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Anti-inflammatory action of a novel orally available peptide 317 in mouse models of inflammatory bowel diseases

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ABSTRACT

Background: The endogenous opioid system constitutes an attractive target in the treatment of GI disorders, including inflammatory bowel diseases (IBD). The aim of our study was to characterize the anti-inflammatory and antinociceptive effect of P-317, a novel cyclic analog of opioid peptide morphiceptin, in animal models of IBD.
Methods: The anti-inflammatory effect of P-317 after intraperitoneal (*ip*) and oral (*po*) administration was assessed in two mouse models of IBD – Crohn's disease, induced by intracolonic instillation of trinitrobenzenesulfonic acid (TNBS) and ulcerative colitis, induced by addition of dextran sodium sulfate (DSS) into drinking water. The antinociceptive action of P-317 was characterized in mice with acute colitis using mustard oil-induced pain test. Real time RT PCR was used to assess semiquantitatively the expression of IL-1β and TNF-α mRNA in mouse colonic samples. To translate our results to clinical conditions, MOP and KOP mRNA were quantified in human colonic biopsies from IBD patients.
Results: P-317 (0.1 mg/kg, *ip* and 1 mg/kg, *po*) alleviated colonic inflammation in TNBS- and DSS-treated mice in the opioid receptor-dependent manner. The anti-inflammatory effect of P-317 was associated with the decrease in mRNA expression of proinflammatory cytokines. The antinociceptive effect of P-317 was observed after *ip* and *po* administration in mice with acute colitis.
Conclusion: Our results show a potent anti-inflammatory and antinociceptive effect of P-317 in mouse models of colitis upon activation of opioid receptors. The unique bioavailability of P-317 after oral administration suggests that it is a promising drug candidate for future treatment of IBD.

Introduction

Inflammatory bowel diseases (IBD), which include Crohn's disease (CD) and ulcerative colitis (UC), are severe chronic disorders of the gastrointestinal (GI) tract [1]. The major symptoms of IBD include inflammation of the colon and abdominal pain, as well as other symptoms, like altered visceral sensation, diarrhea, rectal bleeding, weakness and weight loss [2]. The pathogenesis of IBD is still unknown and so far there is no effective and well-defined first-line therapy. The drugs currently available on the market allow mostly symptomatic treatment and their effectiveness needs to be improved [3]; therefore novel therapeutic strategies for IBD are urgently needed. The major goals to be achieved in IBD patients are the alleviation of inflammation and the attenuation of IBD symptoms, mainly abdominal pain and altered bowel movements [4].

Opioid receptors, namely MOP (μ-opiod receptor), DOP (δ-opioid receptor) and KOP (κ-opioid receptor), belong to a family of G protein-coupled receptors (GPCR) [5]. They are widely distributed in the central and peripheral nervous system and can also be found in non-neuronal cells [6]. The expression of opioid receptors in the GI tract has been reported in neuronal (myenteric and submucosal plexuses), muscular and immune cells [7]. The localization of opioid receptors, including MOP, is closely related to

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the role of opioid ligands in the GI tract – they act as potent antinociceptive and immunomodulatory agents and inhibit GI motility [8]. The application of MOP ligands in the treatment of GI inflammatory disorders may therefore become an attractive alternative or important addition to currently available, mostly inefficient strategies. However, the action of opioids is linked with multiple adverse effects, including CNS-related euphoria and addiction and the extensive research is currently focused on minimization of these side effects and restriction of ligands action to the periphery [9].

In the last two decades a considerable increase in the interest to use peptides as drugs has been observed [10]. Peptides have high potency, good selectivity and low toxicity. However, most of naturally peptides occurring cannot be administered orally, because they are rapidly inactivated by degradation enzymes in the GI tract and serum and thus cannot reach their pharmacological target. To circumvent this problem, novel peptide-based compounds have been synthesized to obtain the most favorable receptor binding affinity, selectivity and resistance to proteolytic degradation. The strategies, which enable conformational and topographical modifications of the peptide structure, include insertion of unnatural amino acids and covalent or noncovalent constraints. Also, design of peptidomimetic ligands, glycopeptides, and N-terminal amidinationed analogs, e.g. incorporating guanidine to increase the penetration through biological membranes has been proposed (for review see [11,12]). Finally, cyclization has been used as a well-established and powerful tool in peptide chemistry to obtain analogs with improved bioactivity and bioavailability. Cyclic peptides, in comparison with linear peptides, have been considered to exert increased chemical and enzymatic stability, improved pharmacodynamic properties and receptor selectivity [13].

Morphiceptin (Tyr-Pro-Phe-Pro-NH_2) is an opioid tetrapeptide isolated from the enzymatic digest of bovine β-casein [14]. Morphiceptin is a selective MOP agonist, with over 1000 times higher selectivity for MOP over DOP [15]. The intracerebroventricular (*icv*) administration of morphiceptin produced supraspinal antinociception [16], but no effect of morphiceptin was observed after peripheral administration, suggesting its relatively rapid degradation [15,16]. Nevertheless, morphiceptin has become a popular template for the design of novel analogs. One of the newly synthesized morphiceptin derivatives, Tyr-Pro-NMePhe-Pro-NH_2, was effective in diarrhea treatment after subcutaneous (*sc*) injection in mice [18]. Another morphiceptin analog, PL-017 is a potent analgesic and antidiarrheal agent [19], suggesting that morphiceptin may find application as a template for drug design in the treatment of GI diseases.

The peptide P-317, Dmt-c(D-Lys-Phe-D-Pro-Asp)NH_2, a cyclic analog of morphiceptin was recently synthesized in our laboratory and its pharmacological profile was characterized *in vitro* and *in vivo*, as described earlier [17]. Promising pharmacokinetics of P-317 encouraged us to proceed further with our studies and to characterize the anti-inflammatory and analgesic action of the peptide in mouse models of IBD. Here we also aimed at elucidating the mechanism of the anti-inflammatory action of P-317 and the possible role of the endogenous opioid system as the target in the treatment of colitis.

Materials and methods

Animals

Male balbC mice (Animal Facility of the University of Lodz, Poland), weighing 22–26 g were used for all experiments. Mice were housed at a constant temperature (22–24 °C) and maintained under a 12-h light/dark cycle with free access to laboratory chow and water ad libitum.

The study was carried out in strict accordance with the institutional recommendations. The protocol was approved by the Local Ethical Committee for Animal Experiments (# 589/2011).

Induction of colitis and assessment of colonic damage

TNBS model

In the animal model of CD, colitis was induced by intracolonic (*ic*) administration of trinitrobenzenesulfonic acid (TNBS), as described previously [20]. Briefly, mice (n = 4–6 per treatment group) were lightly anesthetized with 1% isoflurane (Aerrane, Baxter, Deerfield, USA) and TNBS (4 mg in 0.1 ml of 30% ethanol in 0.9% NaCl) was infused into the distal colon using a catheter inserted 2.5 cm proximally to the anus. Mice were monitored daily for clinical symptoms of colonic inflammation: weight loss, diarrhea and bloody stool. In preliminary experiments the dose of 4 mg for TNBS induced reproducible colitis and therefore was used throughout the study.

On day 4 after TNBS-administration, animals were sacrificed by cervical dislocation. The colon with caecum was quickly removed, opened longitudinally, washed with phosphate-buffered saline (PBS) and immediately examined. The total macroscopic colonic damage was assessed using previously established scoring system [20], based on the extent of ulcerated area, colonic shortening and wall thickness and the presence of hemorrhage, fecal blood, and diarrhea. The total macroscopic score is a sum of the following parameters: for ulcer score: 0.5 points for each 0.5 cm, the adhesion (0–2), the wall thickness measured in mm, the presence of hemorrhage, fecal blood, or diarrhea increased the score by 1 point for each additional feature.

DSS model

Dextran sodium sulfate (DSS) induces significant macrophage infiltration into the colonic epithelium [21]. DSS in the mouse model of ulcerative colitis used in this study, DSS was added to drinking water (4%, w/v) on days 0–5. On days 6–7 the animals received water without DSS. Control animals received tap water throughout the entire experiment. Animal body weight was assessed daily.

On day 7, mice were sacrificed by cervical dislocation. Entire colon without caecum was immediately dissected, measured and weighed. Then the colon was opened along the mesenteric border, placed in the PBS buffer and the fecal pellets were removed by gentle flushing. Total macroscopic damage score was calculated for each animal, based on the following parameters [20]: presence of diarrhea (0 = no diarrhea, 1 = loosely shaped moist pellets, 2 = amorphous, moist, sticky pellets, 3 = diarrhea), the extent of colon damage (0 = no inflammation, 1 = reddening, mild inflammation, 2 = moderate inflammation or more widely distributed, 3 = severe inflammation and/or extensively distributed), colon length (0 = <5% shortening, 1 = 5–14% shortening, 2 = 15–24% shortening, 3 = 25–35% shortening, 4 = > 35% shortening) and weight (0–4, the loss as same as in colon length). The presence (=1) or absence (score = 0) of fecal blood was also included.

Pharmacological treatments

All drugs were dissolved in 5% dimethyl sulfoxide (DMSO) in saline. Control animals received vehicle alone. P-317 was administered intraperitoneally (*ip*) twice daily at the dose of 0.1 mg/kg (TNBS and DSS) and orally (*po*) at the dose of 1 mg/kg (TNBS). The first treatment with P-317 was 15 min before the induction of colitis.

Selective MOP (β-funaltrexamine, β-FNA, 1 mg/kg, *ip*) and KOP (nor-binaltorphimine, nor-BNI, 10 mg/kg, *ip*) and a non-selective opioid receptor (naloxone methiodide, MetNal, 1 mg/kg, *ip*) antagonists were administered 15 min before P-317 injections. All of the used antagonists did not influence the observed parameters when given alone.