P274 Hydrocortisone and noradrenaline down-regulate the expression of the constitutively expressed colonic epithelial antimicrobial peptides
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Introduction: Psychological stress worsens inflammation in inflammatory bowel disease (IBD). In experimental animals, stress increases intestinal mucosal ingress of bacteria [1] and in the skin modulates the expression of antibacterial peptides (ABPs) [2].

Aims: To test the hypothesis that constitutively expressed ABPs are inhibited by stress and predispose to relapse in patients with quiescent IBD, we have assessed the effects of three putative neurohumoral stress mediators, hydrocortisone, noradrenaline and histamine on colonic epithelial expression of human beta defensin-1 (hBD1) and lysozyme in vitro.

Methods: Confluent cells from the human colon cancer-derived cell HT29 line were incubated with the putative stress mediators for 24 hrs. RNA was subsequently extracted, reversed transcribed and gene expression of lysozyme and hBD1 determined using real-time PCR. Gene expression was calculated by relative quantification against the housekeeping gene, GAPDH, and calibrating the samples to untreated controls. For each mediator, 5 samples were included in the analysis, and differences sought using the Mann Whitney U test. Results are expressed as fold reduction (FR) from the vehicle control-treated samples for each mediator.

Results: Hydrocortisone dose-dependently down-regulated expression of both hBD1 and lysozyme. Fold-reductions for hBD1 compared with untreated controls were FR 4.42 (mean ± SEM) for 10−6M, and 32.7 ± 9.9, p < 0.01 for 10−4M hydrocortisone; for lysozyme, the equivalent FRs were 1.19 ± 0.53 for 10−6M and 9.1 ± 2.2, p < 0.01 for 10−4M hydrocortisone. Noradrenaline (10−5M) also reduced hBD1 (FR 4.8 ± 0.9, p < 0.01) and lysozyme expression (FR 6.5 ± 2.0). Histamine (10−5M) had no effect on the expression of either gene.

Conclusions: These results suggest that psychological stress, by releasing hydrocortisone and noradrenaline, may down-regulate the expression of constitutive antibacterial peptides, potentially allowing bacterial ingress and precipitating inflammation in the lamina propria. The inhibitory effects on antibacterial peptide expression of hydrocortisone could underlie its tendency when used therapeutically in IBD and other settings to predispose to sepsis.

Reference(s)

P275 Simvastatin reduces the proliferation and adhesion of intestinal colon lamina propria fibroblasts (CLPF) from fibrotic mucosa of patients with Crohn's disease and modulates the Smad3-mediated TGF-β1 pathway
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Background: A medicamentous treatment of stricturing/fibrotic complications in Crohn's disease (CD) is not established. The HMG-CoA-reductase-inhibitor Simvastatin shows anti-fibrotic effects in rat kidney. Therefore we determined the influence of Simvastatin (S) on cell-behaviour and the TGF-β1 mediated signalling in intestinal colon lamina propria fibroblasts (CLPF) of control (Co) and CD-patients.

Methods: Co- and fibrosis-CLPF were preincubated for 6 h with S and incubated for further 24 h with or without 10 ng/ml TGF-β1. These stimulated CLPF were extracted and checked for adhesion, proliferation, and protein amounts of the TGF-β1 signal pathway.

Results: In Co-CLPF TGF-β1 had no significant effect on adhesion and proliferation. S-preincubation did not influence the TGF-β1-mediated effect. In cells from fibrotic mucosa of CD-patients S-preincubation and TGF-β1 exposition led to an inhibition of proliferation and adhesion to different ECM-proteins. In Co-CLPF S and TGF-β1 exposure reduced the Smad2- and phosphoSmad2-concentration. Smad3, phosphoSmad3, Smad4, and Smad7 were not influenced significantly. In contrast in fibrosis-CLPF S exposure led to a decreased phosphorylation of Smad3 and could reduce the effects of intracellular TGF-β1 pathway.

Conclusions: S exposure reduces proliferation, adhesion and the phosphorylation of Smad3 in CLPF from fibrotic areas and could demonstrate an approach in fibrogenesis.

P276 SGLT-1 activation, a new approach to Crohn's disease
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Aim: Crohn's disease is an ongoing disorder whose treatment, at this time, can help control the disease by lowering the number of times a person experiences a recurrence, but there is no cure. Our recent findings indicate that the activation of SGLT-1, present at apical membrane of enterocytes, interacts with inflammatory signalling and suggest a novel role as an immunological player for this transporter, that orchestrates a local and systemic anti-inflammatory and cytoprotective response, following an inflammatory stimulus, such as LPS. The cornerstone of SGLT-1 activation, resides in the ability to induce reduction of systemic pro-inflammatory signals, such as TNF-α, and induction of endogenous IL-10 production by the host's immune cells. Our studies performed in vitro and in vivo show that SGLT-1 activation is able to provide cytoprotective effects on enterocytes. In the present work, we aim to show that activation of SGLT-1 at intestinal epithelial level may be suggested as a new pharmacological approach for treatment of Crohn's disease.

Materials and Methods: Immunofluorescence reaction was performed to stain occludin and ZO-1 in Caco-2 monolayers exposed to DSS. For in vivo studies, a chemically-induced mouse model of chronic intestinal inflammation was used. Following oral treatment with D-glucose, transepithelial electrical resistance of isolated mouse colon and colon length were evaluated. Inflammatory cytokines IL-12, TNF-α, IL-17, IFN-γ, KC, and anti-inflammatory IL-10 were evaluated by ELISA in plasma and in organotypic cultures of colon.

Results: Crohn's disease is characterized by a disrupted intestinal barrier function, manifested by an increase in intestinal epithelial permeability. This barrier dysfunction is correlated with tight junction (TJ) proteins disruption. Our in vitro studies showed that D-glucose in the medium of Caco-2 cells leads to protection of TJ proteins (occludin and ZO-1) from DSS-induced degradation. Oral in vivo administration of D-glucose, leads to protection of TJ and restores barrier function in our...