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6 β -N-Heterocyclic Substituted Naltrexamine Derivative NAP as a Potential Lead to Develop Peripheral Mu Opioid Receptor Selective Antagonists

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Abstract

A 6 β -N-heterocyclic substituted naltrexamine derivative, NAP, was proposed as a peripheral mu opioid receptor (MOR) selective antagonist based on the *in vitro* and *in vivo* pharmacological and pharmacokinetic studies. To further validate this notion, several functional assays were carried out to fully characterize this compound. In the charcoal gavage and intestinal motility assay in morphine-pelleted mice, when administered 0.3 mg/kg or higher doses up to 3 mg/kg subcutaneously, NAP significantly increased the intestinal motility compared to the saline treatment. The comparative opioid withdrawal precipitation study and the lower locomotor assay demonstrated that NAP showed only marginal intrinsic effect in the central nervous system either given subcutaneously or intravenously: no jumps were witnessed for the tested animals even given up to a dose of 50 mg/kg, while similar noticeable wet-dog shakes only occurred at the dose 50 times of those for naloxone or naltrexone, and significant reduction of the hyper-locomotion only happened at the dose as high as 32 mg/kg. Collectively, these results suggested that NAP may serve as a novel lead to develop peripheral MOR selective antagonist which might possess therapeutic potential for opioid-induced bowel dysfunction (OBD), such as opioid-induced constipation (OIC).

Keywords

MOR; peripheral opioid antagonist; intestinal motility; NAP

There are three subtypes of opioid receptors, designated as mu, kappa and delta,^{1–3} which are located throughout the central nervous system (CNS) as well as within the periphery, such as the gastrointestinal (GI) tract.^{4,5} Among them, the mu opioid receptor (MOR) plays a dominant role in the beneficial analgesic activity of opioids and their adverse GI side

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effects, for example, constipation.^{6–9} Hence, it is highly desirable to develop an agent which may diminish the unwanted GI side effect of opioids without compromising their desired antinociceptional effect. The peripheral selective MOR antagonists may be the answer. Bearing a restricted ability to cross the blood brain barrier (BBB), two drugs, methylnaltrexone (MNTX) and alvimopan (Fig. 1) have been approved by the FDA to treat opioid induced constipation (OIC) in patients with advanced illness and postoperative ileus, respectively.^{10–12} Nevertheless, the relative low efficiency of MNTX to induce spontaneous bowel movement (48–62%) in palliative care patients and myocardial infarction of alvimopan with long-term use call for new members of this family to emerge.^{13–15}

Recently, a 6β -*N*-heterocyclic substituted naltrexamine derivative, NAP (Fig. 1), was identified as a highly selective MOR ligand based on the molecular modeling study.¹⁶ NAP displayed a high binding affinity for the MOR at $K_i = 0.37$ nM with over 700-fold selectivity for the MOR over the DOR and more than 150-fold selectivity over the KOR. It showed only marginal intrinsic efficacy at the MOR compared to the full agonist DAMGO. Additional pharmacological and pharmacokinetic studies suggested that NAP, being a substrate of P-glycoprotein and thus possessing limited ability to penetrate BBB, could serve as a peripheral MOR selective antagonist.^{17,18} To further test this hypothesis, several *in vivo* assays were thus conducted to fully characterize the impact of NAP on the GI tract and the CNS in the animal models.

GI transit time is perhaps the most frequently utilized assay to examine GI function.^{19,20} Hence, it was adopted in the current study to test the effect of NAP on GI motility following the published protocol with minor modification.^{21–23} Briefly, seven groups of five morphine-pelleted (10 mg/kg) mice each received a subcutaneous (s.c.) injection of NAP at different concentrations or saline at time zero. Twenty minutes later, each of them was given a forced meal of charcoal suspension via gavage. Thirty minutes after the meal, mice were euthanized and the intestine was dissected. The distance traveled by the charcoal in the intestine was then measured and expressed as a percentage of the total length of the intestine, from pylorus to rectum (Fig. 2A). To further understand the outcome of NAP on the bowel movement, stool weight was correspondingly measured for the treatment groups that have shown significant intestinal motility increment compared to the saline group. Results were illustrated in Fig. 2B.

As shown in Fig. 2A, administration of morphine pellet diminished the intestine motility (show as the saline bar, as compared with the saline bar in Fig 3A), whereas 0.3 mg/kg of NAP (s.c.) significantly increased the GI transit compared to saline, so did the two higher doses (1 and 3 mg/kg, $P < 0.0001$ for all three doses, One-way ANOVA with posthoc Dunnett's test), whereas the lower concentrations (0.03 mg/kg and lower) seemed not sufficient to antagonize the prolonged GI transit time by morphine. The calculated ED_{50} of NAP is 0.0088 mg/kg (95% C.L., 0.0057–0.0134), nearly 300-fold more potent than MNTX (s.c., $ED_{50} = 2.5$ mg/kg, 95% C.L., 1.5–4.4)²⁴. Associated with this relative high potency of NAP, diarrhea was also observed in one mouse at the dose of 0.3 mg/kg and above.

Interestingly, none of the three doses that effectively improved intestinal motility increased stool weight compared to saline treatment (Fig. 2B). On the contrary, 0.3 mg/kg and 1 mg/kg of NAP significantly decreased the amount of the stool excreted vs. saline ($P = 0.0011$, 0.0193 respectively). It was speculated that the relative short fecal collection period in the study might be the primary reason for this phenomenon. However, other factors, such as occurrence of diarrhea, cannot be fully ruled out. Although not significantly, an apparent stool weight increment from NAP 0.3 mg/kg to 3 mg/kg suggested a positive correlation between stool weight and intestinal motility, i.e. GI transit time, as reported previously.²⁵

To assess the influence of NAP alone on the GI tract, an acute intestinal motility assay was also performed following the aforementioned procedure in morphine “naive” mice (Fig. 3A). Although statistically not significant, the acute intestinal motility assay showed a decreasing trend of the GI transit as the dose of NAP increased. This result was consistent with the previous observation from ^{35}S -GTP[γS]-binding assay in MOR-CHO cells and rat thalamus that NAP acted as a partial agonist of the MOR with relative low efficacy (% maximum stimulation of NAP to the MOR compared to those of 10 μM DAMGO, normalized to 100%, are 22.7 ± 0.8 (MOR-CHO cells), and 16.7 ± 2.0 (rat thalamus), respectively).^{16,17} Thus, a relatively prolonged GI transit was observed with NAP alone.

For comparison purpose, the stool weight for each group in the acute assay was evaluated as well (Fig. 3B). Unlike the morphine-pelleted mice, morphine “naive” ones reacted rather irregularly to NAP treatment as dose increased. Although statistically not significant in contrast to the saline group, the lower and higher doses (0.3 and 30 mg/kg) reduced the amount of stool whereas the medium one (3 mg/kg) raised the mass. As stated previously, the noticeable brief fecal collection interval might complicate the observation.

While NAP seemed to be a potential lead to target the peripheral side effects of opioid analgesics based on the GI motility study, three more assays were carried out to investigate if NAP would precipitate opioid withdrawal (a CNS-mediated mechanism). Most drugs of abuse, such as opioids, can increase locomotion when administered acutely.²⁶ Thus, lower locomotor activity (LLMA, e.g., walking), higher locomotor activity (HLMA, e.g., jumping) and wet-dog shakes were evaluated as three behavior measures for opioid withdrawal symptoms after administration of NAP according to the published procedures.^{17, 27} As shown in Fig. 4, NAP only showed significantly reduced LLMA at a high dose of 32 mg/kg following morphine administration, which was more than 3,600-fold of its ED_{50} to increase intestinal motility. Similarly, NAP did not precipitate escape jumps even up to 50 mg/kg (Fig. 5A) and only modestly exhibited withdrawal symptom in wet-dog shake assays at a dose 50 times higher than those of naloxone and naltrexone (Fig. 5B).

Another concern in developing peripherally acting MOR antagonists is whether they will affect the analgesic effect of opioids. As reported earlier, the constrained ability of NAP to penetrate BBB would limit its access to the CNS,¹⁸ where the antinociception mostly takes place. Moreover, NAP showed an AD_{50} value of 4.51 (95% C.L., 2.45–8.20) mg/kg in the warm-water tail immersion test,¹⁶ which was 500 times higher than the ED_{50} of NAP from the GI transit assay. Hence, one may expect that NAP at doses that might give a beneficial GI effect would unlikely to influence the analgesic effect of other opioids in CNS.

In conclusion, with marginal withdrawal effects in the central nervous system at the dose as high as 10 mg/kg and a relative low potency to block the antinociception of morphine, and most importantly, approximately 300-fold more potent than methylnaltrexone in increasing gastrointestinal transit, NAP seems to be a very promising peripheral mu opioid receptor antagonist. Due to the incidence of diarrhea at doses of 0.3 mg/kg and above, NAP might not be an ideal candidate for further development, but it may be able to serve as a lead to explore other novel agents²⁸ targeting opioid-induced bowel dysfunction, such as constipation.

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Figure 1.
Chemical structures of methylnaltrexone (MNTX), alvimopan, and NAP.

(A)

(B)

Figure 2.

NAP intestinal motility assay in morphine-pelleted mice. (A) charcoal gavage results, **** $P < 0.0001$, comparing to saline; (B) Change in stool weight by effective treatment groups compared with saline, * $P < 0.05$, ** $P < 0.005$, comparing to saline.

(A)

(B)

Figure 3.

NAP acute intestinal motility assay in morphine-naïve mice. (A) charcoal gavage results, * $P < 0.05$, comparing to saline; (B) Change of stool weight, * $P < 0.05$.

(A)

(B)

(C)

Figure 4. Lower locomotor assay in acute morphine exposed mice. NAP: 3.2 mg/kg (A), 10 mg/kg (B), and 32 mg/kg (C).

(A)

(B)

Figure 5. NAP withdrawal assay. (A) Escape jumps in chronic morphine exposed mice; (B) Wet-dog shakes in chronic morphine exposed mice.