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Tenapanor reduces IBS pain through inhibition of TRPV1-dependent neuronal hyperexcitability *in vivo*

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Background

- Irritable bowel syndrome (IBS) is a common gastrointestinal disorder with symptoms including abdominal pain and changes in the pattern of bowel movements.[1]
  - The pathophysiology of abdominal pain in IBS is partly attributed to visceral hypersensitivity.[1]
- Transient receptor potential vanilloid type I (TRPV1) has previously been shown to be involved in the pathogenesis of colonic hypersensitivity in IBS.[2,3]
- Tenapanor, an investigational, minimally absorbed, small-molecule inhibitor of the sodium/hydrogen exchanger NHE3, acts locally on gastrointestinal epithelial cells to inhibit sodium absorption.[4]
- Tenapanor treatment of patients with constipation-predominant IBS (IBS-C) significantly increased the complete spontaneous bowel movement responder rate and significantly reduced abdominal pain scores in a phase 2, randomized, placebo-controlled trial.[5]

Aim

- To investigate the mechanism by which tenapanor reduces abdominal pain in IBS-C, focusing on its effects on colonic hypersensitivity and known pathways of neuronal hyperexcitability in rats.

Methods

Model of IBS-like colonic hypersensitivity in rats

- A neonatal irritation model of IBS-like colonic hypersensitivity was generated by colorectal infusion of 0.5% acetic acid (200 µL) in 10-day-old Sprague–Dawley rats (Harlan Laboratories Inc.), as described previously[6] (**Figure 1A**).
  - Non-sensitized controls were generated by infusion of saline.

Visceral motor reflex response to colorectal distension

- When sensitized and non-sensitized (control) rats were at least 8 weeks old, a pair of electrodes was surgically implanted into their abdominal muscle tissue.
- Sensitized rats were treated orally, twice daily, with vehicle or tenapanor 0.5 mg/kg (n = 7/group) and non-sensitized controls were treated with vehicle (n = 7) for 7 days, starting 1 day after surgery.
  - A laxative control group (n = 7) of sensitized rats received polyethylene glycol 3350 (PEG) 1000 mg/kg twice daily.
- The visceral motor reflex (VMR) response to colorectal distension (CRD) was assessed by electromyography (EMG) 1 day after the end of treatment, as follows.
  - A balloon was inserted into the distal colon under isoflurane anesthesia.
  - After a recovery period of approximately 30 minutes, VMR response to CRD was recorded while 20, 40, 60 or 80 mmHg pressure was applied to the balloon for 20 seconds.
  - VMR responses to CRD were normalized to baseline EMG values measured 20 seconds before CRD.

Stool profiling

- Stools from all treated rats were collected over a period of 5 hours on the last day of treatment and wet and dry stool weights were measured to calculate stool water content.

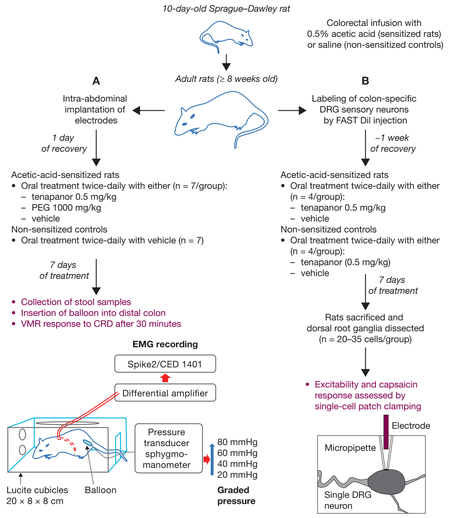
Dorsal root ganglia excitability and response to capsaicin

- Colon-specific dorsal root ganglia (DRG) sensory neurons of adult (8 weeks old) sensitized Sprague–Dawley rats and non-sensitized controls were labeled *in vivo* by injection of FAST DiI (1,1′-dilinoleyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate, 10 mg/mL in methanol) into the distal colon wall (2 µL/site, 10 sites/rat) (**Figure 1B**).
- One week later, rats were treated orally, twice daily, with vehicle or tenapanor 0.5 mg/kg for 7 days (n = 4/group).
- Rats were sacrificed on the day after the end of treatment and DRG were dissected (20–35 cells/group).
- The excitability and capsaicin response of DiI-positive [DiI(+)] DRG neurons were assessed by single-cell patch clamping.

In vitro studies of epithelial cell–neuron crosstalk

- Dissociated DRG neurons from adult non-sensitized Sprague–Dawley rats were incubated for 24 hours in conditioned medium harvested from the basolateral chamber of human colonic enteroid monolayers pre-treated on the apical side with tenapanor 1 µM or vehicle control before single-cell patch clamping experiments.

Figure 1. Design of study investigating the effect of tenapanor in a rat model of IBS using (A) VMR response to CRD and (B) DRG excitability in acetic-acid-sensitized and non-sensitized control rats.



CRD, colorectal distension; FAST DiI, 1,1′-dilinoleyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate; IBS, irritable bowel syndrome; PEG, polyethylene glycol; VMR, visceral motor reflex.

Results

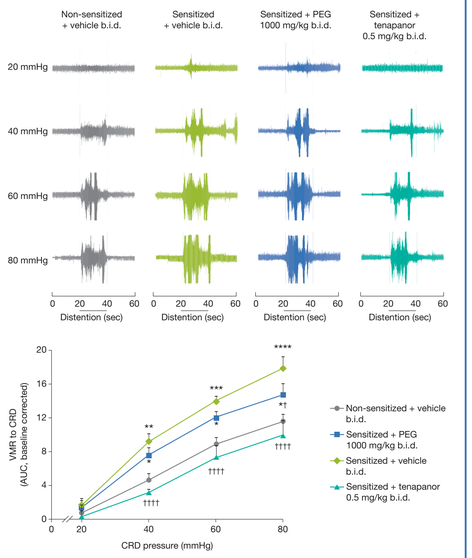
Effect of tenapanor on visceral hyperalgesia in the rat model of IBS

- Vehicle-treated acetic-acid-sensitized rats had significantly increased VMR responses to CRD than vehicle-treated non-sensitized controls, consistent with visceral hypersensitivity in the rat model of IBS (**Figure 2**).
- Tenapanor treatment in sensitized rats significantly reduced VMR responses to CRD compared with the responses in sensitized rats treated with vehicle ($p < 0.001$) or PEG ($p < 0.05$) (**Figure 2**).
  - Treatment with tenapanor resulted in similar VMR responses to CRD to those produced in vehicle-treated non-sensitized controls, suggesting that tenapanor prevents visceral hyperalgesia.
  - In contrast, the VMR responses in sensitized rats treated with PEG were significantly greater than those vehicle-treated non-sensitized controls ($p < 0.05$).

Effect of tenapanor on stool water content in the rat model of IBS

- Tenapanor-treated sensitized rats had significantly increased wet and dry stool weights compared with vehicle- and PEG-treated sensitized rats and vehicle-treated non-sensitized controls ($p < 0.05$), indicating that tenapanor increased stool excretion in rats with IBS-like colonic hypersensitivity (**Figure 3A**).
- Stool water content was significantly higher in tenapanor-treated sensitized rats than in vehicle-treated sensitized rats and vehicle-treated non-sensitized controls (both $p < 0.05$, **Figure 3B**).

Figure 2. Electromyography traces and VMR response curves to CRD in acetic-acid-sensitized rats and non-sensitized controls treated with tenapanor or comparator agents for 7 days.



n = 7/group.
Data are shown as mean + standard error.
Student–Newman–Keuls *post hoc* test: *$p < 0.05$, **$p < 0.01$, ***$p < 0.005$, ****$p < 0.001$ vs non-sensitized rats treated with vehicle; †$p < 0.05$, ††††$p < 0.001$ vs sensitized rats treated with vehicle.
AUC, area under the curve; CRD, colorectal distension; PEG, polyethylene glycol; VMR, visceral motor reflex.

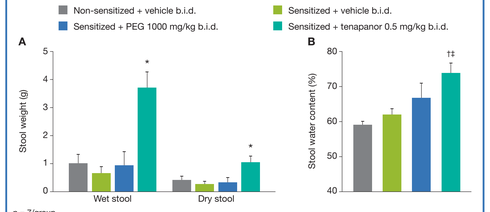
Effect of tenapanor on DRG excitability and capsaicin response in the rat model of IBS

- Colon-specific DiI(+) DRG neurons from vehicle-treated sensitized rats were hyperexcitable compared with those from vehicle-treated non-sensitized controls, as demonstrated by:
  - significantly increased resting membrane potential ($p < 0.05$; **Figure 4A**)
  - significantly reduced rheobase (the minimum current required to trigger an action potential; $p < 0.05$; **Figure 4B**)
  - significantly increased evoked action potential firing ($p < 0.05$; **Figure 4C**)
  - significantly increased current density in response to the TRPV1 agonist capsaicin ($p < 0.05$; **Figure 4D**).
- Tenapanor treatment significantly reduced the hyperexcitability of colon-specific DRG compared with vehicle treatment in sensitized rats, but had no effect in non-sensitized controls (**Figure 4A–D**).

Effect of tenapanor on epithelial cell–neuron crosstalk *in vitro*

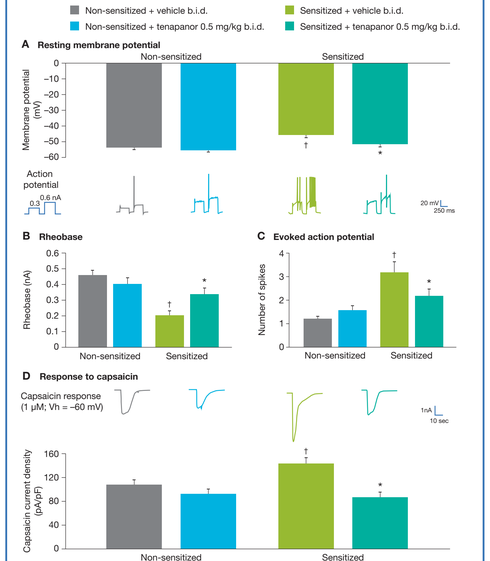
- There were no significant differences in neuron excitability and response to capsaicin in any of the parameters measured in non-sensitized DRG neurons following incubation for 24 hours in a conditioned medium from human colonic enteroid monolayers pre-treated with tenapanor (1 µM) or vehicle control (**Figure 5A–D**).

Figure 3. Stool weight and water content in acetic-acid-sensitized rats and non-sensitized controls treated with tenapanor or comparator agents for 7 days.



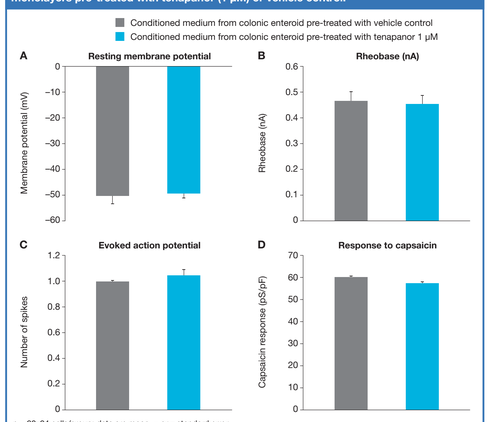
n = 7/group.
Data are shown as mean + standard error.
*$p < 0.05$ vs all other groups; †$p < 0.05$ vs sensitized rats treated with vehicle; ‡$p < 0.05$ vs non-sensitized controls treated with vehicle.
PEG, polyethylene glycol.

Figure 4. Single-cell patch clamping: electrophysiological parameters in DiI(+) DRG neurons from acetic-sensitized rats and non-sensitized controls treated *in vivo* with vehicle or tenapanor for 7 days.



n = 20–35 cells/group; data are mean, + or – standard error.
*$p < 0.05$ vs sensitized rats treated with vehicle; †$p < 0.05$ vs non-sensitized controls treated with vehicle.
DRG, dorsal root ganglia; mV, millivolts; nA, nanoamperes; pA/pF, picoamperes per picofarad; Vh, holding potential.

Figure 5. Single-cell patch clamping: electrophysiological parameters in non-sensitized DRG neurons treated for 24 hours with conditioned medium from human colonic enteroid monolayers pre-treated with tenapanor (1 µM) or vehicle control.



n = 20–24 cells/group; data are mean, + or – standard error.
No significant differences between the tenapanor and control groups.
DRG, dorsal root ganglia; mV, millivolts; nA, nanoamperes; pA/pF, picoamperes per picofarad.

Conclusions

- Oral tenapanor treatment in an established rat model of IBS-like colonic hypersensitivity:
  - reduced visceral hypersensitivity
  - normalized colonic sensory neuronal excitability and TRPV1 currents
  - was associated with increased stool excretion and stool water content.
- These data are the first supporting a mechanistic link between tenapanor and specific nociceptive pathways such as TRPV1 signaling.
- The results provide insights into the mechanism of reduction of abdominal pain in patients with IBS-C treated with tenapanor.

References

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Disclosures

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