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Novel orally available salvinorin A analog PR-38 protects against experimental colitis and reduces abdominal pain in mice by interaction with opioid and cannabinoid receptors

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ABSTRACT

Background: Salvinorin A (SA) is a potent anti-inflammatory diterpene isolated from the Mexican plant S. divinorum. Recently we showed that the novel SA analog, PR-38 has an inhibitory effect on mouse gastrointestinal (GI) motility mediated by opioid and cannabinoid (CB) receptors. The aim of the study was to characterize possible anti-inflammatory and antinociceptive action of PR-38 in the mouse GI tract.
Methods: Macro- and microscopic colonic damage scores and myeloperoxidase activity were determined after intraperitoneal (i.p.), intracolonic (i.c.), and per os (p.o.) administration of PR-38 in the trinitrobenzene sulfonic acid (TNBS) and dextran sodium sulfate (DSS) models of colitis in mice. Additionally, MOP, KOP and CB1 protein expression was determined using Western blot analysis of mouse colon samples. The antinociceptive effect of PR-38 was examined based on the number of behavioral responses to i.c. instillation of mustard oil (MO).
Results: The i.p. (10 mg/kg, twice daily), i.c. (10 mg/kg, twice daily) and p.o. (20 mg/kg, once daily) administration of PR-38 significantly attenuated TNBS- and DSS-induced colitis in mice. The effect of PR-38 was partially blocked by the KOP antagonist nor-binaltorphimine and CB1 antagonist AM 251. Western blot analysis showed a significant increase of MOP, KOP and CB1 receptor expression during colonic inflammation, which was reversed to the control levels by the administration of PR-38. PR-38 significantly decreased the number of pain responses after i.c. instillation of MO in the TNBS-treated mice.
Conclusions: Our results suggest that PR-38 has the potential to become a valuable anti-inflammatory and analgesic therapeutic for the treatment of GI inflammation.
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1. Introduction

Human central and peripheral nervous system, as well as non-neuronal sites are abound in opioid, namely MOP, DOP, KOP and cannabinoid (CB1 and CB2) receptors [1–3]. Various types of opioid and CB receptors are expressed throughout the wall of the gut, particularly in the two ganglionated plexuses, the myenteric and submucosal plexus of the enteric nervous system (ENS) [2–5]. Ganglia of the ENS consist of both, efferent and afferent fibers, as well as interneurons, thus opioid and CB receptors are very likely to be present on all functional classes of enteric neurons. Opioid and cannabinoid ligands play a crucial role in the physiology and pathophysiology of the gastrointestinal (GI) tract; moreover, some of their actions seem to be interconnected, but the exact mechanism of this phenomenon in yet not fully understood (for review see: [2] and [6]).

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Inflammatory bowel diseases (IBD) are acute GI disorders characterized by impaired immunological response, which results in a chronic, progressing and relapsing inflammatory state in the gut. Despite the numerous studies in the field of IBD, its etiology remains unclear. One of the specific features of inflammatory GI disorders is the up-regulation of opioid and CB receptors in the tissues, both at mRNA and protein level [6–8]. This includes MOP, KOP and CB1 receptor protein in the course of colitis in mice [9,10].

The major therapeutic goals in IBD patients are the control of inflammation and the treatment of clinical symptoms, which include abdominal pain and altered bowel movements. Opioid and CB agonists significantly attenuate visceral nociception, as shown in animal models, as well as in human subjects [2,11–13]. Of interest, the antinociceptive effects of MOP, KOP and CB agonists are enhanced in the inflammatory conditions, confirming an up-regulation of opioid and CB systems in the inflamed intestine [14–16]. The use of classical MOP, KOP and CB1 agonists for the clinical treatment of IBD is strongly limited by central and peripheral adverse side effects. Design and synthesis of novel derivatives is thus a promising and considered necessary direction in search for novel drugs for inflammatory diseases of the GI tract.

Previously, our group showed that the novel, orally available analog of salvinorin A (SA), PR-38 has potent effect on GI motility and visceral nociception, which was mediated principally via MOP, KOP and CB1 receptors [17]. In this study we characterized the anti-inflammatory effect of PR-38 in two models of experimental colitis in mice with a particular interest in the actions in different administration routes and dosing regimens. We also used antagonists of MOP, KOP and CB1 receptors to evaluate the mechanism of anti-inflammatory action of PR-38. Furthermore, we determined the effect of PR-38 administration on MOP, KOP and CB1 expression at the protein level. Finally, since visceral pain is one of the most common symptoms in IBD, we also investigated the antinociceptive effect of PR-38 in control and TNBS-treated animals.

2. Materials and methods

2.1. Animals

Experimentally naive male C57B1/6 mice were obtained from the Animal House of the University of Lodz, Poland. All animals used in experiments weighed 22–30 g. The animals were housed at a constant temperature (22 °C) and maintained under a 12-h light/dark cycle (lights on 6:00 a.m.) in sawdust-lined plastic cages with access to chow pellets and tap water *ad libitum*. All animal protocols were in accordance with Polish legislation acts concerning animal experimentation. The experimental protocol was approved by the Local Ethics Committee at the Medical University of Lodz (#590/2012). All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Induction of colitis

2.2.1. TNBS model

Colitis was induced by intracolonic (i.c.) instillation of 2,4,6-trinitrobenzene sulfonic acid (TNBS), as described before [18]. Briefly, mice were slightly anesthetized with 1% isoflurane (Baxter Healthcare Corp., IL, USA) and TNBS (4 mg in 0.1 ml of 30% ethanol in saline) was instilled into the colon through a catheter inserted into the anus (3 cm proximally).

Control animals received vehicle alone (0.1 ml of 30% ethanol in saline). Preliminary experiments demonstrated that the dose of TNBS used in this study induced reproducible colitis.

2.2.2. DSS model

Colitis was induced by the addition of DSS (4% w/v; molecular weigh 40,000; MP Biomedicals, Aurora, OH, Lot No. 5237 K) to drinking water from day 0 to day 5. On days 6 and 7 animals received tap water (without DSS). Control animals received tap water throughout entire experiment. Animal body weight was monitored daily and mean water and food consumption was recorded.

2.3. Pharmacological treatments

PR-38 was administered once daily at the dose of 20 mg/kg (per os, p.o.) in TNBS model, 1, 5 and 10 mg/kg twice daily (intraperitoneally, i.p.) and 10 mg/kg (i.c. in TNBS or DSS models, with the first treatment 30 min before the induction of colitis. Selective MOP (β-FNA, 1 mg/kg i.p.), KOP (norBNI, 10 mg/kg, i.p.), and CB1 (AM 251, 1 mg/kg, i.p.) receptor antagonists were administered 10 minutes before PR-38 [18]. Control animals received vehicle (i.p., i.c. or p.o.) alone. None of the antagonists influenced the observed parameters when given alone.

2.4. Evaluation of colonic damage

2.4.1. TNBS model

Animals were sacrificed by cervical dislocation 3 days after TNBS infusion. The colon was rapidly removed, opened longitudinally, rinsed with phosphate buffered saline (PBS), and immediately examined. Macroscopic colonic damage was assessed by an established semiquantitative scoring system by adding individual scores for ulcer, colonic shortening, wall thickness, and presence of hemorrhage, fecal blood, and diarrhea, as described before [18]. For scoring ulcer and colonic shortening the following scale was used: ulcer—0.5 points for each 0.5 cm; shortening of the colon: 1 point for >15%, 2 points for >25% (based on a mean length of the colon in untreated mice of 8.07 ± 0.20 cm, $n = 6$). The wall thickness was measured in mm. The presence of hemorrhage, fecal blood, or diarrhea increased the score by 1 point for each additional feature.

2.4.2. DSS model

Mice were sacrificed by cervical dislocation 7 days after addition of DSS to the drinking water. The colon was rapidly isolated and weighed with fecal content. Colon was then opened along the mesenteric border and fecal material removed. A total macroscopic damage score was calculated for each animal including stool consistence (0–3), colon epithelial damage considered as number of ulcers (0–3), colon length and weight scores (0–4), where score = 0 means no inflammation [18]. The presence (score = 1) or absence (score = 0) of fecal blood was also recorded.

2.5. Determination of tissue myeloperoxidase activity

The method described by Fichna et al. [18] was used to assess granulocyte infiltration and quantify the myeloperoxidase (MPO) activity. Briefly, 1-cm segments of colon were weighed and homogenized in hexadecyltrimethylammonium bromide (HTAB) buffer (0.5% HTAB in 50 mM potassium phosphate buffer, pH 6.0; 1:20 w/v) immediately after isolation and the homogenate was centrifuged (15 min, 13,200 × *g*, 4 °C). On a 96-well plate, 200 μl of 50 mM potassium phosphate buffer (pH 6.0), containing 0.167 mg/ml of *O*-dianisidine hydrochloride and 0.05 μl of 1% hydrogen peroxide was added to 7 μl of supernatant. Absorbance was measured at 450 nm (iMARK Microplate Reader, Biorad, United Kingdom). All measurements were performed in triplicate.

MPO was expressed in milliunits per gram of wet tissue, 1 unit being the quantity of enzyme able to convert 1 μmol of hydrogen peroxide to water in 1 min at room temperature. Units of MPO activity per 1 min were calculated from a standard curve using purified peroxidase enzyme.

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