Altered Expression of Tight Junction-Related Proteins and Increased Intestinal Permeability in a Rat Model of Post-Infectious Gut Dysfunction

Fernandez JA et al.
Abstract 520
Background and Methods

• Background
  – Alterations of epithelial barrier function (EBF) are a common feature of functional bowel disorders, such as post-infectious irritable bowel syndrome (PI-IBS).

• Objective
  – characterize changes in the expression of barrier function-related TJ proteins, as the basis for EBF alterations, in a rat model of PI-IBS

• Study design
  – Experimental *T. spiralis* infection in rats was used as a model of PI-IBS
  – At days 2, 6, 14 and 30 post-infection (PI), jejunal samples were collected.
  – Histopathology and interleukin (IL)-6 expression were assessed to characterize intestinal inflammation
  – Transepithelial conductance and FITC-dextran (4kD, FD4) flux served to evaluate paracellular permeability
  – Local modulators of TJ proteins, namely mucosal mast cell (MMC)-derived proteinase rMCP-2 and proglucagon (precursor of the barrier-enhancing factor GLP-2), were evaluated by immunostaining and RT-qPCR.
Results

- Upregulation of IL-6 expression were observed beginning on day 2.

- Inflammation-related alterations were maximum on day 14 PI, coinciding with significant alterations of EBF (consistent with increased permeability).

- Histological changes and IL-6 overexpression mostly reverted by day 30 PI, however EBF alterations persisted.

- Expression of TJ proteins associated with the enhancement of the epithelial barrier (claudin-3 and occludin) was reduced.

- Upregulation of the pore-forming protein claudin 2 was observed.

- Overexpression of rMCP-2 was observed from day 2 PI, confirmed by the presence of a MMC hyperplasia that persisted up to day 30 PI.

- Expression of proglucagon, precursor of GLP-2, was reduced by 40%.
Conclusions

- Altered expression of transmembrane TJ proteins is consistent with a loss of epithelial tightness, and provides a molecular mechanism for the long-lasting increased epithelial permeability observed in the *T. spiralis* infection model.

- Enhanced levels of the MMC-derived proteinase, rMCP-2, and the down-regulation of the GLP-2 precursor, proglucagon, are likely to participate in the local rearrangement of tight junctions.
Cytokine and Clinical Response to *Saccharomyces boulardii* Therapy in Diarrhea-Dominant Irritable Bowel Syndrome: A Double-Blind, Randomized, Placebo-Controlled Study

*Abbas Z et al.*
*Abstract Su2036*
Background and Methods

• Background
  – Saccharomyces boulardii is a probiotic yeast
  – Its effect on blood and tissue cytokines in patients with diarrhea-dominant irritable bowel syndrome (IBS-D) has not been extensively investigated.

• Objective
  – Assess the efficacy of S boulardii to improve cytokine profile, symptoms, quality of life, and histology in patients with IBS-D

• Study design
  – Double-blind, placebo-controlled study
  – Patients (N=72) randomized to S boulardii 250 mg orally three times daily or placebo for 6 weeks
  – Pretreatment colonoscopy and end of treatment sigmoidoscopy conducted
  – Rectal biopsies and blood samples were taken pre- and post-treatment for the evaluation of IL-8, IL-10, IL-12, and TNF-α levels
Results

• In the active drug group, there was a significant decrease in blood levels of pro-inflammatory cytokines IL-8 and TNF-α as judged by comparing mean improvement ($P=0.0001$)
• There was an increase in anti-inflammatory cytokine IL-10 level ($P=0.002$).
• Tissue cytokine IL-8 level decreased ($P=0.002$)
• IL-10 and IL-10/IL-12 ratio increased in the active drug group ($P=0.006$, $P=0.008$, respectively)
• Bowel-related symptoms improved in each group without statistical differences except abdominal pain which was less severe at the end of treatment in the placebo group
• Improvement in 14 out of 34 parameters of QOL-IBS in patients who received the active drug (vs 6 parameters with placebo)
• Histologic improvement noted
Conclusions

- *S. boulardii* with Ispaghula husk was superior to placebo with Ispaghula husk in improving cytokine profile and quality of life in patients suffering from IBS-D

- This study, albeit mildly positive, was small; thus, few definitive conclusions can be drawn
Atopic IBS: Is it Really Different From Non-atopic IBS?

Störsrud S et al.
Abstract Mo2042
Background and Methods

• Background
  – Atopic IBS has been proposed to be a new IBS subgroup
  – The presence of allergy has been correlated with more severe IBS symptoms in a recent study
  – Whether atopic IBS patients really differ from non-atopic IBS patients remains to be established

• Objective
  – To assess the presence of atopy in IBS and to characterize the association with symptoms and self-reported food intolerance

• Study design
  – Double-blind, placebo-controlled study
  – Patients (N=197) with IBS assessed for total and specific IgE in serum and blood eosinophilic granulocytes
  – Patients completed questionnaire assessing presence of atopic disease and symptoms after intake of 56 different food items relevant for food intolerance/allergy
  – Also completed IBS symptom severity (IBS-SSS), anxiety and depression (HAD) and somatic symptoms (PHQ-15) measures
Results and Conclusions

• The presence of an atopic disease (eczema, asthma and/or rhinoconjunctivitis) was reported by 92 patients (48%)

• Except for more severe somatic symptoms in IBS patients with atopic disease vs non-atopic IBS (PHQ-15: 13.3±4.5 vs. 11.9±5.0; \( P=0.03 \)), no differences in IBS symptom pattern/severity, anxiety or depression were noted

• Conclusions
  – Atopic disease is common in patients with IBS, but whether this is of relevance for their IBS symptoms is less clear
  – Data do not support major differences in symptom pattern or severity between atopic and non-atopic IBS
FODMAP Diet Modulates Visceral Nociception by Changing Gut Microbiota and Intestinal Inflammation

Zhou S-Y et al.
Abstract 164
Background and Methods

• Background
  – Foods that are high in FODMAPs (fermentable oligo-, di- and mono-saccharides and polys) may exacerbate symptoms of IBS
  – FODMAPs may modulate visceral nocieption by altering gut microbiota and intestinal inflammation

• Study design
  – Wistar rats fed regular, low, or high FODMAP diets
  – Visceral hypersensitivity, gut microbiota, and intestinal inflammation assessed
Results and Conclusions

- High-FODMAP diet caused significant increase in intestinal inflammation (as measured by TNF-alpha and IL-6 levels in ileal mucosa) and gut permeability
- High-FODMAP diet was associated with significant changes in composition of the gut microbiota
  - Decreases in clostridiales (18% vs 70%), Peptostreptococacea (<1% vs 12%) and Lactobacillaceae (<1% vs 12%)
  - Increases in Erysipelotrichaceae (69% vs 5%) and Lachnospiraceae (5% vs <1%)
- Visceral hypersensitivity developed in high-FODMAP but not control groups
- Two weeks of low-FODMAP diet prevented mucosal inflammation, improved gut barrier function, and normalized visceral hypersensitivity
Human Gut Microbiota Modulates Gut Serotonergic Pathway and Motility

Kashyap PC et al.
Abstract 436f
Background and Methods

• Background
  – Serotonin (5-hydroxytryptamine, 5-HT) is a gut-derived neurotransmitter with important roles in regulating visceral sensation, secretion and intestinal motility
  – Serum concentrations of 5-HT are nearly 3-fold lower in germ free mice (GF) compared to conventionally raised mice, however, the functional relationship between the microbiota and gut-derived 5-HT is not well understood

• Study design
  – Germ-free mice, ex-germ-free mice colonized with human feces (humanized) and conventionally raised mice compared
  – Gut transit and motility compared using carmine red dye and colonic manometry
  – Fecal metabolites and changes in gene expression in colonic tissue were measured
Results

- GI transit time (min) was shorter in humanized mice than GF controls ($P<0.05$), but not different from conventionally raised or ex-GF mice colonized with mouse feces.

- Colonic manometry revealed significantly higher contractility in humanized mice compared to GF mice ($P<0.05$).

- Significantly higher concentrations of a metabolite with an m/z ratio corresponding to that of 5-hydroxyindoleacetic acid (5-HIAA) in the feces of humanized mice compared to GF mice.

- Colonic expression of Tph1 mRNA (tryptophan hydroxylase 1, rate limiting for 5-HT biosynthesis) was significantly higher in humanized mice compared with GF mice (1.9-fold and 2.5-fold in the proximal and distal colon, respectively, $P<0.05$).

- Expression of MaoA mRNA (monoamine oxidase A, the main enzyme for enteric catabolism of 5-HT to 5-HIAA) was not significantly different between GF and humanized mice.

- Treatment with a 5-HT3/4 receptor antagonist led to significantly delayed gut transit in humanized mice ($P<0.05$) but had no significant effect in GF mice.
Conclusions

• These data suggest that gut microbiota affect gut motility through a microbe-induced increase in 5-HT production by gut mucosa

• Gut microbiota-induced changes in serotonin pathways represent potential therapeutic targets for treatment of gut motility disorders
Irritable Bowel Syndrome Symptoms Are Related to the Resting Brain's Sensorimotor Network

Chen MP et al.
Abstract 574
Background and Methods

• Background
  – Altered function of the brain’s pain processing pathways have been identified in IBS during task related functional imaging studies, however less is known about the impact of IBS on the resting brain

• Aims
  – To demonstrate the relationship between IBS symptoms and sensorimotor resting state network (SM-RSN) function
  – To assess the effect of successful mind-body interventions (IBS directed hypnosis or educational classes) on the SM-RSN

• Study design
  – Female patients (N=30) with Rome III IBS underwent a 10-minute resting fMRI on a 1.5-Tesla scanner
  – Completed either a series of standardized hypnosis or educational sessions followed by a repeat scan
Results

• IBS-SSS scores decreased from a mean of 336 (SD=65) at baseline to 239 (SD=89) after treatment ($t=5.56, P<.001$)

• Both treatment groups showed a clinically significant response (defined as >50 point decrease) and there was no difference between groups ($P=0.42$) so the groups were combined for the remaining post treatment analysis

• Baseline SM-RSN was well correlated to the template

• Before treatment, IBS-SSS showed a significant correlation to the sensorimotor network functioning at the posterior insular cortex

• Post-treatment connectivity of the posterior insula to the SM-RSN decreased ($T=5.2, P<0.001$)
Conclusions

- Strength of posterior insula connectivity to a resting state sensorimotor network is related to subjective IBS symptoms and that connectivity decreases when symptoms improve
PPI Use and Small Intestinal Bacterial Overgrowth Detected on Lactulose Breath Testing: Results of a Prospective Cross Sectional Study Among U.S. Veterans With Irritable Bowel Syndrome

Jahng AW et al.
Abstract Su2026
Background and Methods

• Background
  – Proton pump inhibitors (PPIs) have been proposed to be the missing link in the controversy surrounding small intestinal bacterial overgrowth (SIBO) and irritable bowel syndrome (IBS)

• Aims
  – To calculate adjusted odds ratios (ORs) for hydrogen (H₂) and methane (CH₄) SIBO based upon PPI
  – To determine the impact of PPI use on lactulose breath testing (LBT) parameters

• Study design
  – Patients (N=149) with IBS included
  – Use of PPIs, fiber, laxatives, probiotics, H2-blockers, anticholinergics, diagnoses of GERD and BMI were obtained from medical records
  – Patients underwent LBT with positivity assessed using different criteria:
    • Two H₂ Peaks (Increase >20 ppm over the baseline by 90 min with a single peak occurring at least 15 min prior to the second peak with a trough after the first peak of > 5ppm)
    • Increase in H₂ by > 20 ppm by 90 min. 3) Any CH₄ > 5 ppm) Rise in CH₄ by > 20 ppm by 90 min
Results

- The prevalence of H$_2$-SIBO using two H$_2$ peak criteria was 36.9% in PPI vs. 17.9% in non-PPI groups (P=0.01)
- Adjusted OR for PPI Use and two-peak H$_2$-SIBO was 4.3 (95% CI 1.4-12.9, P=0.01)
- PPI use of >180 days was found to be associated with two-peak H$_2$-SIBO with OR 3.2 (95% CI 1.2-8.9, P=0.02)
  - ORs for PPI use and SIBO were not statistically significant using the other H$_2$ or CH$_4$ criteria
- There was a trend towards a shorter time to P1 in the PPI group
- A statistically significant difference was found in time to P1 when comparing PPI use of >180 days vs. < 180 days (58.7 vs. 75.7 min, P=0.02)
- Comparisons between PPI vs. Non-PPI groups did not find differences in baseline H$_2$ (0 vs. 0 ppm, p=0.45), CH$_4$ (1 vs.1.2 ppm, P=0.70) or amplitude to peak 1 (50 vs. 45 ppm, p=0.55).
Conclusions

• PPI use is associated with an increased prevalence of H2-SIBO on LBT but only when using the Two H2 Peak criteria
  – This may be caused by an earlier rise in H2 in the proximal small bowel seen with prolonged PPI use
Mechanisms Underlying Linaclotide-induced Inhibition of Colonic Nociception

Castro J et al.
Abstract 743
Background and Methods

• Background
  – Both exogenous extracellular cGMP and linaclotide inhibit colonic nociceptor mechanosensitivity

• Aims
  – To determine the mechanisms underlying inhibition of colonic nociceptor mechanosensitivity by linaclotide

• Study design
  – Mechanosensory responses of colonic splanchnic high-threshold nociceptors were compared in vitro in the presence and absence of linaclotide (1, 30, 100, 300 and 1000nM) applied to the colonic mucosal surface
  – Linaclotide was evaluated in healthy C57BL/6 and GC-C null mice and in mice with chronic visceral hypersensitivity (CVH), 28 days post-TNBS administration, when inflammation had resolved and nociceptors were mechanically hypersensitive
  – Linaclotide-induced effects were also investigated in the presence of the cGMP transport blocker, probenecid (1mM) and the hyperpolarization-activated cyclic nucleotide-gated (HCN) channel blocker ivabradine (3μM)
  – Linaclotide also evaluated in TRPA1 -/- and TRPV4 -/- mice
Results

• In healthy mice, linaclotide significantly and dose-dependently reduced nociceptor mechanosensitivity 100, 300 and 1000nM ($P<.001$)
• In chronic visceral hypersensitivity this effect became more potent, with linaclotide (30, 100, 300 and 1000 nM) significantly reducing nociceptor mechanosensitivity ($P<.001$)
• Linaclotide-induced inhibition was completely lost in GC-C -/- mice ($P>.05$, n=7)
• Linaclotide-induced inhibition was prevented by the prior application of the cGMP transport blocker probenecid ($P>.05$, n=6), or in separate preparations by the prior removal of the colonic mucosal epithelium ($P>.05$)
• Linaclotide remained able to inhibit nociceptors in the presence of the HCN blocker ivabradine ($P<.01$)
Conclusions

• This is a comprehensive set of animal experiments showing a consistent pattern of result that demonstrate that linaclotide reduces visceral pain hypersensitivity by a mechanism involving the release of cGMP, probably from the basolateral membrane.

• The effect requires an intact mucosal epithelium, presumably because this is where the receptors are located that linaclotide targets.

• The effect is independent of TRPV4 and TRPA1 nociceptors.

• The missing piece is the identity and location of the nociceptors which cGMP targets.

• This study provides a compelling explanation for the effects of linaclotide on abdominal pain.