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Propionate Ameliorates Dextran Sodium Sulfate-Induced Colitis by Improving Intestinal Barrier Function and Reducing Inflammation and Oxidative Stress

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Inflammatory bowel disease (IBD)

Multifactorial disorder

- gene susceptibility, immune dysregulation, microbial flora, environmental factors

Two principal types

- Crohn’s disease (entire gastrointestinal tract)
- Ulcerative colitis (colon, rectum)
DSS

- Induction of experimental IBD with Dextran Sulfate Sodium (DSS)
- Loss of tight junction proteins → loss of colonic integrity → inflammation
- DSS binds to Medium-Chain-Length Fatty Acids forming a complex (~200nm) → able to fuse with colonocyte membranes

Common therapy

- Glucocorticoids, sulfasalazine, immunosuppressive drugs

- Clinical application of these substances is limited → adverse effects
Sodium propionate

- Short chain fatty acid
- Produced by anaerobic fermentation
- Reducing the production of pro-inflammatory cytokines
- Enhancing intestinal barrier function
- Inhibition of oxidative stress
Methods:
DSS induced colitis in Animals

- C57BL/6J male mice
- 40 mice randomized to four groups:
  - Control group (drinking water for 14 days)
  - Propionate group (1% in ddH2O for 14 days)
  - DSS group (d1-d6 drinking water, d7-d14 3% DSS in ddH2O)
  - DSS/Propionate group (d1-d6 1% propionate from d7-d14 supplemented with 3% DSS)
Methods:
Histopathological assessment

• Measurement of colon length

• Paraffin embedded → cross-sectioning → HE-stain

• Histopathological evaluation

• 0: no obvious inflammatory reaction
• 1: the presence of low-level inflammatory reaction with a few scattered inflammatory cells
• 2: the presence of moderate inflammatory infiltration
• 3: the presence of severe inflammatory reaction in the colon tissue as represented by increased vascular density and thickness
• 4: the presence of large amounts of inflammation cell infiltration and rupture of goblet cell mass.
Methods: 
In vivo Intestinal Permeability

• Mice were fastened o/n
• FITC-dextran delivered via gavage
• Scarification 4h after administration
• Serum levels of FITC (480 and 520nm microplate flourometer)
Methods:
RNA Isolation and Quantitative RT-PCR

• RNA extraction from colon tissue

• Inflammatory factors:
  • TNFα
  • IL-1β
  • IL-6
Methods:
Immunoblotting

• Protein extraction

• Antibodies used:
  • Anti-ZO-1
  • Anti-occludin
  • Anti-E-cadherin
  • Anti-STAT3
  • Anti-p-STAT3
Methods:
Measurement of Myeloperoxidase (MPO) Level in Colon and Serum

• MPO can modulate hydrogen peroxide

• Measurement of MPO activity

• MPO activity was defined as the quantity of enzyme degrading 1 mmol/ml of peroxide at 37°C
Methods:
Assessment of Macrophages in Colonic Mucosa by Immunofluorescence

- Immunofluorescence of colonic tissue
- Anti-CD68-antibody
Results
Results
Results
Results
Results

A. MPO (Ug colon tissue)

B. Serum MPO (U/L)

C. SOD (U/mg/protein)

D. Serum SOD (U/mL)

E. CAT (U/mg/protein)

F. Serum CAT (U/mL)
Results
Discussion

• Sodium propionate inhibits down-regulation of ZO-1, occluding, E-cadherin

• Sodium propionate reduces the expression of pro-inflammatory cytokines: inflammatory factors TNF-α, IL-1β, and IL-6 mRNA

• Sodium propionate reduces CD68 expression in colonic tissue ↓ macrophages infiltration

• Sodium propionate inhibits oxidative stress reduces MPO activity and enhances SOD and CAT activities in serum

• Sodium propionate inhibits phosphorylation of STAT3