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Methyl-orvinol – dual activity opioid receptor ligand inhibits gastrointestinal transit and alleviates abdominal pain in the mouse models mimicking IBS-D

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Abstract

Background—Diarrhea-predominant irritable bowel syndrome (IBSD) is a functional disorder of the gastrointestinal (GI) tract. The major IBS-D symptoms include diarrhea, abdominal pain and discomfort. High density of opioid receptors (ORs) in the GI tract and their participation in the maintenance of GI homeostasis make ORs ligands an attractive option for developing new anti-IBS-D treatments.

The aim of this study was to characterize the effect of methyl-orvinol on the GI motility and secretion and in mouse models mimicking symptoms of IBS-D.

Materials and methods—In vitro, the effect of methyl-orvinol on electrical field stimulated smooth muscle contractility and epithelial ion transport were characterized in the mouse colon. In vivo, the following tests were used to determine methyl-orvinol effect on mouse GI motility: colonic bead expulsion, whole GI transit and fecal pellet output. An antinociceptive action of methyl-orvinol was assessed in the mouse model of visceral pain induced by mustard oil.

Results—Methyl-orvinol (10⁻¹⁰ – 10⁻⁶ M) inhibited colonic smooth muscle contractions in a concentration-dependent manner. This effect was reversed by naloxone (non-selective opioid antagonist) and β -funaltrexamine (selective MOP antagonist). Experiments with a selective KOP receptor agonist, U50488 revealed that methyl-orvinol is a KOP receptor antagonist in the GI tract. Methyl-orvinol enhanced epithelial ion transport. In vivo, methyl-orvinol inhibited colonic bead

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#Equal contribution

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Disclosures

Designed the research study: MZ, JF

Performed the research: AJ, MZ, AW, GC-K

Analyzed the data: AJ, MZ, SH, JF

Wrote the paper: AJ, MZ, JF

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expulsion and prolonged GI transit. Methyl-orvinol improved hypermotility and reduced abdominal pain in the mouse models mimicking IBS-D symptoms.

Conclusion—Methyl-orvinol could become a promising drug candidate in chronic therapy of functional GI diseases such as IBS-D.

Keywords

abdominal pain; GI motility; methyl-orvinol; opioid receptors; orvinols

Introduction

Endogenous opioid system is composed of opioid peptides and cell surface receptors. ORs are divided into three subtypes μ (mu, MOR), δ (delta, DOR) and κ (kappa, KOR). In the human body ORs are widely distributed in the central and peripheral nervous system and non-neuronal tissues (mainly in the GI system). In the GI tract they are present on smooth muscle cells, at the terminals of sympathetic and sensory peripheral neurons and immune cells, where they are involved in the maintenance of homeostasis through modulation of the intestinal motility, but also fluid and electrolyte secretion/absorption and immune response [1;2]. The role of ORs agonists in pain modulation is well known and determined in numerous animal models of pain. This antinociceptive effect is mediated through either central or peripheral ORs. There are several differences in molecular mechanism of action between types of ORs. It was reported, that activation of MOR and DOR results in inhibition of adenylate cyclase, decrease of cAMP and reduced protein kinase A activation (what leads to reduction of neuronal excitability). Moreover, activation of MOR and DOR results in inhibition of Ca^{2+} channels and activation of K^{+} channels. While the action mediated by KOR is limited to reduction of neurotransmitters release, a result of inhibition of Ca^{2+} channels located on the nerve terminals [2].

In recent years, researchers have investigated the role of the endogenous opioid system in the pathophysiology of GI diseases. For instance, it was evidenced that the relative mRNA expression of MOR and KOR is decreased in patients with IBS-D [3]. IBS-D is a chronic relapsing functional GI disorder, which affects up to 4% of population. IBS-D patients suffer from diarrhea, abdominal pain, cramping, and bloating (for review see: [4]). Their quality of life is poor during symptoms exacerbation. Moreover, patients with IBS-D have psychosocial disturbances and more frequently suffer from depression or affective disorders. Currently available anti-IBS-D therapeutics only alleviate symptoms of the disease. Therefore, ORs appear to be promising pharmacological targets in IBS-D therapy.

Orvinols (6,14-ethenotetrahydrooripavine derivatives), developed by Kenneth Bentley and his group in 1960s, belong to a large group of thebaine derivatives [5]. Orvinols are strong antinociceptive agents (comparable or even more effective than morphine). They were initially designed in the hope of reducing adverse effects common for opioids (i.e. morphine), such as: tolerance and addiction development, respiratory depression or constipation. The primary effects of orvinols are mediated through one or both, MOR and KOR [6].

The structure of orvinols and their interesting pharmacological profile have gained the attention of numerous researchers. It was observed that already minor modifications in certain parts in the structure result in significant changes in their action. The pharmacological profile of orvinols has been characterized in various assays including early work in isolated tissues such as vas deferens isolated from rats and rabbits and more recently in [³⁵S]GTPγS assays [6]. While binding affinity remains high for each ORs, efficacy at MOR and KOR, is strongly influenced by the N-substituent (typically methyl or cyclopropylmethyl groups) and the groups attached to C₂₀ [7]. Similarly, Bentley et al. [5] reported that in the thevinols, 3-O-methyl ether analogues of the orvinols, modification of the C₂₀ group significantly altered antinociceptive potential in the tail pressure test in rats.

Methyl-orvinol, the simplest tertiary alcohol member of the orvinol family, having two methyl groups attached to C₂₀, was characterized in vasa deferentia as a potent MOR and KOR ligand [6]. In our study we determined the effect of methyl-orvinol on GI motility, secretion and abdominal pain in mouse models mimicking IBS-D symptoms.

Materials and methods

Animals

In this study, male Balb/C mice (Institute of Occupational Medicine, Lodz, Poland), weighing from 22 to 26 g were used. Mice were maintained under a 12-h light/dark cycle and at a constant temperature (22–23°C). Mice were housed in sawdust-lined plastic transparent cages with a free access to laboratory chow and tap water. All of the experiments in this study were performed in accordance with respective national guidelines and animal use was approved by the Local Ethical Committee (48/ŁB700/DLZ/2015).

In vitro experiments

Isolated smooth muscle strips—Organ bath studies were performed as described previously [8]. Mice were sacrificed by cervical dislocation. Subsequently, the colon was rapidly removed and full-thickness fragments (0.5 cm) of the distal colon were kept in Krebs solution (NaCl 115.0 mM, KCl 8.0 mM, KH₂PO₄ 2.0 mM, NaHCO₃ 25.0 mM, MgCl₂ 2.4 mM, CaCl₂ 1.3 mM, and glucose 10.0 mM). One end of each colonic fragment was attached to the bottom of the individual organ bath, another end to a FT03 force displacement transducer (Grass Technologies, West Warwick, RI, USA) using a silk thread. Each segment of the colon was placed between two electrodes in organ bath containing Krebs solution (25 ml) oxygenated with 95% O₂ and 5% CO₂ at constant temperature (37°C). The changes in tension were amplified by a P11T amplifier (Grass Technologies, West Warwick, RI, USA) and recorded using the POLYVIEW software (Polybytes Inc., Cedar Rapids, IA, USA). Electrical field stimulation was applied by a S88X stimulator (Grass Technologies, EFS, 8 Hz, 60 V, pulse duration 0.5 ms, train duration 10 s), and delivered through electrodes placed around the tissue.

The tissue preparations were exposed to methyl-orvinol in increasing concentrations (10^{−10} to 10^{−6} M). Methyl-orvinol was added cumulatively into the organ bath for 8 min for each concentration. The effect of tested compound was recorded on a personal computer using the

PolyView software (Polybytes Inc., 108 Cedar Rapids, IA, USA). At first, the mean amplitude of four twitch contractions was measured and treated as an internal control (in control experiments, the effect of the vehicle (DMSO) was assessed). The changes in smooth muscle contractions were reported as the percentage of the internal control. These assays were performed as paired with respective controls, using four organ baths in parallel.

To assess the involvement of ORs, the following OR antagonists were added 10 min prior to methyl-orvinol: naloxone (10^{-6} M, a non-selective OR antagonist) and β -funaltrexamine (β -FNA, 10^{-6} M, to block MOR).

In further *in vitro* experiments we wanted to determine if methyl-orvinol is KOR antagonist. Thus, we assessed the effect of U50488, selective KOR agonist (concentrations ranging from 10^{-10} – 10^{-6} M, added cumulatively), on smooth muscles contractility of the mouse colon, in the presence of β -FNA (10^{-6} M) (added at the beginning of the experiment), and methyl-orvinol (10^{-6} M) (added 10 minutes after MOR antagonist). The results were compared to contractility of tissue strips exposed to U50488 alone (10^{-10} to 10^{-6} M).

Epithelial ion transport—Epithelial ion transport was assessed according to techniques described earlier [9]. The distal colon was removed and kept at 37°C Krebs solution (NaCl 115.0 mM, KCl 8.0 mM, KH_2PO_4 2.0 mM, NaHCO_3 25.0 mM, MgCl_2 2.4 mM, CaCl_2 1.3 mM). Then preparations were opened along the mesenteric border and mounted in Ussing chambers (0.6 cm² opening). Two tissue segments were used per mouse - one as a vehicle control, another exposed to tested compound.

Using WPI EVC-4000 voltage clamp (World Precision Instruments, Sarasota, FL, USA), tissues were subjected to short circuited conditions. At the beginning and the end of each experiment tissues were unclamped to record open potential difference values for the calculation of tissue conductance (mS/cm²). After establishment of baseline I_{sc} (15–30 min), tested drug (10^{-6} M) or vehicle (DMSO) was added (final concentration: 0.1%). Ten minutes later, preparations were challenged with either forskolin (cAMP-dependent secretagogue activator, 10^{-6} M) or veratridine (voltage-dependent Na⁺ channel activator, 3×10^{-5} M).

***In vivo* motility studies**

Colonic expulsion test—The distal colonic expulsion test was performed in mice, which were fasted for 12–14 hours. Briefly, mice were slightly anesthetized by inhalation of 1% isoflurane (Aerrane, Baxter, Deerfield, USA) and a pre-warmed (37°C) glass bead was inserted 2.5 cm into the distal colon using a silicone pusher. After the bead insertion, mice were placed in individual cages on a white sheet and the time to bead expulsion was measured. Animals that did not expel the bead during 30 minutes were killed and the presence of the bead was confirmed in the lumen of the colon.

Methyl-orvinol (0.1, 0.3 or 1 mg/kg), was administered intraperitoneally (*ip*) 15, 45 or 90 min before colon bead insertion. To evaluate if effect of methyl-orvinol *in vivo* is mediated through ORs (particularly MOR), naloxone and β -FNA, respectively, were administered (both at the dose of: 1 mg/kg, *ip*). Quaternary salt of naloxone, methiodide-naloxone (1

mg/kg, *ip*), a non selective ORs antagonist was used to assess if effect of methyl-orvinol is mediated through peripheral ORs.

Whole gastrointestinal transit time—Whole GI transit test in mice was performed as described earlier [8]. Methyl-orvinol or vehicle was injected *ip* 15 min before intragastric (*ig*) administration of coloured marker (0.15 ml of liquid consists of 5% Evans blue and 5% gum Arabic). The colored dye was administered with 18-gauge animal feeding tube. Subsequently, mice were placed to the individual cages placed on a white sheet of paper (in order to facilitate recognition of coloured pellets). The whole GI transit is described as time between *ig* administration of marker and first coloured bolus excreted.

Fecal pellet output and mouse model of hypermotility—One day before the experiment non-fasted animals were placed into individual cages for habituation. On experimental day, methyl-orvinol (0.1 mg/kg *ip*) or vehicle (*ip*) was administered and immediately mice were returned to clean separated cages. Afterwards, fecal pellets excreted over a 60 min period were counted.

The exposition to novel environment was used to mimic stress conditions and induce hypermotility. Mice were not separated for habituation before the experiment. On the day of the experiment, tested compound or vehicle was administered *ip* and immediately after animals were placed to individual cages. Fecal pellets, excreted over a 60 min period were counted and the results were compared between groups.

Mouse model of castor-oil induced diarrhea—The anti-diarrheal effect of methyl-orvinol (0.1 mg/kg, *ip*) was assessed in mouse model of diarrhea induced by castor-oil. Its efficacy was compared to loperamide (1 mg/kg *ip*) and vehicle. Tested compound or vehicle were administered *ip* 15 min prior to castor oil (*ig*, 0.2 ml/mouse). Then, mice were placed into individual cages. The time between the administration of castor oil and excretion of liquid faeces was measured and compared between groups.

Behavioral Pain Responses—Mustard-oil (MO, allyl isothiocyanate) induced pain test was performed as described earlier [10]. Mustard oil (1% in 70% ethanol) was administered intracolonicallly (0.05 ml, *ic*) under isoflurane anaesthesia. Subsequently, mice were placed to individual transparent cages. After 5 min of recovery, spontaneous pain-related behaviours, such as: licking of the abdomen, squashing of the lower abdomen against the floor, stretching the abdomen, and abdominal retractions, were observed for 20 min and counted as 1. The observation was performed by blinded experimenter.

Methyl-orvinol (0.1 mg/kg) or vehicle was injected *ip* before MO administration. Selective MOR antagonist – β -FNA (1 mg/kg *ip*), was injected 15 min before methyl-orvinol. Notably, antagonist injected alone did not affect experiment conditions (data not shown).

Drugs

All reagents and drugs, unless otherwise stated, were purchased from Sigma-Aldrich (Poznan, Poland). Methyl-orvinol was synthesized in the Department of Pharmacy and Pharmacology, University of Bath (Bath, United Kingdom). Naloxone hydrochloride, β -

funaltrexamine hydrochloride were purchased from Tocris Bioscience (Ellisville, MO, USA).

In the *in vitro* experiments all drugs were dissolved in dimethyl sulfoxide (DMSO). In the *in vivo* tests, drugs were dissolved in 5% DMSO in saline, which was used as vehicle in control group. The vehicle given alone had no effects on the observed parameters.

Statistics

Statistical analysis was performed using Prism 5.0 (GraphPad Software Inc., La Jolla, CA, USA). The data are expressed as mean $\pm$ SEM. In the *in vitro* experiments, n indicates the number of individual tissue samples from 3 different animals. While, in the *in vivo* studies n stands for the number of animals. Student's t -test was used to compare single treatment means with control means. Analysis of one way variance (ANOVA) followed by Newman–Keuls *post hoc* test was used for analysis of multiple treatment means. p values < 0.05 were considered as statistically significant.

Results

Methyl-orvinol inhibited smooth muscle contractility in vitro

In organ bath studies, we evaluated the effect of methyl-orvinol on smooth muscle contractility in isolated segments of the mouse colon. We observed that methyl-orvinol inhibited EFS-induced colonic smooth muscle contractility in a concentration-dependent manner (10^{-10} to 10^{-6} M), Fig. 2A. This inhibitory effect was mediated through ORs, particularly MOR, because both naloxone (10^{-6} M) and β -FNA completely reversed methyl-orvinol action in the colon.

We also determined whether methyl-orvinol can act as a KOR antagonist. In order to do that, colon tissues were pre-treated with β -FNA (10^{-6} M), which was used to block MOR in the colon. Methyl-orvinol reduced the inhibitory effect of U50488, a selective KOR agonist (10^{-10} – 10^{-6} M) on smooth muscle contractions, Fig 2B.

Methyl-orvinol increased epithelial ion transport

In further studies, we characterized the impact of methyl-orvinol on the epithelial ion transport, Fig. 3. Forskolin, through adenylate cyclase activation, promoted Cl^- and H_2O secretion and impaired ion absorption. We observed that methyl-orvinol (10^{-6} M) increased ion transport in the colonic segments stimulated with forskolin, when compared to control (1.47 ± 0.02 vs. 0.43 ± 0.15 mA/cm², respectively), Fig. 3A. Moreover, we compared the effect of methyl-orvinol and loperamide on epithelial ion transport, as loperamide is an effective and well tolerated opioid anti-diarrheal agent. In contrast to methyl-orvinol, loperamide did not have any significant effect on transepithelial ion transport (0.53 ± 0.15 vs. 0.43 ± 0.15 mA/cm² for loperamide and control, respectively).

Veratridine, a voltage-dependent Na^+ channel activator, caused enteric neurons depolarization inducing Cl^- secretion through the colonic epithelium [11]. In the tissue stimulated with veratridine, methyl-orvinol (10^{-6} M) significantly increased transport of ions as compared to control (2.1 ± 0.78 vs. 0.32 ± 0.03 mA/cm²), Fig. 3B. Notably, in the

colonic tissue exposed to loperamide (10^{-6} M) significant changes in epithelial ion transport were also observed in comparison with control (0.72 ± 0.14 vs. 0.32 ± 0.03 mA/cm²).

Methyl-orvinol significantly inhibited GI motility

Colonic bead expulsion test was used to evaluate the action of methyl-orvinol after *ip* administration at the doses ranging from 0.1 to 1 mg/kg, Fig. 4A. Tested compound produced a significant inhibitory effect on the colonic motility already at the dose of 0.1 mg/kg (420.00 ± 60.22 vs. 47.30 ± 3.36 s in control group) at 15 min after drug administration.

The inhibitory potency of methyl-orvinol was dose-dependent and reached maximum 15 min after methyl-orvinol administration. Methyl-orvinol remained active up to 90 min after administration. The mean time to bead expulsion, 15 min after methyl-orvinol injection, was 1.6 fold- and 4.8 fold-higher at the doses of 0.3 and 1 mg/kg, respectively.

To investigate the involvement of ORs in the action of methyl-orvinol, classical OR antagonist naloxone, quaternary salt of naloxone (naloxone methiodide) and a selective MOR antagonist, β -FNA, were used. We observed that the inhibitory effect of methyl-orvinol in colonic bead expulsion test was mediated through ORs (295.00 ± 49.71 vs. 103.00 ± 23.06 s for methyl-orvinol-treated animals and mice pre-treated with naloxone). Then, we found that the action of methyl-orvinol is mediated mainly through MOR, as β -FNA alleviated this inhibitory effect, Fig. 4B. Of note, using the quaternary salt of naloxone (naloxone methiodide, 1 mg/kg *ip*), we determined that the methyl-orvinol action on GI motility resulted from activity at peripheral ORs.

Subsequently, we assessed the effect of methyl-orvinol on whole GI transit time. We observed that methyl-orvinol at the dose of 0.1 mg/kg (*ip*) delayed excretion of coloured dye (78.50 ± 4.31 vs. 62.30 ± 5.05 min for methyl-orvinol-treated group and controls, respectively), Fig. 4C.

Methyl-orvinol reduced fecal pellet output in mice

In fecal pellet output test, we evaluated the effect of methyl-orvinol on the GI motility in mice under physiological and pathophysiological conditions, Fig 5A. We observed a significant difference in action of methyl-orvinol between non-stressed and stressed mice. There was no statistically significant difference between methyl-orvinol-treated and control group in non-stressed mice (mean values: 2.29 ± 0.56 vs. 3.50 ± 0.62). However, the tested compound reduced hypermotility in stressed mice (exposed to novel environment), as compared to control (9.00 ± 0.82 vs. 13.67 ± 1.08 in control group).

Methyl-orvinol is deprived of antidiarrheal potency

Castor oil, administered *ig*, induces irritation in the intestinal mucosa and leads to acute diarrhea. Methyl-orvinol did not affect time to occurrence of diarrhea (64.63 ± 2.46 min in methyl-orvinol treated mice vs. 76.86 ± 5.00 min in control group). In contrary, in loperamide-treated group, time to diarrhea was over 2-fold longer than in control group (167.25 ± 5.32 vs. 76.86 ± 5.00 min, respectively), Fig 5B.

Methyl-orvinol exerted antinociceptive effect in mice

Mustard oil is an irritant agent which causes visceral hypersensitivity and therefore induces visceral pain in the colon (when administered *ic*). Methyl-orvinol at the dose of 0.1 mg/kg, administered *ip*, significantly alleviated visceral pain (40% reduction of total number behavioral pain responses). Interestingly, pre-treatment with β -FNA (1 mg/kg *ip*) reversed the antinociceptive potency of methyl-orvinol (62.90 ± 5.14 vs. 82.10 ± 2.191 for number of pain-related behaviors in methyl-orvinol-treated animals and mice pre-treated with β -FNA, respectively), Fig. 6.

Discussion

IBS-D is a chronic relapsing functional GI disorder combined with diarrhea, abdominal pain and discomfort, which significantly decrease patient's quality of life. Currently available therapies rely mainly on alleviation of symptoms and control of the disease course. Patients with IBS-D often use classical anti-diarrheal drugs such as loperamide or diphenoxylate, whose anti-diarrheal effect is mediated through MOR. However, these opioids do not normalize GI peristalsis in a long-term therapy and are deprived of antinociceptive action. Therefore, researchers focus on developing new drugs, which would combine normalization of bowel movement without constipating effect and alleviation of visceral pain.

In our study we observed that methyl-orvinol exerted an inhibitory effect on smooth muscle contractility *in vitro* and was active *in vivo*, in physiological and pathophysiological conditions. Finally, we defined methyl-orvinol as a potent analgesic drug.

In vitro, we observed that the inhibitory effect of methyl-orvinol in the colon relied on the interaction with MOR, which is in line with other studies on the role of this receptor in the gut. For example, a selective MOR agonist DAMGO inhibited smooth muscle contractility in the rat colon [12]. It was also reported by our group that the mixed MOR and KOR agonist, P-317 significantly reduced contractions in mouse ileum and colon [13]. We also found that a MOR and DOR agonist, biphallin exerted inhibitory effect on smooth muscle contractions in the mouse colon [14]. Eluxadoline, which is a MOR agonist and a DOR antagonist, inhibited EFS-induced contractions in guinea pig ileum in a concentration dependent manner [15]. The activation of MOR is crucial in the modulation of the GI motility. However an addition of another component of KOR or DOR or nociceptin (NOP) receptors (agonist or antagonist) may improve action of opioid drugs in the GI system. Noteworthy, we showed a dual activity of methyl-orvinol in the mouse colon, namely agonism at MOR and antagonism at KOR. In earlier studies, Lewis et al. [7] showed that methyl-orvinol revealed similar activity at MOR and KOR in vas deferens isolated from rat (RVD) and rabbit (LVD). Moreover, this group evaluated the expression of ORs in vasa deferentia and found that RVD exhibited high expression of MOR, while the LVD was abundant in KOR. RVD and LVD are characterized as tissues with low sensitivity, only highly potent agonists exhibit activity in this assay. Methyl-orvinol in RVD was characterized as a potent antagonist of normorphine at MOR and LVD revealed its antagonism at KOR.

In vivo, methyl-orvinol inhibited colonic motility at a relatively low dose (0.1 mg/kg *ip*). Another representative of orvinol family, buprenorphine - a partial MOR agonist - did not prolong the GI transit in rats [16]. In contrary, Zhou et al. [17] observed that buprenorphine significantly inhibited the GI transit in mice. Moreover, they characterized the effect of thienorphine, a buprenorphine analog with long-acting MOR and KOR agonist activity and good oral bioavailability. Thienorphine produced potent inhibitory effect in the GI tract, however, its effect was not as strong as buprenorphine [17].

We also observed that methyl-orvinol significantly inhibited colonic motility through peripheral ORs, suggesting that a peripherally restricted version of methyl-orvinol may have good clinical efficacy and reduced adverse effects related to the central nervous system activation (including: somnolence, sleep disturbances, respiratory depression and memory deficits). A 'classical' anti-diarrheal opioid loperamide poorly penetrated the blood-brain-barrier and its action was also related to peripheral MOR. However, loperamide did not exert antinociceptive potency and therefore is not the best choice for IBS-D therapy [18].

As constipation is one of the most frequent adverse effect of anti-diarrheal drugs, it is important to develop compounds which would normalize bowel movements without complete inhibition of GI motility. In the fecal pellet output test, we noted that methyl-orvinol strongly inhibited hypermotility in stressed mice, while in group of non-stressed mice there were no statistically significant changes in GI motility. Our results may indicate that methyl-orvinol is effective in pathophysiology (when peristalsis is accelerated) but does not impair regular peristalsis. These results are promising, because stress is one of the major triggers of motility acceleration in IBS-D and thus it is involved in the pathogenesis of this dysfunction. Noteworthy, the course of IBS-D includes periods of exacerbation and periods of lack of symptoms, occurring alternately. Therefore limitation of the drug action to the periods of symptom exacerbation is anticipated, as it leads to reduction of adverse effects (constipation) during chronic therapy.

Our studies revealed that methyl-orvinol did not possess anti-diarrheal effect. Hence, the lack of efficacy of methyl-orvinol in castor oil induced diarrhea suggests that its action in the GI tract may be atypical and the molecular mechanism needs to be elucidated. We evaluated the effect of methyl-orvinol in the tissue exposed to forskolin (adenylate cyclase activator) or veratridine (a voltage-dependent Na^+ channel activator which causes enteric neurons depolarization), respectively. Adenylate cyclase activation enhances intracellular cAMP production, what promotes protein kinase A activity and further phosphorylation of ion channel proteins and thus affects transmucosal ion transport. Notably, cAMP modifies electrolytes transport through Na^+/Cl^- ion channel what results in increased Cl^- and H_2O secretion and impaired ion absorption [19]. Adenylate cyclase activation and cAMP mediated secretion constitute some of the mechanism proposed to explain the castor oil induced diarrhea [20;21]. We observed that methyl-orvinol, in contrast to loperamide, increased the intestinal secretion in tissues exposed to forskolin or veratridine. Our results are in line with DeHaven-Hudkins et al. [22], who observed that loperamide in CHO cells inhibited cAMP accumulation. Epple et al. [23] reported that loperamide exerted anti-secretory effect in the colonic epithelial cells (HT-29/B6) stimulated with forskolin. Loperamide entailed the reduction of intestinal fluid volume accumulation after *ig*

administration of castor oil in rats [24]. Interestingly, Cai et al. [25] examined the effect of endomorphin analogues on forskolin-stimulated cAMP production in HEK-293 cells (expressing all ORs) and compared the results to reference ORs agonists. They observed that potent, selective agonists such as DAMGO (MOR), DPDPE (DOR) and U69593 (KOR) produced significant inhibitory action on cAMP accumulation in HEK-293 cell line, expressing respective ORs type. 3,4-diTnp characterized as potent MOR agonist and KOR, DOR antagonist, demonstrated similar to methyl-orvinol effect on forskolin-stimulated cAMP accumulation. In cells expressing MOR, 3,4-diTnp displayed potent agonistic effect – it inhibited cAMP accumulation, while in cells expressing DOR and KOR, respectively, cAMP accumulation was significantly increased [25].

The antinociceptive properties of orvinols were broadly described in many reports. In our study methyl-orvinol had a strong antinociceptive effect, partially reversed by β -FNA. As methyl-orvinol is a dual activity ligand with an antagonism at KOR, the inhibition was not complete and did not achieve control level. For instance, TH-030148, an orvinol with non-selective ORs agonist and NOP receptor activity, displayed strong antinociceptive action in writhing and hot plate tests, its effect was reversed by naloxone [26]. Two buprenorphine analogs, *i-butyl* and *i-pentyl*, which are mixed ORs agonists with partial activity at MOR and high efficacy at KOR in the [35 S]GTP γ S assay, were effective in para-phenylquinone abdominal stretch assay in mice (ED₅₀ 0.02 mg/kg *sc*). Their effect was reversed by norbinaltorphimine (nor-BNI, a selective KOR antagonist) and partially by β -FNA [6].

Our results suggest that the dual action of methyl-orvinol - MOR agonism and KOR antagonism – is crucial, yet beneficial for its effect on GI motility and its antinociceptive properties. Prevalent MOR activation results in significant inhibition of GI motility. Consequently, KOR antagonism prevents complete inhibition of GI motility, and thus may decrease the risk of constipation, a primary adverse side effect of many anti-IBS drugs. Even more importantly, the KOR antagonism of methyl-orvinol does not disrupt its antinociceptive potency.

Conclusion

Our studies revealed that methyl-orvinol, due to its dual MOR agonist and KOR antagonist action, elicits a potent inhibitory effect on GI motility and displays antinociceptive potential. Therefore methyl-orvinol is a promising drug candidate in chronic therapy of functional GI diseases associated with accelerated peristalsis and abdominal pain, such as IBS-D.

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Highlights

- Diarrhea-predominant irritable bowel syndrome (IBS-D) is a chronic gastrointestinal disorder.
- Opioid receptors (ORs) maintain the homeostasis in the GI tract.
- ORs ligands are an attractive option for developing new drugs in IBS-D therapy.
- Methyl-orvinol is MOP receptor agonist and KOP receptor antagonist in the GI tract.
- Methyl-orvinol reduced abdominal pain in mouse models mimicking IBS-D symptoms.

Figure 1.
The structure of methyl-orvinol.

Figure 2.

(A) The inhibitory effect of methyl-orvinol (10^{-10} – 10^{-6} M) on electrical field stimulation (EFS)-induced longitudinal smooth muscle contractions in mouse colon alone and in presence of non-selective opioid receptor antagonist – naloxone and MOR-selective antagonist β -funaltrexamine (β -FNA) (both 10^{-6} M). Data represent mean $\pm$ SEM ($n = 6$). *** $p < 0.001$, compared to control, ### $p < 0.001$, as compared to methyl-orvinol alone.

(B) Methyl-orvinol (10^{-6} M) revealed antagonistic effect on KOR. In the presence of β -FNA (10^{-6} M), it reversed inhibitory effect of U50488 (KOR-selective agonist, 10^{-10} – 10^{-6} M)

on smooth muscle contractility in the colon. Data represent mean $\pm$ SEM (n = 6). *** p < 0.001, compared to control, ### p < 0.001, as compared to U50488 alone.

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Figure 3.

The effect of methyl-orvinol (10^{-6} M) or loperamide (10^{-6} M) serosal application on (A) forskolin (10^{-5} M) or (B) veratridine (3×10^{-5} M)-stimulated short-circuit current (Isc) in mouse distal colon. Data are mean $\pm$ SEM, n = 6. * p < 0.05, *** p < 0.001, as compared with control, ### p < 0.001, as compared with methyl-orvinol.

Figure 4.

Methyl-orvinol (0.1–1 mg/kg, injected *ip*) elicited inhibitory effect on colonic bead expulsion time in mice (15, 45 and 90 min after injection) (A). Its inhibitory action was reversed by naloxone (1 mg/kg, *ip*), β -funaltrexamine (β -FNA, 1 mg/kg, *ip*) and by methiodide-naloxone (met-naloxone, 1 mg/kg, *ip*) (B). Moreover, methyl-orvinol at the dose of 0.1 mg/kg, injected *ip*, prolonged whole GI transit time in mice (C). Data represent mean $\pm$ SEM of $n = 8$. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ as compared to control and ## $p < 0.01$, ### $p < 0.001$, as compared to methyl-orvinol-treated mice.

Figure 5.

(A) The effect of methyl-orvinol (0.1 mg/kg, *ip*) on fecal pellet output in non-stressed (left side of the figure) and stressed mice (right side). (B) Comparison of anti-diarrheal potency of methyl-orvinol (0.1 mg/kg, *ip*) and loperamide (1 mg/kg *ip*) in castor oil-induced diarrhea model. Results shown as $\pm$ SEM of $n = 8$ mice per group. *** $p < 0.001$ as compared to control.

Figure 6.

The effect of methyl-orvinol (0.1 mg/kg, *ip*) alone and in the presence of β -funaltrexamine (β -FNA, 1 mg/kg, *ip*) on a total number of behavioral pain responses induced by *ic* administration of mustard oil solution in mice. Data shown as $\pm$ SEM of $n = 8$ mice per group. *** $p < 0.001$ as compared to control (vehicle-treated mice), ### $p < 0.001$ as compared to methyl-orvinol treated mice.