, MCP-3 mRNA and protein expression was analyzed by primer dropping RT-PCR and immunohistochemistry in epithelial cell lines (HT-29, Caco-2, T-84) stimulated with IL-1β, IL-6 and TNF-α. MCP-3 protein was detected in epithelial cells, both in patients with IBD and in controls. MCP-3 staining was particular pronounced in areas of active mucosal inflammation. A similar MCP-3 staining was found in biopsies derived from controls and patients with IBD, provided that the latter were obtained from macroscopic normal mucosal sites. The intensity of MCP-3 staining and the extent of epithelial destruction was positively correlated (both expressed semiquantitatively using a score system). In human epithelial cell lines, MCP-3 protein expression could be increased by stimulation with proinflammatory cytokines such as TNF-α, IL-1β or IL-6. Furthermore, enhanced MCP-3 mRNA expression was detectable after stimulation with these cytokines. In conclusion the data demonstrate that human intestinal epithelial cells are capable of producing MCP-3, and that MCP-3 production is enhanced during chronic inflammatory conditions. Therefore, MCP-3 may be involved in the regulation of recruitment and activation of immunocompetent and inflammatory cells in the intestinal tract.

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THE PREVALENCE OF OSTEOPOROSIS IN PATIENTS WITH GASTROINTESTINAL ALLERGY. M Weidenhiller, S Winterkamp, D Schwab, M Raithel, J Hensen, EG Hahn; I. Department of Medicine, Functional Tissue Diagnostics, University of Erlangen-Nuremberg, FRG

Introduction: Osteoporosis (OP) is often due to malabsorption and deficiencies in calcium and vitamin D. The clinical picture of gastrointestinal allergies (GA) can vary and the severity is graded from I (gastrointestinal symptoms only) to IV (severe, systemic anaphylactic reaction). The main symptoms of intestinal manifestations are diarrhoea, malabsorption and abdominal pain. Therefore, we investigated the prevalence of OP in patients with GA.

Methods: We examined 19 patients (8 male, 11 female, mean 48 years, range 25 to 68 years) with a diagnosis of GA confirmed by oral provocation. Provocation was done placebo-controlled (double- or single-blind). All patients complained of diarrhoea. We excluded a history of steroid use or hyperthyroidism and classified female patients regarding hormone replacement therapy. Moreover, lactose intolerance or celiac disease were accounted for. Patients underwent osteodensitometry scanning of femur neck and lumbar spine with Lunar DPX-L bone scan. Bone density was measured as bone mineral density in g/cm². OP was defined as bone density below 2,5 standard deviations of peak bone mass of young adults (WHO classification).

Results: Of the 19 patients tested, 3 (16%) had OP, all of them female (mean 49 years, range 38 to 68 years). The patient with the most severe OP was 68 years old, not on hormone replacement therapy and suffered from lactose intolerance. The second patient had a diagnosis of lactose intolerance. The OP was not associated with the severity of the GA, also patients with GA grade I and intestinal symptoms had a normal bone mineral density.

Discussion: Our preliminary data indicate that OP is a rare feature of GA. The prevalence and grade of OP is not correlated with the grade of the GA, but other conditions like postmenopausal state and lactose intolerance seem to play a more important role. Although diarrhoea and malabsorption are common features in GA, these mechanisms do not result in a decreased bone density. GA was not found to be a precipitating factor for osteoporosis, because it is mostly mediated by TH2-cytokines (like IL-4, -5, -10). These cytokines are not thought to be involved in bone demineralizing actions in contrast to other proinflammatory cytokines like IFN-gamma, TNF-alpha or interleukin-1 (as found in inflammatory bowel disease).

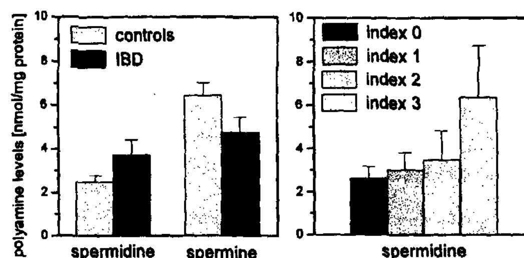
● G1761

POLYAMINE LEVELS IN COLONIC EPITHELIAL CELLS DURING INFLAMMATORY BOWEL DISEASE (IBD). T. Weiss[1], M. Cetto[1], H. Zirngibl[1], J. Schölmerich[2], K.W. Jauch[1], G. Rogler[2]. Dept. of Surgery ([1]) and Internal Medicine I ([2]), University of Regensburg, Germany

Background: Polyamines (e.g. putrescine, spermidine, spermine and their acetylated derivatives) are considered to be essential for proliferation and differentiation of the rapidly renewing intestinal mucosa. Whereas spermine is more important in differentiation, putrescine and spermidine are necessary for proliferation. A dysregulation of polyamine synthesis could lead to an impaired epithelial barrier and induce chronic intestinal inflammation.

Methods:. Polyamine levels were determined in isolated epithelial cells from endoscopical biopsies from 40 controls and 26 patients with inflammatory bowel disease by a new high-resolution reversed-phase high-performance liquid chromatography method (Analytical Biochemistry 1997, 247: 294-304).

Results: N^8-acetyl-spermidine (N^8-Ac-Spd) and spermidine (Spd) levels were significantly higher in IBD patients (N^8-Ac-Spd: 1.72 ± 0.26 nmol/mg protein in IBD vs. 1.14 ± 0.2 in controls; Spd: 3.73 ± 0,69 vs. 2.49 ± 0.26), whereas spermine levels (4.75 ± 0.68 nmol/mg prot. in IBD vs. 6.44 ± 0.55 in controls) were significantly lower compared to controls ($p < 0.05$). When the polyamine levels were correlated to the inflammatory index (0=without inflammation, 3=severe inflammation) of the patients a clear correlation of highest levels of putrescine, N^8-acetyl-spermidine and spermidine with most active inflammation (inflammatory index 3) could be found.



Conclusion: These data indicate a significant increase of polyamines important for proliferation and a decrease of polyamines necessary for differentiation in IBD-mucosa. As differentiation of cells only can take place when proliferation is sufficient orally administered polyamines could support epithelial regeneration in IBD.

● G1762

EVIDENCE FOR NOVEL MECHANISMS THAT SILENCE IL-8 GENE TRANSCRIPTION. X.M. Wen and G.D. Wu. Division of Gastroenterology, University of Pennsylvania School of Medicine, Philadelphia, PA.

Cis-acting DNA elements and their cognate binding proteins which are capable of transactivating genes at an extended distance from the structural gene have been well characterized and are known as enhancers. In contrast, relatively few examples of mechanisms which silence gene transcription at a distance have been described. In order to identify potentially novel anti-inflammatory mechanisms, we have been investigating various cell culture model systems in which cytokine genes are transcriptionally repressed. The mRNA and protein expression of the proinflammatory cytokine, interleukin 8 (IL-8), is inhibited by spontaneous differentiation of the Caco-2 colon cancer cell line by growth to a postconfluent state. Nuclear run-on studies demonstrate that the IL-8 gene is inhibited at the transcriptional level in post-confluent Caco-2 cells. All known transcriptional elements which regulate the IL-8 gene reside within the first 135 bp of the 5'-flanking region. The element(s) responsible for inhibiting IL-8 gene transcription in post-confluent Caco-2 cells, however, are not located in the immediate 5'-flanking region of the IL-8 gene. Functional analysis of this region using stabily integrated