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Prevention of apomorphine- or cisplatin-induced emesis in the dog by a combination of methylnaltrexone and morphine

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Abstract *Purpose*: Morphine can have either an emetic or an antiemetic effect. The emetic effect of morphine can be blocked by methylnaltrexone (MNTX), a quaternary opioid antagonist with peripheral action. In this study, we tested the hypothesis that administering MNTX to block the peripheral emetic effect of morphine would unmask the central antiemetic effect of the morphine. The net result, we hypothesized, would be a reduction in apomorphine- or cisplatin-induced emesis. *Methods*: MNTX 0.25 mg/kg and morphine 1 mg/kg were administered to conscious dogs which were then challenged with the potent emetic agents apomorphine or cisplatin. Emesis was assessed by the presence of characteristic retching motions accompanied by the regurgitation of gastric contents. *Results*: We observed that apomorphine challenges of 0.1 mg/kg and of 0.03 mg/kg produced 100% emesis in control animals. After pretreatment with MNTX and morphine, the frequency of emesis with the larger dose of apomorphine was reduced to 50% and with the smaller dose to 22%. MNTX alone did not block apomorphine-induced emesis. In animals challenged with cisplatin 3 mg/kg, the emetic response was 100%. Emesis did not occur in animals pretreated with MNTX 0.25 mg/kg and morphine 1 mg/kg before cisplatin. *Conclusions*: Our results demonstrate that MNTX combined with morphine reduces apomorphine-induced emesis and blocks cisplatin-induced emesis. These results support the hypothesis that the emetic effect of morphine is peripheral and its antiemetic action is central. In combination, MNTX and morphine may have a clinical role in the treatment of chemotherapy-induced emesis.

Keywords Morphine · Methylnaltrexone · Cisplatin · Apomorphine · Chemotherapy-induced emesis

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Introduction

Emesis is a complex process involving afferent stimuli from such diverse structures as the chemoreceptor trigger zone (CTZ) in the area postrema of the brain, the vestibular apparatus, and stretch and chemosensitive receptors in the gut and cerebral cortex [4, 12]. The afferent stimuli must be interpreted to generate a coordinated response, primarily in the medullary retching and vomiting centers, adjacent to the respiratory center and connected via the nucleus tractus solitarius. Emesis is then mediated through efferent voluntary and involuntary nervous pathways to the muscles of the gut, abdomen, and thorax. The mechanism of this response has been studied with drug-induced emesis, and agents to block many of the key points in the path have been developed [4, 5].

Opioids like morphine can have a biphasic action on this system, i.e. they can induce and prevent emesis [3, 15]. Morphine has been shown to induce emesis in many animal models, and its emetic effect is blocked by ablation of the CTZ [24], by the nonspecific opioid

receptor antagonist naloxone [7], or by the peripheral opioid receptor antagonist methylnaltrexone (MNTX) [9, 10]. In contrast, opioids also can block the emetic effects of agents that act on opioid or nonopioid receptors to induce emesis [3]. Thus, in addition to their peripheral emetic action, opioids may block the vomiting center via a central antiemetic receptor [3]. MNTX, the tertiary *N*-methyl derivative of naltrexone, is an opioid antagonist which blocks only receptors peripheral to the blood-brain barrier. In this study, we tested the hypothesis that the combination of MNTX (blocking the peripheral emetic action of the morphine) and morphine would block emesis induced by the emetic compound apomorphine or the chemotherapy agent cisplatin [17, 18].

Materials and methods

All protocols were approved by the Institutional Animal Care Committee and were conducted following the guidelines for animal care of the National Institute of Health.

Animals

Adult male mongrel dogs (15–25 kg) in individual cages were observed in a quiet room. All animals had free access to food and water until the morning of the study. They were offered 500 ml of food (a mix of canned and dry food, or moistened dry food) during a 15-min acclimatization period before the start of the study, but they were restricted from eating or drinking during the study period. Emesis was assessed by the presence of characteristic retching motions accompanied by the regurgitation of gastric contents. Time to first emesis and number of emetic episodes were recorded. The animal pens were examined the morning after the experiment for evidence of delayed emesis.

The absence or presence of opioid effects (alertness, sedation, or unsteady gait) were also observed, and a sedation score was assigned every 15 min based on the following observations: A = none, dog appeared alert and spontaneously inquisitive; B = mild, dog was quiet, but aroused easily, possibly unsteady on feet; C = moderate, dog had difficulty standing, was arousable but quietened quickly; D = severe, dog could not be aroused.

Repeat trials in an individual animal were separated by at least 3 days.

Antiemetic studies

Three emetic challenge groups were studied. Each animal was given apomorphine 0.1 mg/kg (Apo-high), apomorphine 0.03 mg/kg (Apo-low) or cisplatin 3.0 mg/kg (Cisp). The Apo-high group was divided into two treatment subsets: no pretreatment ($n = 4$) or MNTX 0.25 mg/kg at 0 min followed by morphine 1.0 mg/kg at 15 min ($n = 4$). The Apo-low group was divided into three treatment subsets: no pretreatment ($n = 7$), MNTX 0.25 mg/kg at 0 min alone ($n = 4$), or MNTX 0.25 mg/kg at 0 min followed by morphine 1.0 mg/kg at 15 min ($n = 9$). Animals in the Apo-high and Apo-low groups were observed for at least 1 h after challenge or until completely recovered.

The Cisp group was pretreated either with MNTX 0.25 mg/kg plus morphine 1.0 mg/kg at 0 min and then with MNTX 0.25 mg/kg plus morphine 0.5 mg/kg at 90 min ($n = 5$) or with MNTX 0.25 mg/kg alone at 0 min and again at 90 min ($n = 5$). Animals in the Cisp group were challenged with cisplatin 3 mg/kg over 10–15 min as an infusion at 30 min. The dogs also received at least 20 ml/kg of normal saline or lactated Ringer's solution. Animals were observed for 5 h after challenge.

Drugs

All drugs were mixed daily and diluted to an appropriate concentration for administration of 0.1 ml/kg. Drugs were administered into a foreleg vein intravenously. MNTX (*N*-methylnaltrexone bromide) was from H. Merz, Boehringer Ingelheim, Ingelheim, Germany. Morphine (morphine sulfate) was from Mallinckrodt Chemical, St. Louis, Mo. Apomorphine was from Research Biochemicals, Natick, Mass. Cisplatin (Platinol) was from Bristol Laboratories, Syracuse, N.Y., and was mixed at a concentration of 1 mg/ml.

Statistics

The results were analyzed using Fisher's exact test with $P < 0.05$ considered significant.

Results

Apomorphine challenges: Apo-high group and Apo-low group

Without morphine pretreatment, apomorphine at both doses produced emesis in all animals (100% incidence, Apo-high and Apo-low groups). The onset of emesis was within 1–4 min with two to six episodes; one animal had 15 emetic episodes. Episodes were completed in 5–10 min. Animals without morphine pretreatment were graded score A (none) on the sedation scale.

In the Apo-high group (apomorphine 0.1 mg/kg) pretreatment with the combination of MNTX and morphine reduced the incidence of emesis to 50% (two of four animals), but the reduction was not statistically significant because of the small sample size. MNTX alone had no antiemetic effect against the smaller (0.03 mg/kg) dose of apomorphine. MNTX/

Table 1 Inhibition of apomorphine-induced emesis by pretreatment with MNTX and morphine

Group	Pretreatment	Apomorphine challenge (mg/kg)	*n*	Animals with emesis *n* (%)
Apo-high	None	0.1	4	4 (100)
	MNTX + morphine	0.1	4	2 (50)
Apo-low	None	0.03	7	7 (100)
	MNTX alone	0.03	4	4 (100)
	MNTX + morphine	0.03	9	2 (22)*

*$P < 0.01$ compared with no pretreatment

Table 2 Inhibition of cisplatin-induced emesis by pretreatment with MNTX and morphine

Group	Pretreatment	Cisplatin challenge (mg/kg)	*n*	Animals with emesis *n* (%)
Cisp	MNTX alone	3.0	5	5 (100)
	MNTX + morphine	3.0	5	0 (0)*

*$P < 0.01$ compared with MNTX alone

morphine significantly prevented emesis at this smaller dose, reducing the incidence to 22% (two of nine animals, $P < 0.01$ compared with no pretreatment; Table 1).

All animals given morphine showed signs of sedation (score B or C, no scores D). No animal vomited during the 15 min between morphine and apomorphine administration. All animals recovered without apparent sequelae. There were no gross behavioral effects attributable to MNTX alone.

Cisplatin challenges: Cisp group

Cisplatin after MNTX alone as pretreatment produced 100% emesis ($n = 5$). Cisplatin-induced emesis was characterized by onset within 120–150 min with 11–18 emetic episodes; one animal had only four emetic episodes. No delayed emesis during the 5–24 h period was observed. Pretreatment with the combination of MNTX and morphine completely prevented the emetic response to cisplatin ($n = 5$, $P < 0.05$; Table 2).

Animals not pretreated with morphine had score A on the sedation scale. All animals pretreated with morphine showed signs of sedation (score B or C, no scores D). No animal vomited during the 30 min between pretreatment with MNTX and morphine and cisplatin administration. There were no gross behavioral effects attributable to MNTX alone.

Discussion

Morphine and other opioids exhibit both emetic and antiemetic effects. The antiemetic effects of morphine were noted early in this century [15]. After administering morphine or naloxone systemically and intracerebroventricularly to cats, Costello and Borison demonstrated that the opposing emetic and antiemetic actions of opioids occur at different sites in the brain [7]. The emetic effect is thought to be at the superficially located CTZ, whereas the antiemetic effect is at the vomiting center located more deeply in the medulla. The CTZ is contained in the area postrema on the caudal margin of the IV ventricles. The vomiting center is located immediately beneath the solitary tract of the caudal brainstem. Morphine can have both an emetogenic effect at the CTZ and an antiemetic effect at the deeply located vomiting center. We postulate that the increased permeability of the blood-brain barrier at the CTZ allows MNTX to antagonize the morphine effects at the CTZ, but MNTX is unable to penetrate to the vomiting center. Thus, if this hypothesis is correct, MNTX unmasks the antiemetic effect of the morphine.

Both the emetic and antiemetic effects of morphine can be blocked by naloxone [7]. The tertiary compound, naltrexone, readily crosses the blood-brain barrier. The quaternary amine structure of the MNTX molecule increases its polarity and lowers its lipid solubility, thus limiting its activity to the peripheral side of the blood-brain barrier. In rodents, MNTX is metabolized via demethylation, but demethylation is not significant in the dog at the doses used in this study [14]. The peripheral site of action of MNTX has been shown. MNTX does not affect nociception or precipitate opiate withdrawal [6]. Recently, we have demonstrated that MNTX does not reverse morphine-induced analgesia using the cold-pressor test in human volunteers [30]. Experiments designed to evaluate MNTX effects on opioid subtype receptors are underway.

In dogs, MNTX can block morphine-induced emesis [10]. We hypothesized that MNTX would be able to antagonize the emetic effect of morphine at the CTZ even though MNTX does not penetrate the deeper structures of the brain. The CTZ, located in the area postrema, is considered a privileged site because of its leaky capillary structure [25–27]. Electrophysiological studies have shown that some neurons in the area postrema receive gastric vagal inputs [29], and the neurons respond to noxious, excessive distension of the stomach, and produce nausea [23]. The antiemetic effect of MNTX has been demonstrated by a dose-dependent reduction in morphine-induced emesis from 88.9% of control animals to 0% of animals treated with intravenous MNTX 0.2 mg/kg or intramuscular MNTX 0.25 mg/kg [10].

Butorphanol, a mixed opioid agonist, has been shown to have antiemetic effects in cisplatin-induced emesis in ferrets and dogs [21]. Blancquaert et al. [3] compared the emetic and antiemetic effects of *mu*-, *delta*- and *kappa*-agonists. These investigators showed that morphine induces emesis but it also blocks apomorphine-induced emesis. More lipid-soluble *mu*-agonists such as fentanyl and methadone do not elicit emesis, but do prevent apomorphine-induced vomiting. *Delta*-agonists ([D-Ala_2,Met_5]enkephalinamide and [Leu_5]enkephalin) induce emesis, but do not show antiemetic activity. The *kappa*-agonists bremazocine and ethylketocyclazocine do not induce emesis but do block apomorphine-induced emesis. Naloxone blocks both emetic and

antiemetic effects of the opioids. The investigators concluded that the *delta*-receptor is involved in the (peripheral) emetic effect and a *mu*- or *kappa*-receptor in the (central) antiemetic effect of opioids. *Delta*-agonists do not produce sedation at the doses used, and no assessment of their penetrance into the central nervous system was made.

Apomorphine is a dopamine-2 agonist that triggers emesis [5, 8, 17]. The control animals in this study demonstrated a pattern of emesis similar to that described by others [13]. The effects of apomorphine are not reversed by naloxone [20] and were not altered by the administration of MNTX alone in this study. However, when combined with morphine, MNTX was able to reduce apomorphine-induced emesis significantly. Our results are consistent with those obtained by Blancquaert et al. [3], who observed emesis after morphine alone. In our study MNTX prevented morphine-induced emesis in a similar paradigm.

Cisplatin-induced emesis is preventable by ablation of the CTZ [2, 16]. The latency period with cisplatin-induced emesis dictates multiple dosing to ensure prevention of delayed emesis. The mechanism of delayed emesis is unclear, but suggested etiologies include products of the agents, products of altered metabolism or cell damage, or other functional disturbances of the emetic system [4].

In this study, cisplatin-associated emesis was completely blocked by the combination of MNTX and morphine. Results with this combination compare favorably with the results using other agents tested in the same model, including chlorpromazine, haloperidol, metoclopramide, AL-1612, nabilone and butorphanol [11, 21]. Antiemetic effects of ondansetron in different animal models have also been reported [19, 22, 28]. It would be interesting to compare the effects between MNTX/morphine and 5-HT3 antagonists in future experiments.

The side-effect profile of the MNTX/morphine combination is unclear. Our animals were sedated, which may prove to be an obstacle or a benefit in clinical use in oncology patients, although sedation alone has been demonstrated to be ineffective in preventing vomiting [3]. This sedation effect must be compared with the side effects associated with other antiemetics in use, including sedation, dysphoria, restlessness, and dystonia [1].

Future studies might include agents such as copper sulfate, known to act on emetic triggers in the gut rather than on the CTZ. Studies of the dosing and kinetics of the MNTX/morphine combination in dogs would be useful in evaluating the minimal levels needed to prevent emesis, but such studies may have limited relevance to other species. The doses of morphine required in the dog for analgesia or sedation are much higher than those generally used in humans. Human studies are needed.

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