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

Ji Wang,1 Muriel Larache,2 Matthew Siege,1 Andrew J King,1 Emeran A Mayer,1 Kirsten Tillisch,1 Jennifer Labus,2 Lin Chang,3 Jeremy S Caldwell4

1Ardelyx, Inc., Fremont, CA, USA; 2G. Oppenheimer Center for Neurobiology of Stress and Resilience, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

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 content 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), significantly reduces abdominal pain and increases complete, spontaneous bowel movement responder rates in patients with IBS-C.4
- 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.5
- Fecal samples were obtained from 9 patients with IBS-C according to Rome III criteria (6 women, 3 men) and from 10 healthy controls (6 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) or fecal supernatants from healthy controls or patients with IBS-C (1:100) in the presence of 1 µM tenapanor or vehicle (dimethyl sulfoxide [DMSO]).
- Trans-epithelial electrical resistance (TER) of the monolayer cultures was measured with a voltohmeter, 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 TER and an increase in 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 TER (Figure 3a & b) and a time-dependent increase in FITC-dextran-measured permeability (Figure 3c & d).
- 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 treatment (vehicle control), tenapanor attenuated the permeability increase caused by fecal supernatants both from patients with IBS-C and healthy controls (Figure 4).

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.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Average ± SEM (pg/mL)</th>
<th>p</th>
<th>*p vs DMSO-measured permeability (IBS-C vs healthy control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>250 ± 15</td>
<td></td>
<td>&lt; 0.001 (interaction)</td>
</tr>
<tr>
<td>2</td>
<td>200 ± 10</td>
<td></td>
<td>&lt; 0.001 (time)</td>
</tr>
<tr>
<td>3</td>
<td>180 ± 10</td>
<td></td>
<td>&lt; 0.001 (time)</td>
</tr>
<tr>
<td>4</td>
<td>160 ± 10</td>
<td></td>
<td>&lt; 0.001 (time)</td>
</tr>
</tbody>
</table>

Figure 1. Treatment of human colon monolayer cultures with cytokines TNF-α and IL-6 resulted in a concentration- and time-dependent decrease in TER and an increase in FITC-dextran-measured permeability (IBS-C fecal supernatant – TEER = 0.22 ± 0.06; vehicle (DMSO) control – TEER = 0.34 ± 0.06) (p < 0.001, Student’s two-tailed t-test; n = 6 per group). Data are mean values with standard deviations.

Figure 2. Treatment with tenapanor significantly attenuated the increase in FITC-dextran-measured permeability caused by cytokines (a) IL-6 and (b) TNF-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 following stimulation with a variety of insults, using primary human intestinal epithelial monolayer cultures as a model system.

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.

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 TER and increase 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 hypersensitivity of sensory neurons.

References


Acknowledgments

Medical writing support was provided by Richard Clay, PhD, of PharmaGenesis London, London, UK, and was funded by Ardelyx.

Disclosures

This study was funded by Ardelyx. Ardelyx-eligible authors are current or former employees of Ardelyx and may have shares in Ardelyx. The other authors have no conflicts of interest to declare.