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Olorinab (APD371), a Peripherally Acting, Highly Selective, Full Agonist of the Cannabinoid Type 2 Receptor (CB_2), Reduces Visceral Hypersensitivity in Animal Models

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INTRODUCTION

- Abdominal pain is a key symptom of inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS)
  - In patients with IBD, abdominal pain is reported up to 60% of the time, is associated with lower quality of life, and is severe enough to require pain treatment in most cases[1]
  - Even in apparent remission of inflammation, pain, bloating, and erratic bowel habits are noted in 25%-46% of patients with IBD[2]
  - Recurrent abdominal pain is a requirement for a diagnosis of IBS[3] and causes distress in a substantial proportion of IBS patients, reducing quality of life and decreasing work productivity[4]
- There is an unmet need for effective nonopioid treatment options for abdominal pain in both disorders[1]
- The cannabinoid type 2 receptor (CB_2) provides an attractive target for abdominal pain associated with IBD and IBS[5-7]
  - CB_2 is expressed throughout the gastrointestinal tract and may be upregulated in states of disease[5-7]
  - CB_2 agonists decreased visceral hypersensitivity in preclinical models of colitis[8,9]
- Olorinab (APD371) is a full agonist of CB_2 designed to provide pain relief with lowered risk of psychoactive effects and dependence[10]
  - Olorinab was shown to activate endogenous CB_2 in primary rat splenocytes, human leukemia (HL)-60 cells, and primary human B cells[10]
  - Olorinab exhibited >1000-fold selectivity for CB_2 over CB_1,[10,11] which minimizes potential for activation of CB_1 located in the brain, and sustained efficacy in several animal models of chronic pain[10,11]
  - Olorinab showed low blood-brain barrier penetration in rats[11]
- Olorinab is in clinical development for visceral pain associated with gastrointestinal diseases[10,12]

OBJECTIVE

- To investigate the potential antinociceptive effects and mechanisms of action of olorinab in animal models of IBD and IBS

METHODS

In Vivo Visceromotor Response Study Design

- Olorinab or vehicle (0.5% methylcellulose) was administered in an IBD-like rat model of colitis, an IBS-like mouse model of chronic visceral hypersensitivity (CVH), and in healthy control animals
- Treatment:
  - Colitis or control rats: olorinab 3 or 30 mg/kg twice-daily (BID) by oral gavage for 5 days, starting 1 day before induction of colitis
  - CVH or control mice: olorinab 3, 10, or 30 mg/kg BID on days 24 to 28 after induction of colitis

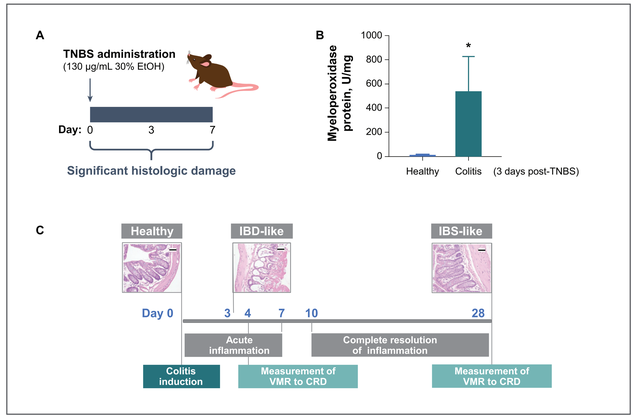
In Vitro Colonic Nociception Study Design

- Olorinab and/or a CB_2 antagonist (SR144528) were applied to the surface of the mucosal epithelium of splanchnic colonic afferent nerves from colitis mice, CVH mice, and healthy control animals
- After baseline firing rate was recorded in response to mechanical stimulation with von Frey filaments (2g), treatment was applied as follows, with recordings lasting 10 minutes at each concentration:
  - 0 (Baseline), 10, 100, 1000, 10000 nM olorinab
  - Baseline, 1000 nM SR144528, 1000 nM SR144528 plus 1000 nM olorinab

Animal Models of Colitis and CVH

- Colitis was induced in mice and rats as previously described in Hughes et al[13] (**Figure 1**)
  - Male 6- to 7-week-old Sprague Dawley rats were administered an intracolonic enema of 2,4,6-trinitrobenzene sulfonic acid (TNBS) 12 mg in 35% ethanol (0.3 mL volume)
  - Male 13-week-old C57 BL/6 mice were administered an intracolonic enema of 2,4-dinitrobenzene sulfonic acid (DNBS) using DNBS 6.5 mg in 30% ethanol (0.1 mL) per mouse
- Colitis animals were allowed to recover and were studied at 4 days following administration of TNBS or DNBS (**Figure 1**); measurement of Myeloperoxidase protein was used to assess inflammation
- CVH was induced in male 10- to 11-week-old C57 BL/6 mice using DNBS 7 mg in 30% ethanol (0.1 mL) administered rectally
- CVH mice were allowed to recover and were studied at 28 days following DNBS administration (time of CVH) (**Figure 1**)

Figure 1. Induction of Colitis (A) Results in Acute (IBD-like) (B-C) and Chronic (IBS-like) (C) Visceral Hypersensitivity

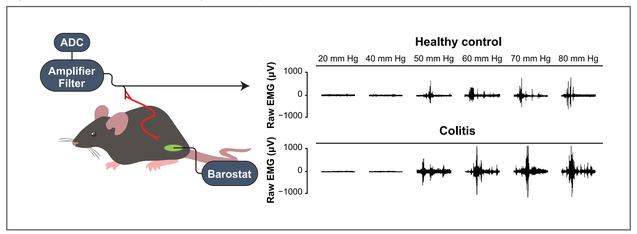


CRD, colorectal distension; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; TNBS, 2,4,6-trinitrobenzene sulfonic acid; VMR, visceromotor response. Histology images adapted with permission from Hughes et al.[13]

Pain Assessment In Vivo

- Visceral mechanosensitivity was assessed in vivo by quantifying visceromotor responses (VMR) to colorectal distension (CRD; 0 to 80 mm Hg)
- Noxious distension of the colorectum triggers the VMR, a nociceptive brainstem reflex consisting of the contraction of the abdominal muscles,[14] used as an indicator of pain
- After TNBS treatment in rats (colitis model) and DNBS in mice (CVH model), CRD was induced using a barostat, and VMR was measured using an amplifier connected to an analog-to-digital converter (**Figure 2**)

Figure 2. Pain Assessment In Vivo by VMR Response to CRD in Rats and Mice

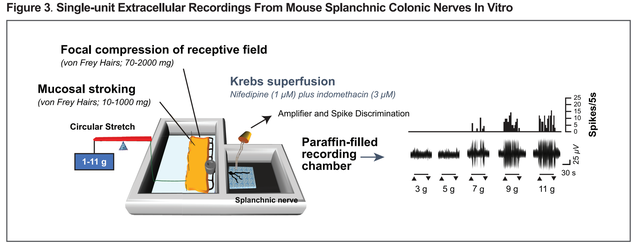


ADC, analog-to-digital converter; CRD, colorectal distension; EMG, electromyogram; VMR, visceromotor response.

Assessment of Colonic Mechanosensitivity In Vitro

- Single-unit extracellular recordings from splanchnic colonic afferent nerves were performed as previously described[13] (**Figure 3**)

Figure 3. Single-unit Extracellular Recordings From Mouse Splanchnic Colonic Nerves In Vitro



Spike recording image (right) reproduced with permission from Hughes et al.[13]

Statistical Analysis

- Generalized estimating equations (SPSS software) were used for analysis with $P < 0.05$ considered significant

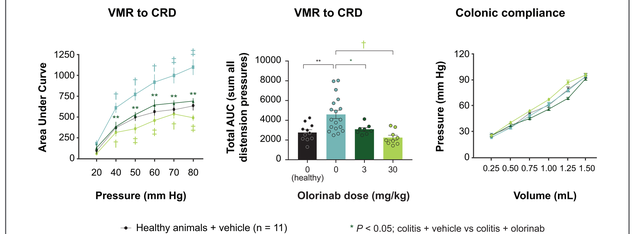
RESULTS

Colonic Inflammation in the Rat Model of Colitis

- TNBS-treated rats displayed significant colonic inflammation (increased myeloperoxidase activity), compared with controls ($P < 0.01$, N = 9/group; **Figure 1B**)

Visceral Hypersensitivity and Colonic Compliance in the Rat Model of Colitis

Figure 4. Treatment with 3 and 30 mg/kg Olorinab in Rats with Colitis Prevented Visceral Hypersensitivity without an Effect on Colonic Compliance



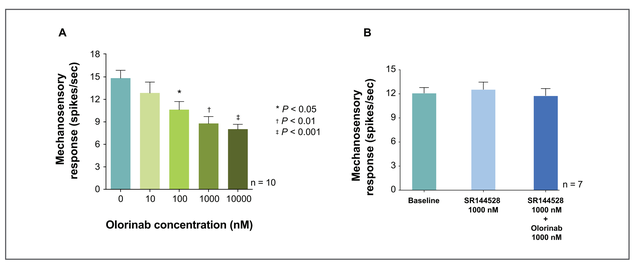
AUC, area under the curve; CRD, colorectal distension; VMR, visceromotor response.
All comparisons were done with a post hoc generalized estimating equation using least significant difference. AUC was calculated as the difference of area values obtained pre-distension (20s) minus those obtained during distension (20s). Data are presented as mean ± standard error of the mean.

- Healthy control rats treated with olorinab (30 mg/kg) did not show altered visceral sensitivity to CRD or changes in colonic compliance (data not shown)

Colonic Nociceptor Mechanosensory Recordings in the Mouse Model of Colitis

- Nociception of mechanical stimuli was heightened in colitis animals versus controls (data not shown)

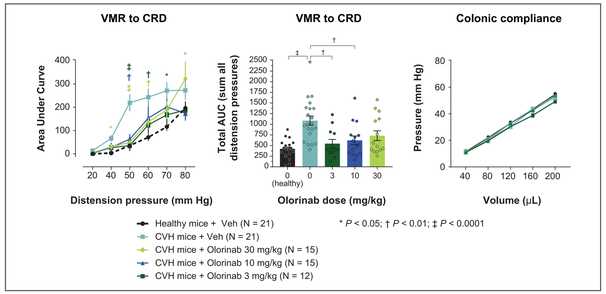
Figure 5. In Colitis Mice, Olorinab Reduced Colonic Nociceptor Hypersensitivity (A) and a CB_2 Antagonist Prevented this Therapeutic Effect (B)



CB_2, cannabinoid receptor type 2. Tests of significance were performed using 1-way analysis of variance with Bonferroni post hoc tests. Coloring represents x-axis labels. Data are presented as mean ± standard error of the mean.

Visceral Hypersensitivity and Colonic Compliance in the Mouse Model of CVH

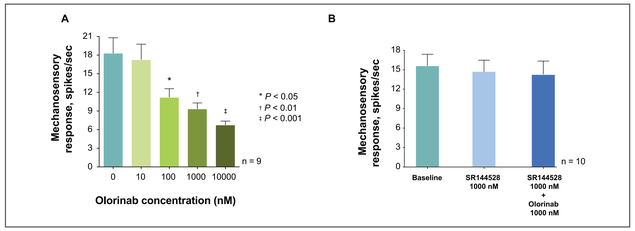
Figure 6. In CVH Mice Olorinab Decreased Visceral Hypersensitivity in Response to Colonic Distension without Changing Colonic Compliance



AUC, area under the curve; CVH, chronic visceral hypersensitivity; Veh, vehicle.
AUC was calculated as the difference of area values obtained pre-distension (20s) minus those obtained during distension (20s). The colors for *P* value symbols represent comparisons of the olorinab dose group of the same color versus CVH + vehicle. Data are presented as mean ± standard error of the mean.

Colonic Nociceptor Mechanosensory Recordings in the Mouse Model of CVH

Figure 7. In CVH Mice, Olorinab Reduced Colonic Nociceptor Hypersensitivity (A) and a CB_2 Antagonist Prevented this Therapeutic Effect (B)



CB_2, cannabinoid receptor type 2; CVH, chronic visceral hypersensitivity.
Coloring represents x-axis labels. Data are presented as mean ± standard error of the mean.

CONCLUSIONS

- Olorinab reduced visceral hypersensitivity in animal models of IBD and IBS but not in healthy controls, suggesting that activation of CB_2 causes antinociceptive actions in visceral sensory pathways in models of IBD and IBS
- The CB_2 antagonist SR144528 prevented olorinab-induced inhibition of colonic nociceptor hypersensitivity, which further supports the role of CB_2 in nociception
- Olorinab, through its selectivity and peripheral action, is designed to provide pain relief without psychotropic effects and without the potential for dependence
- These data support clinical study of olorinab as a nonopioid therapy for IBD- and IBS-associated abdominal pain

REFERENCES

1. Zeitz J et al. *PLoS One.* 2016;11:e0156666.
2. Halpin SJ, Ford AC. *Am J Gastroenterol.* 2012;107:1474-1482.
3. Basnayake C. *Aust Prescr.* 2018;41:145-149.
4. Paré P et al. *Clin Ther.* 2006;28:1726-1735.
5. Wright KL et al. *Br J Pharmacol.* 2008;153:263-270.
6. Wright KL et al. *Gastroenterology.* 2005;129:437-453.
7. Cremon C et al. *Aliment Pharmacol Ther.* 2017;45:909-922.
8. Kikuchi A et al. *J Pharmacol Sci.* 2008;106:219-224.
9. Iwata Y et al. *Bioorg Med Chem Lett.* 2015;25:236-240.
10. Adams JW et al. Presented at: American Pain Society Scientific Summit; March 4-6, 2018; Anaheim, California. Poster #100.
11. Han S et al. *ACS Med Chem Lett.* 2017;8:1309-1313.
12. Jones RCW et al. Presented at: American Pain Society Scientific Summit; March 4-6, 2018; Anaheim, California. Poster #286.
13. Hughes PA et al. *Gut.* 2009;58:1333-1341.
14. Ness TJ, Gebhart GF. *Brain Res.* 1988;450:153-169.

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DISCLOSURES

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