**Neurokinin-1 receptor blocker CP-99 994 improved emesis induced by cisplatin via regulating the activity of gastric distention responsive neurons in the dorsal motor nucleus of vagus and enhancing gastric motility in rats**

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**Abstract**

**Background:** Nowadays, chemotherapy induced nausea and vomiting (CINV) is still common in patients with cancer. It was reported that substance P mediated CINV via neurokinin-1 (NK₁) receptor and antagonists of NK₁ receptor has been proved useful for treating CINV but the mechanism are not fully understood. This study aimed to examine the role of NK₁ receptor blocker, CP-99 994, when administrated into dorsal motor nucleus of vagus (DMNV), on the cisplatin-induced emesis in rats and the possible mechanism.

**Methods:** Rats' kaolin intake, food intake, and bodyweight were recorded every day; gastric contraction activity was recorded in conscious rats through a force transducer implanted into the stomach; gastric emptying was monitored using the phenol red method; single unit extracellular firing in the DMNV were recorded.

**Key Results:** DMNV microinjection of CP-99 994 reduced the changes of increased kaolin consumption and suppressed food intake in cisplatin-treated rats; enhanced the gastric contraction activity dose-dependently in control and cisplatin-treated rats but enhanced gastric emptying only in cisplatin-treated rats; reduced the firing rate of gastric distention inhibited (GD-I) neurons but increased the firing rate of GD excited (GD-E) neurons in the DMNV. The effects of CP-99 994 on gastric motility and neuronal activity were stronger in cisplatin-treated rats than those of control rats.

**Conclusions and Inferences:** Our results suggested that CP-99 994 could improve emesis induced by cisplatin by regulating gastric motility and gastric related neuronal activity in the DMNV.

**KEYWORDS**
dorsal motor nucleus of vagus, emesis, gastric distention responsive neurons, gastric motility, NK₁ receptor antagonist

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**INTRODUCTION**

Despite pharmacological prophylaxis, more than half of patients are still suffered from severe emesis when undergoing emetogenic chemotherapy and this may lead to their refusing the continuation of the therapy. So it is urgent to investigate the mechanism of emesis and look for more effective methods to improve the life quality of patients that suffered from cancer. Cisplatin is one of the high potential anticancer drugs that induces the acute (present within 24 hours after treatment) and delayed (persistent for several days after treatment)
emesis. Rats do not vomit but kaolin ingestion behavior “pica” in rats is equivalent to vomiting.1,3-5 Rats treated with cisplatin represent a good emesis animal model.

The medullary dorsal vagal complex (DVC), comprising the nuclei of the solitary tract (NST), the area postrema (AP), and the dorsal motor nucleus of the vagus (DMNV), is the dominant component of the vago-vagal reflex circuits that control gastric motility.6 It has been well demonstrated in multiple species that vagal neurocircuitry is important during the generation of nausea and vomiting.7-9 Particularly, preganglionic parasympathetic visceral motor neurons in the DMNV play a critical role through their innervations of abdominal postganglionic neurons in the stomach and intestines. Cholinergic preganglionic neurons in the DMNV may send projections to intragastric cholinergic neurons that mediate gastric contraction, whereas other neurons in the DMNV may innervate intragastric nitric oxide (NO) or vasoactive intestinal polypeptide (VIP) neurons that regulate gastric relaxation.7,10–12 Therefore, motoneurons that regulate emesis and vomiting.

Substance P is a peptide formed with eleven amino acids, belonging to the tachykinin family of peptides. Substance P and its agonist regulate emesis and vomiting.13-15 Its effects are predominantly mediated through Neurokinin-1 (NK1) receptor.16 NK1 receptor antagonists, aprepitant and fosaprepitant, are used for preventing the development of CINV and postoperative nausea and vomiting (PONV).14 In addition, the NK1 receptor blocker CP-99 994, improved both acute and delayed emesis and displayed an effective role against peripheral and central emetics.17 So the NK1 receptor may be used as a therapeutic target for emesis. NK1 receptor is enriched in neurons of the DMNV.18 Furthermore, the NK1 receptor is expressed in DMNV neurons which send projections to the greater curvature of the stomach.19 However, to our knowledge, there is no report about the role of NK1 receptor blocker on the electrophysiological activity of neurons in the DMNV in vivo and the consequent effect on gastric motility in cisplatin-treated rats.

Therefore, in this study, we aimed to use saline-treated rats and cisplatin-treated rats to (i) observe the role of microinjection of NK1 receptor blocker, CP-99 994, into the DMNV on kaolin intake, food intake, and bodyweight, (ii) study the role of microinjection of CP-99 994 into the DMNV on the gastric motility and gastric emptying, and (iii) explore the effect of CP-99 994 on the firing activity of neurons in the DMNV.

2 | MATERIALS AND METHODS

2.1 | Animals

Male Wistar rats at 250-300 g (Qingdao Marine Drug Institution, Shandong, China) were used in this study. Animals were housed in individual home cages with room temperature and room humidity at 25±2°C and approximately 50%, respectively. The room was maintained under 12:12 hours light-dark cycle (light on at 8:00AM). Standard laboratory chow, water, and kaolin pellets were available to animals ad libidum during the habituation period. All animal experiments were performed in accordance with the Institutional Animal Care and Use Committee at Qingdao University.

2.2 | Chemicals

Cisplatin (Qilu Pharmaceutical Co. Ltd, Hainan, China) was dissolved in sterile saline (2 mg/mL) for intra-peritoneal injection (i.p.) CP-99 994 ([+]-[2S,3S]-3-[2-methoxybenzylamino]-2-phenylpiperidine) (Sigma, St. Louis, MO, USA) was dissolved in distilled water. Urethane was purchased from Sigma. Other chemicals were obtained locally.

2.3 | Food intake, kaolin intake, and bodyweight measurement

Rats were immobilized in a stereotaxic apparatus following anesthesia with chloral hydrate (400 mg/kg, i.p.). A stainless steel guide cannula (26 gauge) was implanted above the DMNV (AP: −13.2 mm from the bregma, ML: +0.6 mm or −0.6 mm from the midline, DV: −6.0 mm from the outer surface of the skull) (Paxinos and Watson, 2007). Stainless steel screws and dental acrylic were used to fix the cannula to the skull. A stainless steel stylet was placed into the cannula to prevent the dust.

Rats were allowed to recover for 1 week before testing. During this period, rats were given free access to chow and kaolin pellets and were handled several times by the experimenter to reduce the stress. Rats’ food intake, kaolin intake, and bodyweight were measured at 10:00AM (2 hours after the start of the light cycle) 1 day prior to receiving drug injections. On the experiment day at 10:00AM, the rats were injected with either cisplatin (3 mL/kg, i.p.) or the same volume of saline. 5 minutes later, either CP-99 994 (10 pmol 500 nL) or 0.9% NaCl was microinjected into the DMNV. For DMNV injection, a 34-gauge injection cannula 2 mm longer than the guide cannula was inserted into the guide cannula, and microinjections of 500 nL solution were delivered at 30 psi for 20 seconds with a pneumatic pico-pump (model...
PV830; World Precision Instruments, New Haven, CT, USA) through a polyethylene tubing attached to the injection cannula. Food intake, kaolin intake, and bodyweight were measured at 24 hours and 48 hours after i.p. injection. A second microinjection of CP-99 994 or saline was given on the second day after measurement.

2.4 Measurement of gastric contraction activity in awakened rats

Sixty-four rats were fasted overnight. Following anesthesia with thiobutabarbital (100 mg/kg, ip), a midline laparotomy was performed to expose the abdominal cavity. Contractile force transducers were sutured onto the gastric antrum serosa with 0.5 cm posterior to the pyloric ring to measure circular muscle motility. The lead wires of the transducers were threaded under skin and protruded 2-3 cm from the nape of the neck via a small cut between the scapulae. The abdomen was then closed. Next, the rats were immobilized in a stereotaxic frame. Guide cannula was embedded above the DMNV as described before. After recovery for 3 days, rats received either saline (n=32) or cisplatin (n=32, 6 mg/3 mL/kg) treatments by i.p. injection at 8:00 AM. After fasted overnight, DMNV injections were carried out at 8:00 AM on the next day. On the day of the measurement, to prevent the interference of the novel surroundings, rats were allowed to explore the experimental area for 30 minutes prior to the measurement. During the recording, rats were allowed to move freely with free access to water but not food.

A polygraph (3066-23; Chengdu Precision Instruments, Sichuan, China) was used to store the recordings of gastric contraction activity. After recording baseline activity for 30 minutes, either 500 nL CP-99 994 (0.1, 1.0, 10 pmol for each group, eight rats per group) or 500 nL 0.9% NaCl (eight rats) was slowly injected through the injection cannula as described before. The drugs were delivered to the DMNV in 2 minutes and the injection cannula was left in place for another 3 minutes to allow solution diffusing from the injection site. Effects of agents on gastric contraction activity, including gastric amplitude and gastric frequency were calculated as percentage changes. Data were averaged every 5 minutes after agent infusion and compared to the 5 minutes baseline right before the administration.

The motility index (MI) was determined by calculating the area under the manometric trace using data-acquisition software (LabChart v7; AD instruments). The basal MI was the area under the manometric trace before drug administration. The change in MI (%MI) was calculated as: %MI = (area under the manometric trace after CP-99 994 treatment)/(basal MI) × 100%. %MI was used to compare the effect of different agents on the contraction activity of gastric antrum. Gastric contraction activity was recorded for at least 30 minutes before microinjection and lasted for 1-2 hours after microinjection.

2.5 Measurement of gastric emptying

Gastric emptying of a nonnutrient viscous meal was measured using the phenol red approach.20 Cannula implantation and drugs administration were the same as described before. 64 rats were fasted overnight. 0.1 pmol, 1 pmol or 10 pmol CP-99 994 or vehicle was microinjected into the DMNV of saline- or cisplatin-treated rats, respectively. Ten minutes later, the rats were fed with 1.5% carboxymethylcellulose sodium salt containing 0.05% phenol red (1.5 mL/rat) by gavage. After 30 minutes, rats were killed and gastric contents were collected to measure the concentration of phenol red. Phenol red collected from an animal sacrificed immediately after gavage was used as the standard sample. Gastric emptying (%) was calculated as: (1−concentration of phenol red in test sample/concentration of phenol red in standard sample)× 100%.

At the end of the experiment, 500 nL pontamine sky blue was injected through the cannula to verify the injection sites (Figure 1A,B). Rats were fixed with 4% paraformaldehyde through transectional perfusions. Brains were then taken out, frozen, and sectioned at 50 μm. All the injection sites were visualized under light microscope. If the injection site was not in the DMNV, the data were discarded.

2.6 Surgery and extracellular recording of GD responsive neurons in DMNV

2.6.1 Abdominal and cranial surgeries

Urethane (1 g/kg, i.p.) was used to anesthetize rats. Following midline laparotomy, a small incision was made into the gastric fundus and the stomach content was cleaned. Subsequently, a balloon connected with a plastic cannula was inserted into the stomach and fixed with purse string suture so that the stomach can be distended by water inflation of gastric balloon. The abdomen was then closed. Next, the rat was immobilized in a stereotaxic apparatus. A small hole was made to the skull to expose brain. The brain surface was then covered with warm agar, which allow for stable neuronal recording. Body temperature was maintained at −37°C by heating pad.

2.6.2 Extracellular single unit recording

Microelectrodes with three barrels (total tip diameter: 3-10 μm, resistance: 15-20 MΩ) were stereotaxically placed into the DMNV (AP: −13.2-13.8 mm from the bregma, ML: 0.4-0.8 mm from the midline, DV: −7.8-8.1 mm from the skull surface) (Paxinos and Watson, 2007) for recording and drug microinjection. The recording barrel contained 0.5 M sodium acetate and 2% pontamine sky blue. The other two barrels contained vehicle and CP-99 994 (5 μM diluted in distilled water) respectively and were connected to micropressure ejection device.

The single unit recording started when the microelectrode was lowered down to the DMNV. Signals were amplified with MEZ2801 (Nihon Kohden, Tokyo, Japan) and displayed on an oscilloscope (VC-II, Nihon Kohden, Tokyo, Japan). Meanwhile, amplified signals were sent to SUMP-PC bioelectric signal processing system. Data were then stored in a computer. Once baseline firing rate was stable for ≥10 minutes, the gastric balloon was filled with 3-5 mL 37°C water at 0.5 mL/s and maintained for 10-30 seconds to identify GD
responsive neurons. Depending on the increase or decrease in firing rate beyond 20%, the GD responsive neurons were named as GD-excitatory (GD-E) neurons and GD-inhibitory (GD-I) neurons, respectively. After that, drugs were ejected onto the surface of neurons with short pulse gas pressure (1500 ms, 5.0-15.0 psi) (Xue et al., 2007). The baseline firing rate was calculated by averaging the firing rate of 120 seconds baseline prior to drug ejection. The maximal change in frequency within 50 seconds following drug administration was taken as drug effect. The firing rate changed beyond 20% was considered to be significant. Drug administration was performed once per neuron and at least 30 minutes interval was given between two recordings in the same track.

After each single-unit recording, the recorded site was labeled by pontamine sky blue using iontophoresis (10 μA, 20 minutes) (Figure 1C,D). If the recorded neuron was not in the DMNV, the data were discarded.

2.7 | Statistics

All data were presented as mean±SEM and the statistics were performed with SPSS 17.0 (SPSS Inc., Chicago, IL, USA). For behavioral DMNV and i.p. injection experiments, two-way ANOVAs were used to compare different treatments on food intake, kaolin intake and bodyweight. For electrophysiological experiments, paired t test was used to compare the firing rates before and after a treatment in the same neuron; unpaired t test was used to compare data of two unrelated groups. For gastric motility experiments, one-way ANOVA and posthoc Bonferroni’s tests were used to compare data of multiple groups. Significance level was presented at 0.05.

3 | RESULTS

3.1 | Effect of CP-99 994 administrated into the DMNV on kaolin intake, food intake, and bodyweight

3.1.1 | Effect of CP-99 994 administrated into the DMNV on kaolin intake

The animals ate approximately 0-0.5 g kaolin from the day of habituation period. In saline-treated rats, the following administration of vehicle or CP-99 994 into the DMNV had no effect on the amount of kaolin intake. However, in cisplatin-treated rats, kaolin intake increased significantly in the first and second day (P<.01) when vehicle injected into the DMNV and CP-99 994 could attenuate this effect obviously (P<.01 on first day and P<.05 on the second day. Figure 2A).
3.1.2 | Effect of CP-99 994 administrated into the DMNV on food intake

The rats ate approximately 20-25 g food in habitation period and in saline-treated rats, administration of CP-99 994 increased food intake obviously compared with vehicle (P<.05, on the first and second day). In cisplatin-treated rats, food intake decreased significantly compared with saline-treated rats (P<.01 on the first and second day) and this effect was attenuated obviously by CP-99 994 (P<.05-.01 compared with vehicle group Figure 2B).

3.1.3 | Effect of CP-99 994 administrated into the DMNV on bodyweight change

In bodyweight change study, the weight increased about 6-7 g every day in control rats, and administration of CP-99 994 into the DMNV had no significant influence on this effect. Cisplatin led to the bodyweight loss in vehicle-treated rats, and CP-99 994 could not attenuate this suppressive effect (P>.05, Fig. 2C).

3.2 | Effect of CP-99 994 administrated into the DMNV on gastric contraction activity

3.2.1 | Effect of CP-99 994 administrated into the DMNV on gastric contraction activity of saline-treated rats

As we expected, administration of 0.1 pmol, 1 pmol, and 10 pmol CP-99 994 (n=8 in each group) into the DMNV could increase gastric contraction activity, including gastric frequency and gastric amplitude in saline-treated rats, in a dose-dependent manner (P<.05-P<.01, Figure 3A,B). The increase in gastric amplitude induced by different concentration of CP-99 994 had about 5-10 minutes latency and lasted for approximately 10, 20, and 35 minutes, respectively. It was higher in the 10 pmol group than 1 pmol group in the sixth and the seventh 5 minutes after CP-99 994 administration and was higher than 0.1 pmol CP-99 994 group from the fourth to the seventh 5 minutes after CP-99 994 administration. The effect of CP-99 994 on gastric frequency was also dose-dependent. The effect of CP-99 994 on gastric frequency had a latency about 10-15 minutes and lasted for approximately 5, 20, and 40 minutes in 0.1 pmol, 1 pmol, and 10 pmol group, respectively. It was higher in the 10 pmol group than 0.1 pmol group from the second to the sixth 5 minutes after CP-99 994 administration. (P<.05-.01)

3.2.2 | Effect of CP-99 994 administrated into the DMNV on gastric contraction activity of cisplatin-treated rats

In this study, kaolin intake increased more obviously on the first day following cisplatin administration. So we chose the time point of 24 hours after cisplatin administration to study the effect of CP-99 994 on gastric motility. The data showed that the gastric frequency...
was 4.02±0.89 times/min and the gastric amplitude was about 7.01±1.02 g/min in saline group. However, the gastric contractility weakened markedly in the cisplatin-treated rats. 24 hours after cisplatin was used, the average gastric frequency decreased to 2.01±0.39 times/min (P<.05 vs saline-treated group) and average amplitude reduced to 4.41±0.56 g/min (P<.05 vs saline-treated group).

Just like in saline-treated group, administration of 0.1 pmol, 1 pmol, and 10 pmol CP-99 994 (n=8 in each group) into the DMNV could also increase gastric contractility dose-dependently (P<.05-.01, Figure 3C,D) in cisplatin-treated group. No significant difference was observed in latency and duration of effect of CP-99 994 on gastric amplitude and gastric frequency between cisplatin-treated rats and saline-treated rats. In 10 pmol CP-99 994 treated group, the frequency increased to 3.45±0.47 times/min and the amplitude increased to 5.92±0.51 g/min. Although the average amplitude and frequency of gastric contraction were still lower but had no statistic difference (P>.05) compared with the basal contractility of saline-treated rats (frequency: 4.02±0.89 times/min; amplitude: 7.01±1.02 g/min). The gastric frequency and amplitude still lower than saline-treated rats in 0.1 and 1 pmol CP-99 994 group (data not shown).

According to the result of %MI study, the increased gastric contractility induced by 10 pmol was elevated in the cisplatin-treated rats compared to that of saline-treated rats (P<.05, Figure 4).

3.3 Effect of CP-99 994 administrated into the DMNV on the gastric emptying of rats

Although CP-99 994 improved gastric contraction activity, whether it could enhance gastric emptying is unknown. As gastric emptying has closer relationship with emesis, we next investigated the role of CP-99 994 on gastric emptying. Our results showed that in cisplatin-treated rats, a significant reduction in the gastric emptying was observed when compared with saline-treated rats in vehicle-injected groups (48.2±3.7% vs 82.9±4.3%, P<.001). CP-99 994 with any dose

![FIGURE 3](image-url) Effect of CP-99 994 microinjected into DMNV on gastric contraction activity in saline and cisplatin-treated rats. CP-99 994 increased gastric amplitude and gastric frequency in saline-treated rats (A) and (B) and cisplatin-treated rats (C) and (D) both with a dose-dependent manner. Data are presented as mean±SEM. N=8 in each group.*P<.05, **P<.01 vs control group; #P<.05, ##P<.01 vs 0.1 pmol CP-99 994 group; †P<.05, ††P<.01 vs 1 pmol CP-99 994 group.

![FIGURE 4](image-url) Effect of CP-99 994 on gastric motility index change (%MI) in saline-treated and cisplatin-treated rats. CP-99 994 increased %MI in a dose-dependent manner both in saline-treated and cisplatin-treated rats. The effects of 1pmol CP-99 994 group and 10 pmol CP-99 994 group on %MI in cisplatin treatment rats were markedly enhanced than those effect in saline-treated rats. Data are represented as mean±SEM, %MI = (area under the manometric trace after CP-99 994 treatment)/(area under the manometric trace before CP-99 994 treatment) × 100%. *P<.05, **P<.01 vs control group; #P<.05, ##P<.01 vs 0.1 pmol CP-99 994 group; †P<.01 vs 1 pmol CP-99 994 group. *P<.05, compared between saline- and cisplatin-treated rats.
had no obvious effect on gastric emptying in saline-treated rats (P>.05, Figure 5). Nevertheless, in cisplatin-treated rats, CP-99 994 dose-dependently increased gastric emptying (Figure 5). A total of 1 pmol and 10 pmol CP-99 994 enhanced gastric emptying to 67.0±4.6% and 78.9±3.2%, which were significant increased compared with vehicle-injected group (48.2±3.7%, P<.01 and P<.001, respectively). Compared with saline-treated rats, gastric emptying decreased significantly in vehicle, 0.1 pmol and 1 pmol groups (P<.001-P<.01, Figure 5) but not in 10 pmol group (P>.05, Figure 5) in cisplatin-treated rats. This result suggested that the enhanced effect of CP-99 994 on gastric emptying only occurred in cisplatin-treated rats.

3.4 | Effect of CP-99 994 on the spontaneous discharge of neurons in the DMNV

3.4.1 | Effect of CP-99 994 on the spontaneous discharge of neurons in the DMNV in saline-treated rats

Autonomous firing activities of 121 neurons in the DMNV were collected from 42 saline-treated rats. Among 121 neurons, 70 neurons were sensitive to gastric distension (70/121, 57.9%) with 55 (55/70, 78.6%) GD-I neurons and 15 (15/70, 21.4%) GD-E neurons.

Of 55 GD-I neurons, 32 (32/55, 58.1%) were inhibited by CP-99 994 with firing rates decreased from 1.69±0.23 Hz to 1.14±0.16 Hz (n=32, P<.001, Figure 6A,C). The average reduction (36.1±3.1%) was statistically different (P<.01) from that of vehicle group (basal: 1.54±0.18 Hz; NS: 1.58±0.20 Hz, average change: 4.1±2.6%). The latency of this inhibitory effect was 23.1±5.2 seconds
and the duration was 145.8±15.3 seconds. The remaining 23 GD-I neurons had no significant responses to CP-99 994. Of 15 GD-E neurons, five were activated by CP-99 994 with an increase in firing rates from 1.24±0.49 Hz to 1.64±0.63 Hz (P<.05, Figure 6B,C). The average increase (36.9±4.8%) was statistically different (P<.05) from that of vehicle group (basal: 1.42±0.28 Hz; NS: 1.52±0.21 Hz, average change: 7.2±3.1%). The latency of this excitatory effect was 35.1±8.3 seconds and the duration was 107.5±9.4 seconds. The remaining 10 GD-E neurons had no significant responses to CP-99 994.

3.4.2 | Effect of CP-99 994 on the spontaneous discharge of neurons in the DMNV 24 hours after cisplatin administration

Autonomous firing activities of 97 DMNV neurons were obtained from 37 cisplatin-treated rats. Among 97 neurons, 54 neurons were responsive to gastric distension (54/97, 55.7%) with 42 (42/54, 77.8%) GD-I neurons and 12 (12/54, 22.2%) GD-E neurons. There was no significant difference in the ratio of number of GD-I neurons to that of GD-E neurons in cisplatin-treated rats compared to saline-treated rats.

Of 42 GD-I neurons, 25 (25/42, 59.5%) were inhibited by CP-99 994 with an decrease in firing rates from 1.84±0.23 Hz to 0.91±0.19 Hz (P<.001, Figure 7A,C). The average decrease (55.6±5.5%) was statistically distinct (P<.01) from that of vehicle group (basal: 1.48±0.20 Hz; NS: 1.52±0.21 Hz, average change: 3.1±1.7%) and was also much stronger than the inhibitory effect of CP-99 994 on GD-I neurons in saline-treated group (55.6±5.5% vs 36.1±3.1%, P<.01,

![Figure 7](image)

**FIGURE 7** Effect of CP-99 994 on the spontaneous firing rate of GD responsive neurons in the DMNV in cisplatin-treated rats. Typical frequency histogram illustrating that CP-99 994 had a remarkable inhibitory effect on GD-I neurons (A) and excitatory GD-E neurons (B). Pooled data summarize the effects of CP-99 994 on GD-I and GD-E neurons in cisplatin-treated rats (C). Data are represented as mean±SEM. ***P<.001, *P<.05

Of 12 GD-E neurons, five were activated by CP-99 994 with an increase in firing rates from 1.20±0.38 Hz to 1.69±0.46 Hz (P<.05, Figure 7B,C). The average increase (46.0±13.7%) was statistically distinct (P<.05) from that of vehicle group (basal: 1.57±0.19 Hz; NS: 1.52±0.27 Hz, average change: 5.4±2.7%). No significant difference was observed between the effect Figure 8). The latency of this inhibitory effect was 20.4±8.2 seconds and the duration was 125.8±14.3 seconds. The remaining 17 GD-I neurons had no significant responses to CP-99 994. Of 12 GD-E neurons, five were activated by CP-99 994 with an increase in firing rates from 1.20±0.38 Hz to 1.69±0.46 Hz (P<.05, Figure 7B,C). The average increase (46.0±13.7%) was statistically distinct (P<.05) from that of vehicle group (basal: 1.57±0.19 Hz; NS: 1.52±0.27 Hz, average change: 5.4±2.7%). No significant difference was observed between the effect Figure 8). The latency of this inhibitory effect was 20.4±8.2 seconds and the duration was 125.8±14.3 seconds. The remaining 17 GD-I neurons had no significant responses to CP-99 994. Of 12 GD-E neurons, five were activated by CP-99 994 with an increase in firing rates from 1.20±0.38 Hz to 1.69±0.46 Hz (P<.05, Figure 7B,C). The average increase (46.0±13.7%) was statistically distinct (P<.05) from that of vehicle group (basal: 1.57±0.19 Hz; NS: 1.52±0.27 Hz, average change: 5.4±2.7%). No significant difference was observed between the effect
of CP-99 994 on GD-E neurons in cisplatin-treated rats and that in saline-treated rats (46.0±13.7% vs 36.9±4.8%, P>0.05, Figure 8). The latency of this excitatory effect was 31.7±6.9 seconds and the duration was 100.3±6.8 seconds. The remaining seven GD-E neurons had no significant responses to CP-99 994.

4 | DISCUSSION

Our present study revealed that NK₁ receptor blocker, CP-99 994, exhibited anti-emetic effect in both acute and delayed phase of emesis in rats. When CP-99 994 was microinjected into the DMNV, the gastric contraction activity was enhanced dose-dependently in saline- and cisplatin-treated rats but gastric emptying was enhanced only in cisplatin-treated rats. CP-99 994 decreased the firing rate of GD-I neurons and increased the firing rate of GD-E neurons in the DMNV in both saline- and cisplatin-treated rats. All the effects of CP-99 994 on gastric motility and neuronal activity were stronger in cisplatin-treated rats compared to those in saline-treated rats. Our results suggest that CP-99 994 improved the symptoms of emesis by regulating neuronal activity as well as gastric motility via NK₁ receptors in the DMNV.

Antagonist of NK₁ receptor, CP-99 994, prevented the emesis caused by various emetic challenges including morphine, apomorphine, cisplatin, nicotine, copper sulphate, and provocative motion. However, in these studies, CP-99 994 was given all by systemically administration. The NK₁ receptors are dense in the central nervous system including NST, AP, and the DMNV in ferrets and rats. Few researches focus on the direct effect of central NK₁ receptors on emesis. In this study, our results showed that i.p. injection of cisplatin with 6 mg/kg induced increased kaolin intake in rats and when CP-99 994 microinjected into the DMNV, the kaolin intake induced by cisplatin reduced markedly. This result provided direct evidence that NK₁ receptors in the DMNV participated in the pathogenesis of emesis and blockage of NK₁ receptors took a role in anti-emesis. Our results also showed that CP-99 994 reduced the kaolin intake not only in the first 24 hours but also in the second 24 hours after cisplatin was used suggested that blockage of NK₁ receptors in the DMNV could inhibit both acute and delayed emesis. Furthermore, our study demonstrated the CP-99 994 microinjected into the DMNV could increase food intake in saline- and cisplatin-treated rats. This observation is consistent with a previous study that another NK₁ receptor blocker, GR205171, increased food intake in cisplatin-treated rats. However, CP-99 994 had no obvious effect on bodyweight change in both of saline- and cisplatin-treated rats. Therefore, we draw a conclusion that CP-99 994 microinjected into the DMNV not only improved the symptoms of emesis but also improved the anorexia symptoms in rats treated with cisplatin.

Chemotherapy is often complicated by delayed gastric emptying symptoms and markedly alters neuro-muscular gastric function. Although it is still a controversial issue about the relationship between gastric emptying and nausea and vomiting, there are some gastric prokinetic drugs do take anti-emesis effect in patients with nausea and vomiting. For example, low doses of erythromycin could anti-emesis through increasing in gastric motility and regulation of vagal reflex circuits responsive for emesis. In animal models of gastroparesis and dyspepsia, activation of ghrelin receptors promotes gastric emptying and attenuates symptoms of anorexia and vomiting. Metoclopramide and dazoxpride have the effect of antagonizing cisplatin-induced emesis in dogs through enhancing gastric motility. More importantly, substance P in the DMNV inhibits gastric motility via NK₁ receptors in rat. Thus, we wonder if antagonism of NK₁ receptors in the DMNV could relieve vomiting by increasing gastric motility. As we expected, in our study, when CP-99 994 was microinjected into the DMNV, gastric contraction activity was enhanced both in control and cisplatin-treated rats. This result suggested that in physiological state, endogenous substance P inhibited gastric contractility via NK₁ receptors. Furthermore, effect of gastric contractility increased by CP-99 994 was stronger in cisplatin-treated rats compared to that in saline-treated rats. The possible explanation for this phenomenon may be that studies showed that the substance P-producing preprotachykinin-1 (PPT1) mRNA levels peaked at 4 and 24 hours in the brain correlating with emesis induced by cisplatin. Yamamoto et al. reported that cisplatin administration dramatically increased substance P release to about six fold of basal levels within 18 hours of its application and another investigation also showed that cisplatin administration in the least shrew markedly increased NK₁ receptor mRNA and NK₁ receptor protein in the brainstem. Therefore, the enhanced activity of substance P system would make the blocking effect of NK₁ receptor stronger in cisplatin-treated rats compared to that in saline-treated rats. This mechanism also could be used to explain our gastric emptying result that CP-99 994 did not have significant effect on saline-treated rats but could enhance gastric emptying in cisplatin-treated rats. Our results provided a new evidence for the improvement of emesis by enhancing gastric motility.

Next, we investigated the effect of CP-99 994 on the spontaneous firing of stomach-related neurons in the DMNV to determine the mechanism of NK₁ receptors in modulating gastric motility. Gastric distention inhibited 78.6% and excited 21.4% neurons in the DMNV in saline-treated rats. This result is consistence with previous studies. Activated vagal afferents by gastric distension excite NST neurons, which in turn release GABA to DMNV neurons. So most neurons’ activities in the DMNV are inhibited by gastric distention, which were defined as GD-I neurons. For the neurons excited by gastric distention, which were termed as GD-E neurons, the excitation is possibly due to that glutamate released from the vagal afferents directly activates its receptors on these neurons. Immunohistochemistry revealed that NK₁ receptors were expressed in the DVC, particularly enriched in the medial NST and rostral DMNV. In this study, NK₁ antagonist CP-99 994 inhibited the GD-I neurons and excited GD-E neurons in the DMNV in control rats suggest that in the physiological state, endogenous substance P regulates the neuronal activity and has an excitatory effect on GD-I neurons via NK₁ receptors. This hypothesis is supported by other researches, for example, intracellular recordings showed that...
substance P may regulate vagal output particularly through increasing neuronal excitability in the DMNV\textsuperscript{37} and whole cell patch-clamp recordings showed that substance P can induce inward currents in different subclasses of DMNV neurons that project to the gastrointestinal tract, which were partially attributed to the activation of NK\textsubscript{1} receptors on the postsynaptic DMNV neurons.\textsuperscript{38} On the other hand, microinjection of substance P into the DMNV reduced the tonic pressure and phasic activity of the stomach.\textsuperscript{39} Further studies indicated that NK\textsubscript{1} receptor activation is solely responsible for the effect seen with substance P administration in the DMNV.\textsuperscript{25} In our study, when the effect of endogenous substance P on NK\textsubscript{1} receptors was blocked by CP-99 994, there is a decrease in the firing rate of GD-I neurons and this inhibitory effect on DMNV neurons may be the reason for an enhanced gastric motility in rats. Unfortunately, we cannot observe the direct effect of GD-I neurons on gastric motility because GD responsive neurons were identified by single unit recording, and we are unable to observe the change in gastric motility by activating GD-I neurons at the same time with the current technique. However, other studies demonstrated that excitation of DMNV neurons by substance P could inhibit gastric motility via activation of NK\textsubscript{1} receptors.\textsuperscript{25,37-39} In this study, there are about 60\% of GD-I neurons responded to CP-99 994 which means NK\textsubscript{1} receptors were expressed in these neurons. So we postulate that some of GD-I neurons maybe involved in the inhibitory regulation of gastric motility by activating GD-I neurons at the same time with the current technique. However, other studies demonstrated that excitation of DMNV neurons by substance P could inhibit gastric motility via activation of NK\textsubscript{1} receptors. In addition, the inhibitory effect of CP-99 994 was stronger in cisplatin-treated rats than that in saline-treated rats. The possible reason may be the overexpression of NK\textsubscript{1} receptors in the brainstem of cisplatin-treated animals.\textsuperscript{34} The increased expression of NK\textsubscript{1} receptors led to the enhanced effect of NK\textsubscript{1} receptors antagonist in cisplatin-treated animals.

As for GD-E neurons, we postulate that these neurons have opposite role to GD-I neurons in modulating gastric motility. They may inhibit the activity of GD-I neurons or may have a direct excitatory effect on gastric motility. So when CP-99 994 was microinjected into the DMNV, they were activated and then enhanced the gastric motility by regulating activity of GD-I neurons or releasing acetylcholine to postganglionic neurons in gastric myenteric nerve plexus. The exact mechanisms need us to explore in the future.

In summary, we demonstrated that inhibition of NK\textsubscript{1} receptors in the DMNV could alleviate symptoms of emesis induced by cisplatin and this effect may have relation to regulating spontaneous firing of gastric related neurons in the DMNV and then enhancing gastric motility.

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DISCLOSURES

No competing interests declared.

AUTHOR CONTRIBUTION

Both authors LX and XS were responsible for the conception, design, and revision of the article. Authors XS, FG, SG, XL acquired the data. Author WL undertook the statistical analysis and interpretation of data, and author XS wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

REFERENCES


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