

Olorinab, a Peripherally Acting, Highly Selective, Full Agonist of the Cannabinoid Receptor 2 (CB₂), Reduces Visceral Hypersensitivity in Mice

Joel Castro,¹ Jessica Maddern,¹ Sonia Garcia-Caraballo,¹ Gudrun Schober,¹ Beatriz Lindstrom,² Shirdi Schmiel,² Stuart M. Brierley¹

¹Visceral Pain Research Group, Flinders University, SAHMRI, Adelaide, SA, Australia; ²Arena Pharmaceuticals, Inc, San Diego, CA, USA

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INTRODUCTION

- Abdominal pain is a key symptom of inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS)^{1,2}
 - Up to 70% of patients with IBD experience pain and up to 50% experience chronic pain for 5 years or more, resulting in a profound impact on patients' quality of life in most cases.¹ Furthermore, in patients with IBD who achieve clinical remission, approximately 35% continue to experience IBS-like symptoms, including abdominal pain,³ and thus require additional pain-specific treatment
 - More than 90% of patients with IBS experience abdominal pain weekly, and 39% of patients report that abdominal pain always or often interferes with their daily activities¹
- Currently available treatment options for abdominal pain in IBD and IBS are suboptimal for many patients largely because of inadequate pain relief response and the potential for treatment-related side effects^{4,5}

INTRODUCTION (CONTINUED)

- The endocannabinoid system and its receptors, cannabinoid receptor 1 (CB₁) and cannabinoid receptor 2 (CB₂), have emerged as potential modulators of somatic and visceral pain⁶
 - CB₁ is widely distributed and highly expressed in the brain and mediates the psychoactive effects of cannabis⁶
 - CB₂ is mainly expressed in immune cells and peripheral tissues, including the gastrointestinal tract, and is upregulated in disease states, such as inflammation⁷⁻⁹
- CB₂ is an attractive target for the treatment of abdominal pain because it is increased in the colon of patients with IBD and IBS⁹⁻¹¹ and has been shown to modulate visceral sensitivity in animal models¹²⁻¹⁵
- Olorinab (APD371) is a highly selective, peripherally acting, full agonist of the CB₂ receptor^{16,17}
 - Olorinab exhibited >1000-fold functional selectivity for CB₂ over CB₁,^{16,17} minimizing the potential for CB₁ activation
 - Olorinab is peripherally acting, showing low brain penetration in rats,¹⁷ thus reducing the risk of central nervous system effects
 - Olorinab was shown to activate endogenous CB₂ in primary rat splenocytes, human HL-60 cells, and primary human B cells¹⁶ and has demonstrated sustained efficacy in several animal models of chronic pain^{16,18}

OBJECTIVES

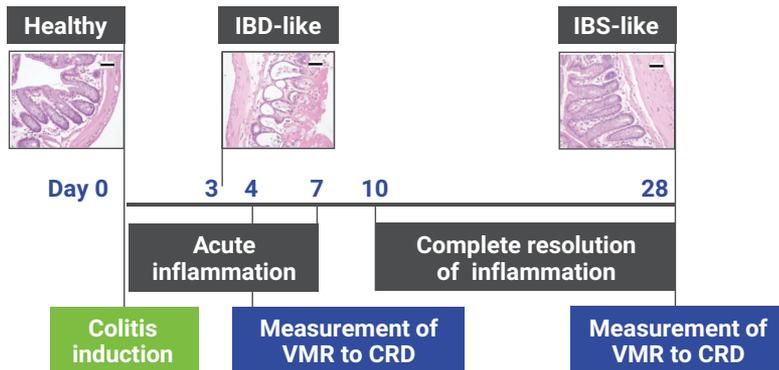
- To investigate the potential antinociceptive effects and mechanisms of action of olorinab in animal models of IBD and IBS
- To identify potential sites of olorinab activity by determining the expression of CB₁ and CB₂ in the colon and dorsal root ganglia (DRG) in animal models of IBD and IBS and in human DRG from donors with IBD and healthy donors

METHODS

ANIMAL MODELS OF COLITIS (IBD-LIKE) AND CHRONIC VISCERAL HYPERSENSITIVITY (CVH; IBS-LIKE)

- Colitis was induced in mice and rats as previously described in Hughes et al¹⁹ (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 C57BL/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
- CVH was induced in male 10- to 11-week-old C57BL/6 mice using an intracolonic enema of DNBS 6.5 mg in 30% ethanol (0.1 mL) (Figure 1)

Figure 1. Induction of Colitis and CVH for Assessing VMR to CRD



CRD, colorectal distension; CVH, chronic visceral hypersensitivity; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; VMR, visceromotor response. Histology images adapted with permission from BMJ Publishing Group Limited (Post-inflammatory colonic afferent sensitisation: different subtypes, different pathways and different time courses. Hughes PA, et al. Volume 58, Issue 10, Pages 1333-1341, Copyright 2009).¹⁹ Scale bar, 20 μ m.

IN VIVO PAIN ASSESSMENT BY VISCEROMOTOR RESPONSE (VMR) TO COLORECTAL DISTENSION (CRD)

- Visceral hypersensitivity was assessed in vivo by quantifying VMR to CRD (0 to 80 mm Hg) on Day 4 (colitis) and Day 28 (CVH) after TNBS/DNBS administration (Figure 1)
 - Noxious distension of the colorectum triggers the VMR, a nociceptive brainstem reflex consisting of the contraction of the abdominal muscles,²⁰ used as an indicator of pain
- After TNBS/DNBS administration in rodents, CRD was induced using a barostat, and VMR was measured using an amplifier connected to an analog-to-digital converter
- Olorinab or vehicle (0.5% methylcellulose) was administered to
 - Colitis or healthy control rodents at 3 or 30 mg/kg twice daily (BID) by oral gavage for 5 days, starting 1 day before induction of colitis
 - CVH or healthy control rodents at 1, 3, 10, or 30 mg/kg BID on days 24 to 28 after induction of colitis

IN VITRO MECHANOSENSORY RESPONSE ASSESSMENT OF COLONIC NOCICEPTORS

- Single-unit extracellular recordings from splanchnic colonic afferent nerves were performed as previously described¹⁹
- Olorinab and/or a CB₂ antagonist (SR144528) were applied to the surface of the mucosal epithelium of splanchnic colonic afferent nerves from colitis, CVH, 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:
 - Baseline (0), 0.01, 0.1, 1.0, 10 μ M olorinab
 - Baseline, 1.0 μ M SR144528, 1.0 μ M SR144528 plus 1.0 μ M olorinab

METHODS (CONTINUED)

MEASUREMENT OF CB₁ AND CB₂ EXPRESSION BY QUANTITATIVE REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION (qRT-PCR)

- RNA was isolated for qRT-PCR from the following sources:
 - Colonic tissue (mucosa or muscle + enteric nervous system [ENS]) from healthy, colitis, and CVH mice
 - DRG (thoracolumbar [T10-L1] and lumbosacral [L6-S1]) from healthy, colitis, and CVH mice
 - Human DRG from healthy donors (T10-L1; AnaBios, San Diego, CA) and from donors with IBD (T11; AnaBios)

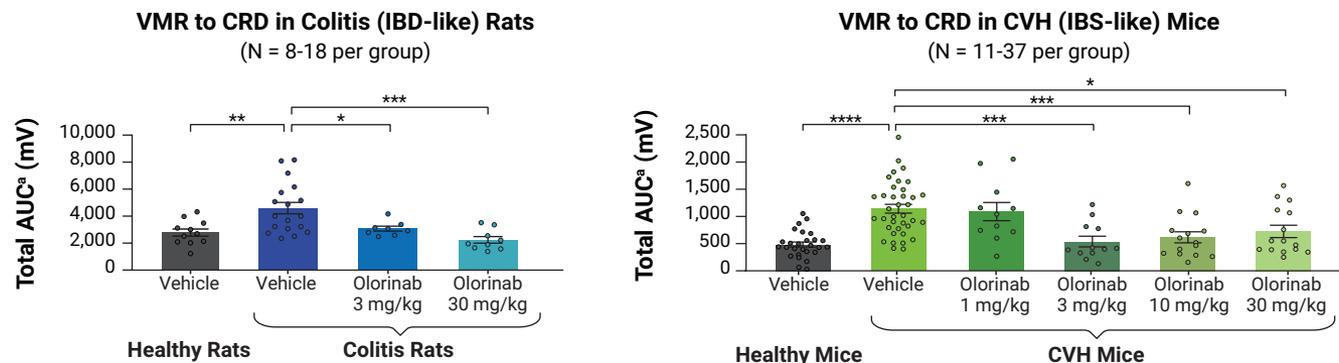
- Mouse and human CB₂ genes have 2 distinct promoter regions, resulting in differential tissue expression (CB_{2A} and CB_{2B}).²¹ Therefore, expression of each CB₂ isoform was assessed individually (where possible) and combined (CB_{2A+B})
- Real-time qPCR was performed using TaqMan® probes for genes coding for the CB₁ and CB₂ proteins in mice and humans (*CNR1*, *CNR2_{A'}*, *CNR2_B* [only mouse probe available], and *CNR2_{A+B}*) and reference genes for β-actin, peptidylprolyl isomerase A (PPIA), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH)
- Results were analyzed using the delta cycle threshold (Ct) method to calculate relative expression levels:
$$N(0) = 2^{(Ct_{[\text{geometric mean of reference genes}] - Ct_{[\text{target}]})}$$

RESULTS

IN VIVO VISCERAL HYPERSENSITIVITY

- VMR to CRD was significantly increased in colitis and CVH rodents compared with healthy animals (**Figure 2**)
- Olorinab significantly attenuated the increased visceral sensitivity in colitis and CVH animals at doses ≥3 mg/kg, reducing the VMR to CRD in colitis and CVH animals to levels similar to those in vehicle-treated healthy animals (**Figure 2**)
 - Healthy animals treated with olorinab (30 mg/kg) did not show altered VMR to CRD (data not shown)

Figure 2. VMR to CRD in Colitis and CVH Rodents With and Without Olorinab Treatment



AUC, area under the curve; CRD, colorectal distension; CVH, chronic visceral hypersensitivity; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; VMR, visceromotor response.

^aSum of all distension pressures.

All comparisons were done with a post hoc generalized estimating equation using least squares difference. AUC was calculated as the difference of area values obtained before distension (20 seconds) minus those obtained during distension (20 seconds). All data are presented as mean ± standard error of the mean. **P* < 0.05; ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001.

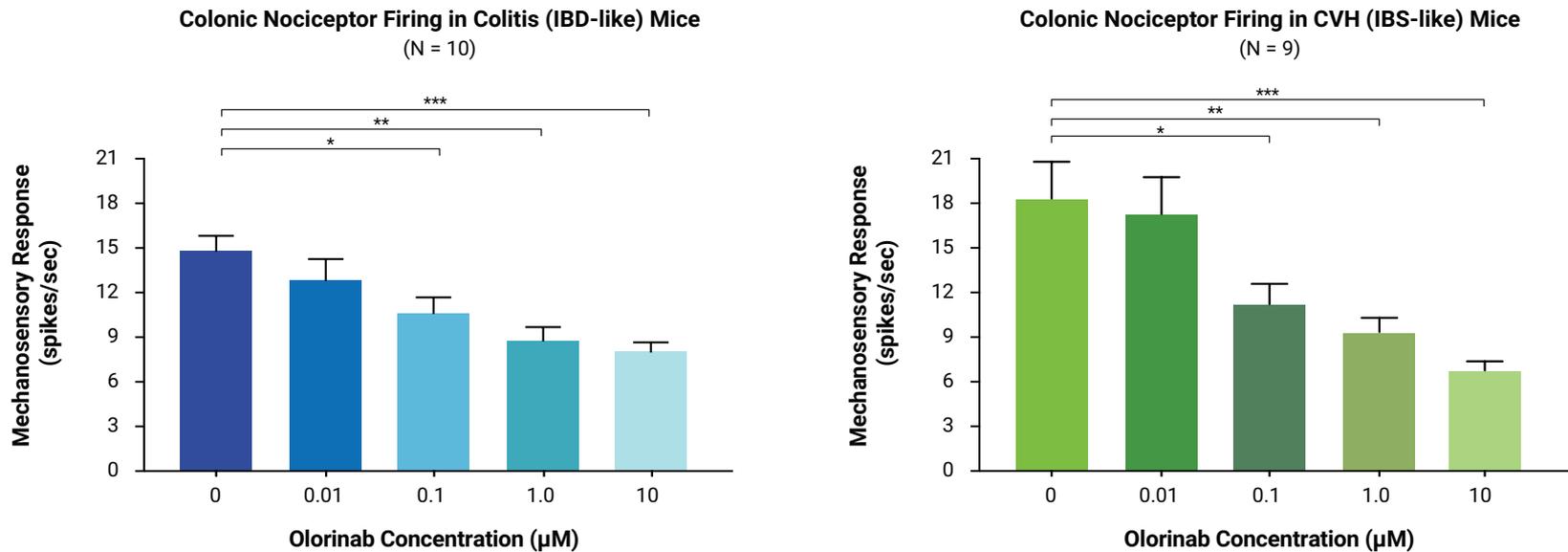
- No changes in colonic compliance were observed in healthy, colitis, or CVH animals treated with olorinab at any dose¹⁸

RESULTS (CONTINUED)

IN VITRO COLONIC NOCICEPTION

- Nociception of mechanical stimuli was heightened in colitis and CVH animals when compared with healthy controls (data not shown)
- Direct application of olorinab to the mucosal surface of animals with colitis and CVH caused a concentration-dependent reduction in mechanosensory responses of colonic splanchnic afferents at doses $\geq 1.0 \mu\text{M}$ (**Figure 3**)
 - The effect of olorinab on colonic nociceptors was inhibited by the CB_2 antagonist SR144528,¹⁸ confirming the activity of olorinab is mediated by the CB_2 receptor

Figure 3. Colonic Nociceptor Mechanosensory Response in Colitis and CVH Rodents With and Without Olorinab Treatment



CVH, chronic visceral hypersensitivity; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome. All data are presented as mean \pm standard error of the mean. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

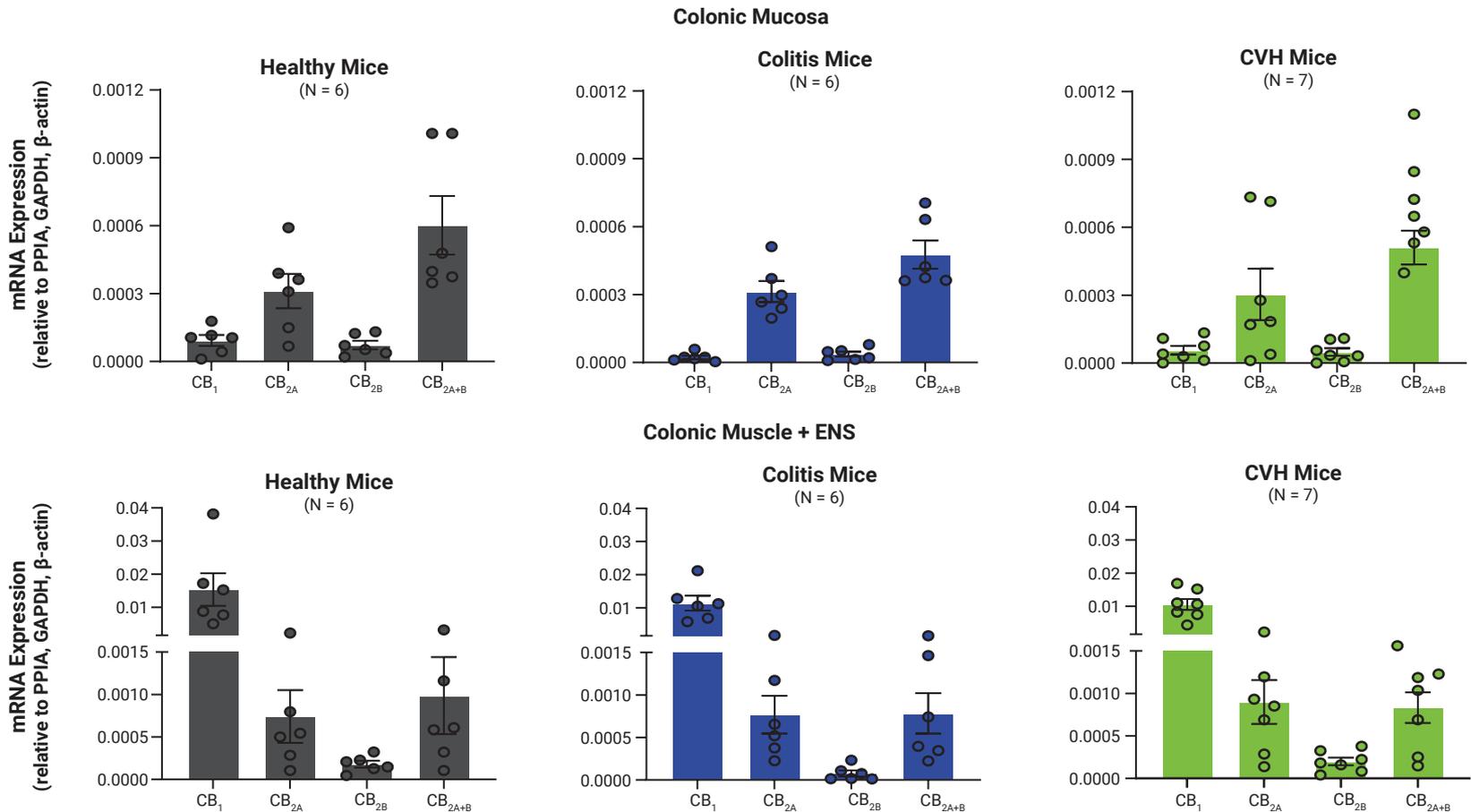
- Olorinab (0.01-10 μM) had no effect on healthy colonic nociceptor mechanosensory response²²

RESULTS (CONTINUED)

RELATIVE mRNA EXPRESSION OF CB₁ AND CB₂ IN COLONIC TISSUE AND DRG

- CB₂ was the predominant cannabinoid receptor transcript in the colonic mucosa, while CB₁ was the predominant transcript in the colonic muscle + ENS and DRG across all states (**Figure 4**)
- Relative mRNA expression levels of the individual and combined CB₂ isoforms were similar in the colonic mucosa, colonic muscle + ENS, and DRG in healthy versus colitis and CVH mice (**Figure 4**)

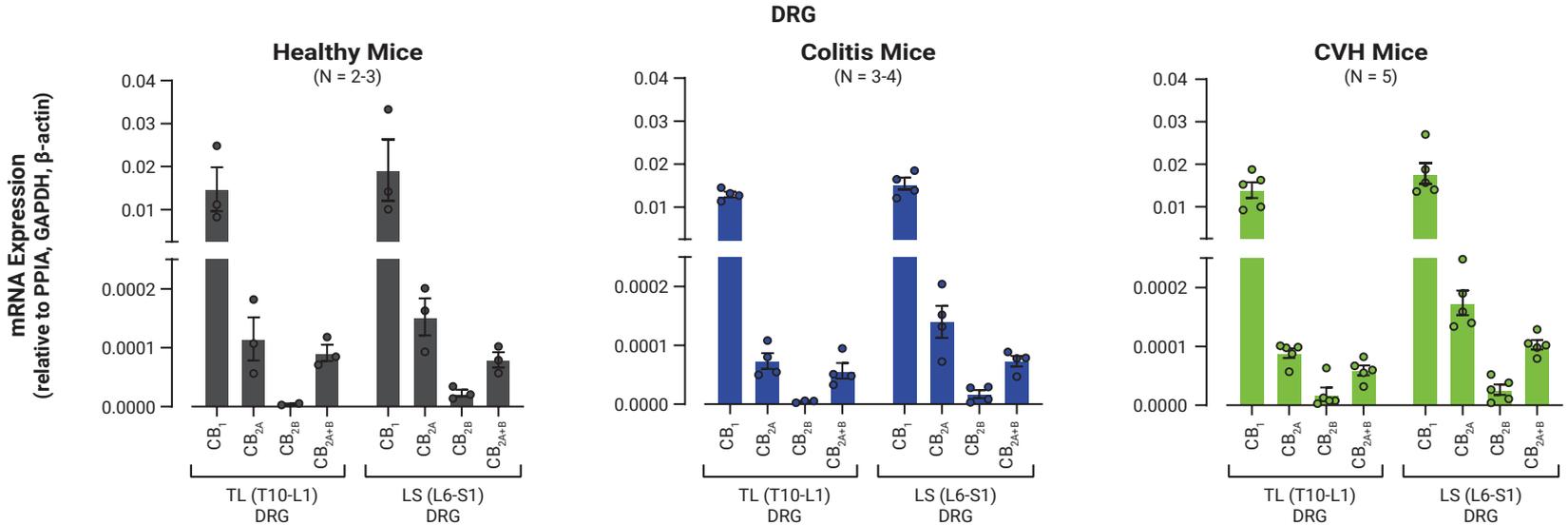
Figure 4. CB₁ and CB₂ Relative mRNA Expression in Healthy, Colitis, and CVH Mice



CB₁, cannabinoid receptor 1; CB₂, cannabinoid receptor 2; CVH, chronic visceral hypersensitivity; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; mRNA, messenger RNA; PPIA, peptidylprolyl isomerase A. All data are presented as mean \pm standard error of the mean.

RESULTS (CONTINUED)

Figure 4. CB₁ and CB₂ Relative mRNA Expression in Healthy, Colitis, and CVH Mice (Continued)

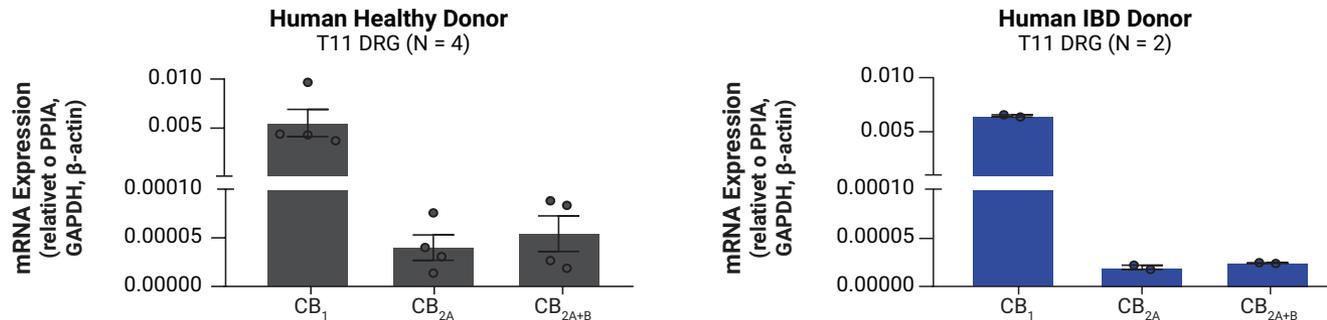


CB₁, cannabinoid receptor 1; CB₂, cannabinoid receptor 2; CVH, chronic visceral hypersensitivity; DRG, dorsal root ganglia; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; LS, lumbosacral; mRNA, messenger RNA; PPIA, peptidylprolyl isomerase A; TL, thoracolumbar.

All data are presented as mean ± standard error of the mean.

- Similar to our findings in rodent DRG, CB₂ was expressed in human DRG, but CB₁ was the predominant receptor (Figure 5)
- CB₂ expression levels were comparable in human DRG from donors with IBD and healthy donors (Figure 5)

Figure 5. CB₁ and CB₂ Relative mRNA Expression in Human DRG From Healthy and IBD Donors



CB₁, cannabinoid receptor 1; CB₂, cannabinoid receptor 2; DRG, dorsal root ganglia; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IBD, inflammatory bowel disease; mRNA, messenger RNA; PPIA, peptidylprolyl isomerase A.

All data are presented as mean ± standard error of the mean.

CONCLUSIONS

Olorinab reduced visceral hypersensitivity in a dose- and CB₂-dependent manner in animal models of IBD and IBS but not in healthy controls, suggesting that activation of CB₂ causes antinociceptive effects in visceral sensory pathways in disease states

CB₂ was observed at low levels in all tissues examined and was more predominant than CB₁ in the colonic mucosa, supporting the hypothesis that olorinab activates CB₂ receptors located in the colon to decrease visceral hypersensitivity

In line with previous research,²³ this analysis found that, though CB₁ was the predominant cannabinoid receptor expressed on DRGs, CB₂ was also expressed on DRGs, a peripheral site of action that may partially account for the antinociceptive effects of olorinab

CB₂ expression levels were similar in healthy and disease states, suggesting that subtle differences in CB₂ expression patterns may not be captured by measuring whole tissue mRNA, and more sensitive methods to assess localization of CB₂ on specific cell types may be required

These data support the further clinical development of olorinab as a novel therapeutic approach for the management of chronic visceral pain in gastrointestinal disorders

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