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Tenapanor attenuates increased macromolecule permeability in human colon monolayer cultures induced by inflammatory cytokines and human fecal supernatants

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Background

- Patients with constipation-predominant irritable bowel syndrome (IBS-C) have abdominal pain associated with decreased bowel movement frequency, difficult stool passage or both.[1]
- Increased intestinal permeability has been observed in patients with irritable bowel syndrome (IBS), and is associated with low-grade inflammation, visceral hypersensitivity and pain.[2]
- Elevated numbers of intestinal mast cells in proximity to nerve fibers have been reported in IBS, which correlate with abdominal pain and increased intestinal permeability; this potentially enables luminal contents to trigger immune activation and neuronal excitation and contribute to the genesis of IBS pain.[3]
- Tenapanor, a first-in-class, minimally absorbed, small-molecule inhibitor of sodium/hydrogen exchanger 3 (NHE3),[4] significantly reduces abdominal pain and increases complete, spontaneous bowel movement responder rate in patients with IBS-C.[5]
- The aim of this study was to determine the effect of tenapanor on colonic epithelial permeability to macromolecules following stimulation with a variety of insults, using primary human intestinal epithelial monolayer cultures as a model system.
  - Cultures were treated with the cytokines tumor necrosis factor α (TNF-α) or interleukin 6 (IL-6) to simulate inflammation, or with fecal supernatants either from patients with IBS-C or from healthy controls to simulate colonic contents in IBS-C or healthy states.
  - The impact of these insults on permeability of the monolayer cultures to macromolecules was assessed in the presence and absence of tenapanor.

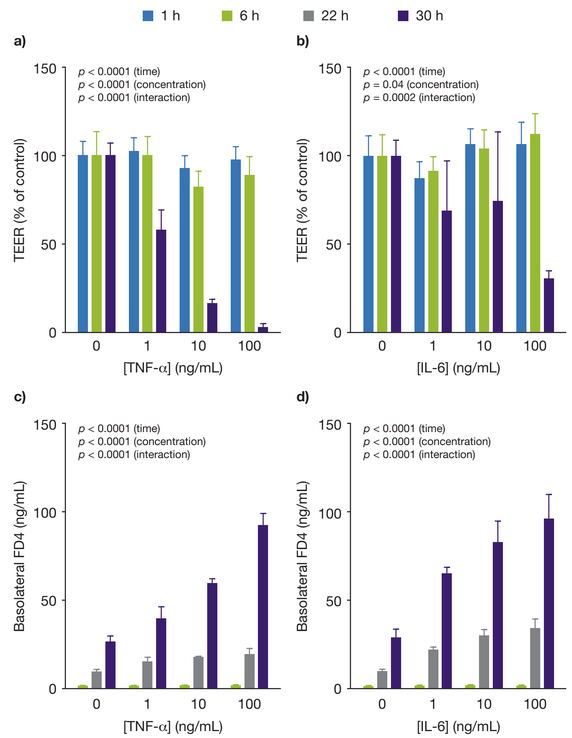
Methods

- Primary human colon monolayer cultures were established using cells derived from three-dimensional human intestinal organoids, which were grown from human colon biopsies, as described previously.[6]
- Fecal samples were obtained from 9 patients with IBS-C according to Rome III criteria[1] (6 women, 3 men) and from 10 healthy controls (5 women, 5 men). The patients and healthy controls were matched for age and weight.
- Fecal supernatants were prepared by dissolving fecal samples at a concentration of 0.3 g/mL in oxygenated Krebs–Ringer buffer and homogenizing on ice with a Polytron homogenizer (30 s, 26 000 rpm). After centrifugation (10 000 × g, 10 min, 4°C), the supernatants were recovered, and coarse particles were separated by filtration using a 100 µm-size filter.
- The human colon monolayer cultures were treated for 24–48 h with either recombinant TNF-α (1–100 ng/mL), IL-6 (1–100 ng/mL) or fecal supernatants from healthy controls or patients with IBS-C (1:4) in the presence of 1 µM tenapanor or vehicle (dimethyl sulfoxide; DMSO) control.
- Trans-epithelial electrical resistance (TEER) of the monolayer cultures was measured with a volt–ohmmeter, and their permeability to macromolecules was measured by apical–basolateral 4 kDa fluorescein isothiocyanate-dextran flux at the indicated time points.

Results

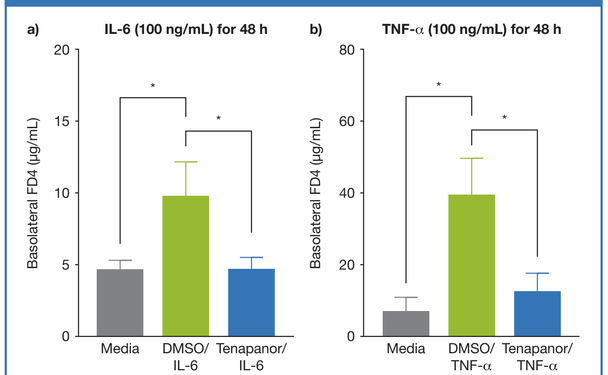
- Treatment of human colon monolayer cultures with TNF-α or IL-6 resulted in a concentration- and time-dependent reduction in TEER and an increase in fluorescein isothiocyanate (FITC)-dextran-measured permeability (Figure 1).
- Tenapanor treatment significantly attenuated the increase in permeability caused by either TNF-α or IL-6, compared with DMSO treatment (vehicle control) (Figure 2).
- Fecal supernatants both from patients with IBS-C and healthy controls also caused a concentration- and time-dependent decrease in TEER (Figures 3a & b) and a time-dependent increase in FITC-dextran-measured permeability (Figures 3c & d; Figure 4).
  - There were no group differences in FITC-dextran-measured permeability caused by fecal supernatants from patients with IBS-C compared with those from healthy controls (Table 1).
- Compared with DMSO (vehicle control), tenapanor attenuated the permeability increase caused by fecal supernatants both from patients with IBS-C and healthy controls (Figure 5).

Figure 1. Treatment of colon monolayers with cytokines TNF-α and IL-6 resulted in (a, b) a concentration- and time-dependent decrease in TEER and (c, d) an increase in FITC-dextran-measured permeability.



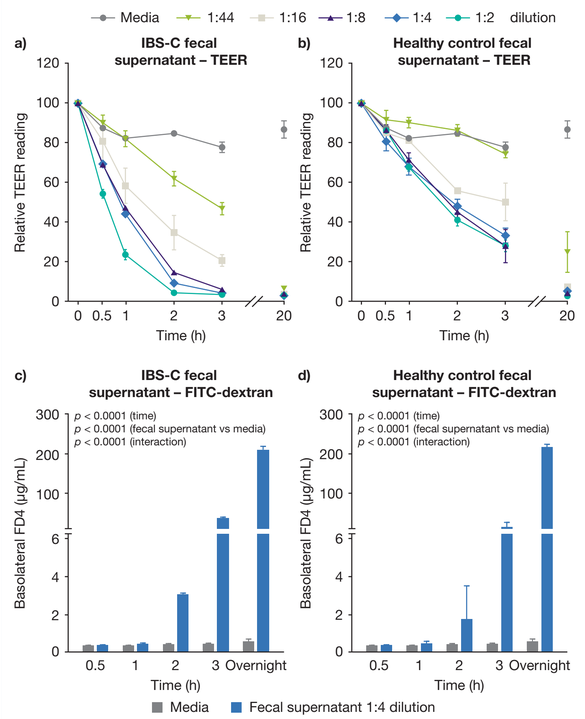
Data are mean values with standard deviations (n = 4 per group); *p* values calculated using a two-way, repeated-measures analysis of variance.
FD4, 4 kDa fluorescein isothiocyanate-dextran; FITC, fluorescein isothiocyanate; IL-6, interleukin 6; TEER, trans-epithelial electrical resistance; TNF-α, tumor necrosis factor α.

Figure 2. Treatment with tenapanor significantly attenuated the increase in FITC-dextran-measured permeability caused by cytokines (a) IL-6 and (b) TNF-α.



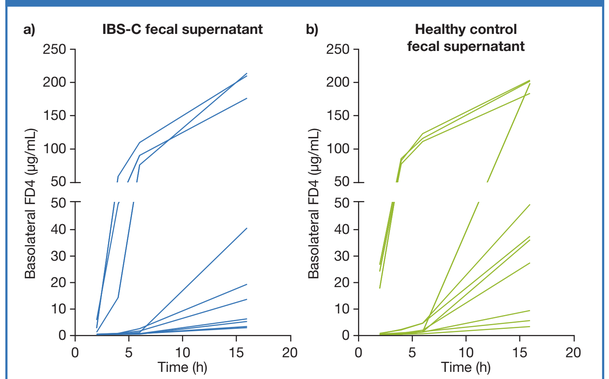
Data are mean values with standard deviations (n = 6 per group); *$p < 0.001$ (Student's two-tailed t-test).
DMSO, dimethyl sulfoxide; FD4, 4 kDa fluorescein isothiocyanate-dextran; FITC, fluorescein isothiocyanate; IL-6, interleukin 6; TNF-α, tumor necrosis factor α.

Figure 3. (a, b) A concentration- and time-dependent reduction in TEER was caused by fecal supernatants from patients with IBS-C and healthy controls. (c, d) The decrease in TEER correlated with an increase in FITC-dextran-measured permeability.



Data are mean values with standard deviations (n = 3 per group); *p* values calculated using a two-way, repeated-measures analysis of variance.
FD4, 4 kDa fluorescein isothiocyanate-dextran; FITC, fluorescein isothiocyanate; IBS-C, constipation-predominant irritable bowel syndrome; TEER, trans-epithelial electrical resistance.

Figure 4. Fecal supernatants from both (a) patients with IBS-C and (b) healthy controls induced a time-dependent increase in FITC-dextran-measured permeability.



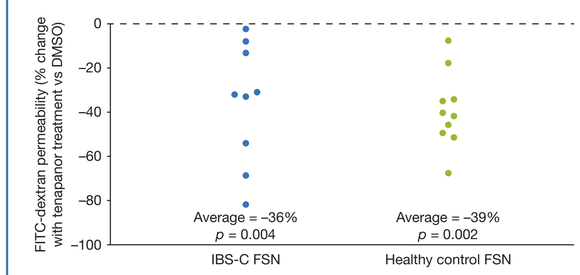
Data are for fecal supernatants from 9 patients with IBS-C and 10 healthy controls.
FD4, 4 kDa fluorescein isothiocyanate-dextran; FITC, fluorescein isothiocyanate; IBS-C, constipation-predominant irritable bowel syndrome.

Table 1. There were no group differences in FITC-dextran-measured permeability caused by fecal supernatants from patients with IBS-C compared with those from healthy controls.

Time, h	*p* value for FD4-measured permeability (IBS-C vs healthy control)
2	0.50
4	0.28
6	0.24
16	0.50

p values calculated using Wilcoxon rank sum test.
FD4, 4 kDa fluorescein isothiocyanate-dextran; FITC, fluorescein isothiocyanate; IBS-C, constipation-predominant irritable bowel syndrome.

Figure 5. Treatment with tenapanor attenuated the increase in FITC-dextran-measured permeability caused by fecal supernatants from patients with IBS-C and from healthy controls.



Data shown are the mean of three values each for fecal supernatants from 9 patients with IBS-C and 10 healthy controls; *p* values calculated by comparing DMSO with 1 µM tenapanor for the indicated fecal supernatants using Wilcoxon matched-pairs signed rank test.
DMSO, dimethyl sulfoxide; FD4, 4 kDa fluorescein isothiocyanate-dextran; FITC, fluorescein isothiocyanate; FSN, fecal supernatant; IBS-C, constipation-predominant irritable bowel syndrome.

Conclusions

- Inflammatory cytokines TNF-α and IL-6, and fecal supernatants both from patients with IBS-C and healthy controls disrupted the integrity of colonic monolayer cultures, resulting in time- and concentration-dependent reductions in TEER and increases in permeability to macromolecules, as measured by FITC-dextran flux.
- Tenapanor attenuated the increase in permeability of human colonic epithelial monolayers to macromolecules caused by cytokines or human fecal supernatants.
- The beneficial effect of tenapanor on abdominal pain in patients with IBS-C may be a result of its ability to reduce colonic permeability to luminal macromolecules, which may reduce hyperexcitability of sensory neurons.

References

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Disclosures

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