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Methotrexate produces delayed emesis in dogs: a potential model of delayed emesis induced by chemotherapy

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Abstract

We investigated the emetic effects of cisplatin and methotrexate in dogs, the effects of ondansetron on cisplatin-induced vomiting, and the effects of ondansetron, dexamethasone and a combination of the two on the vomiting induced by methotrexate. Ondansetron was administered 30 min before cisplatin administration. Ondansetron, dexamethasone or a combination of the two was administered 8, 24 and 48 h after methotrexate administration. Cisplatin (3 mg/kg, i.v.) induced acute vomiting but failed to induce delayed vomiting. The acute vomiting was markedly inhibited by ondansetron (3 mg/kg, p.o.; 1 mg/kg, i.v.). Methotrexate (2.5 mg/kg, i.v.) caused delayed vomiting, which was partly inhibited by ondansetron (1 mg/kg, i.v.) or dexamethasone (2.5 mg/kg, i.v.). The combination of the two agents was more effective. These results suggest that methotrexate-induced emesis in dogs would be useful for studying delayed emesis.

Keywords: Delayed vomiting; 5-HT_3 receptor antagonist; Dexamethasone; (Dog)

1. Introduction

In patients with cancer, nausea or vomiting is one of the most undesirable side-effects of chemotherapy, although the introduction of 5-HT_3 receptor antagonists for the treatment of emesis evoked by cancer chemotherapy has made a significant improvement. Cisplatin chemotherapy induces a biphasic pattern of emesis, which is characterized by an acute phase and a delayed phase. Following the initial peak of intense emesis, which occurs within 8 h after cisplatin administration (De Mulder et al., 1990; Marty et al., 1990), there is a secondary, delayed emesis phase, which is defined as emesis or nausea occurring more than 24 h after cisplatin administration (Kris et al., 1985). The delayed emesis is severest at 48–72 h after drug administration (Kris et al., 1985). 5-HT_3 receptor antagonists are highly effective for controlling the acute emesis induced by cisplatin. However, the delayed phase of emesis is not satisfactorily controlled by antiemetics. Although delayed emesis is less severe than acute emesis (Kris et al., 1985), it may adversely affect patients' quality of life and contribute to the occurrence of anticipatory nausea and emesis with further courses of chemotherapy. The control of delayed emesis, especially after cisplatin administration, remains an important therapeutic challenge.

Ferrets and piglets have been used as experimental models to elucidate the pathophysiology of delayed emesis. Rudd et al. (1994) recently developed a model of cisplatin-induced delayed emesis in the ferret: intraperitoneal administration of cisplatin at a low dose (5 mg/kg) causes an initial peak of emesis within the first 16 h, followed by a delayed emesis after 32–72 h. In the piglet, cisplatin (5.5 mg/kg, i.v.) induces both the acute and delayed phases of the emetic response without any lethality (Milano et al., 1995). Acute emesis is observed from 2–16 h after dosing; delayed emesis starts at 18 h after dosing and lasts until 58 h. The pattern of the acute and delayed emesis in ferrets and piglets is similar to that observed in humans (Kris et al., 1985). However, ferrets die within 1 week after intraperitoneal administration of cisplatin at doses of 5 mg/kg and above (Veyrat-Follet et al., 1997; Fukui and Yamamoto, unpublished data), suggesting that this model may be accompanied by general prostration before death. In the piglet, delayed emesis induced by cisplatin occurs at a non-lethal dose, but the emesis is completely inhibited by repetitive treatment with a 5-HT_3 receptor antagonist, granisetron (Grélot et al.,

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1996). This complete inhibition by the 5-HT_3 receptor antagonist is not the case in humans, as described above. Therefore, we considered that we needed a model of delayed emesis using another species to clarify the mechanism of delayed emesis and to find a new antiemetic agent. It is known that the dog is a species particularly sensitive to emetic stimuli. In the present study, we found that methotrexate, an anticancer drug, could cause delayed emesis in dogs, and we examined the suitability of canine methotrexate-induced delayed emesis as a model of the delayed emesis induced by chemotherapy in humans.

2. Materials and methods

2.1. Animals and surgical procedure

2.1.1. Animals

Thirty-six Beagle dogs of either sex weighing 6.9–13.0 kg were used. The dogs were housed in metal cages in an animal room under controlled conditions: room temperature, 20–26°C; relative humidity, 40–70%; and air exchange, 8–25 times/h. Each animal was given dog food (CD-5, Clea Japan) once daily, was allowed tap water ad libitum, and was fasted for at least 18 h before surgery or administration of methotrexate or cisplatin.

2.1.2. Vagotomy and greater splanchnic nerve section

Four dogs underwent surgery after pretreatment with atropine sulfate (0.05 mg/kg, s.c.) and xylazine hydrochloride (2 mg/kg, s.c.), and anesthesia with ketamine hydrochloride (50 mg/kg, i.m.). The surgery was performed under aseptic conditions. The dorsal and ventral vagal trunks, coursing along the supradiaphragmatic esophagus, and the left and right greater splanchnic nerves were ligated and sectioned. For a few days after surgery, all animals were given penicillin intramuscularly (0.2 million units/animal), and gentamicin sulfate ointment was applied to the suture sites to prevent infection. The animals were allowed 7–10 days to recover from surgery before they were used in experiments. Neither spontaneous vomiting nor other abnormal behavior was seen in any dog with these abdominal lesions.

2.2. Assessment of the effects of ondansetron on acute vomiting induced by cisplatin

The effects of oral or intravenous administration of ondansetron on the vomiting induced by cisplatin (3 mg/kg, i.v.) were examined using 13 dogs. Ondansetron was administered 30 min before cisplatin at a dosage of 3 mg/kg for oral administration and 1 mg/kg for intravenous administration. The dosages of ondansetron were selected on the basis of previous studies with dogs, which demonstrated that oral (1–3 mg/kg) or intravenous (0.1–1 mg/kg) administration was highly effective in inhibiting vomiting induced by cisplatin (3 mg/kg, i.v.) (Sagrada et al., 1991; Haga et al., 1993; Eglen et al., 1993, 1995). All animals treated with cisplatin were observed for survival once daily at least for 2 weeks after cisplatin administration.

2.3. Assessment of the effects of ondansetron, dexamethasone, a combination of the two drugs, and visceral nerve section on delayed vomiting induced by methotrexate

As a control, methotrexate was administered intravenously at 2.5 mg/kg. Ondansetron (10 mg/kg, p.o. or 1 mg/kg, i.v.) or dexamethasone (2.5 mg/kg, i.v.) was administered 8, 24 and 48 h after administration of methotrexate (2.5 mg/kg, i.v.). In the combination treatment, both ondansetron (1 mg/kg, i.v.) and dexamethasone (2.5 mg/kg, i.v.) were administered simultaneously 8, 24 and 48 h after administration of methotrexate. This timing of ondansetron and dexamethasone administration was selected based on the clinical dose regimen of the drugs (once daily) to prevent delayed emesis. The dosages of these drugs were determined as follows. The dosage of methotrexate was selected on the basis of a preliminary dose-finding study in which 1 mg/kg i.v. failed to induce vomiting in one group of animals, whereas other dogs receiving 10 mg/kg i.v. died after showing more than 100 vomiting episodes (data not shown). The dosages of ondansetron were based on the results in which oral (3 mg/kg) or intravenous (1 mg/kg) administration of ondansetron inhibited cisplatin-induced vomiting in dogs, as described above. The dosage of dexamethasone was set according to the results of studies with ferrets, in which a combination of dexamethasone (2.5 mg/kg, i.v.) and ondansetron (0.1 mg/kg, i.v.) reduced significantly the number of retching and vomiting episodes, with an increased latency period to first retch or vomit (Rudd et al., 1996). The effects of abdominal visceral nerve section on the vomiting induced by methotrexate (2.5 mg/kg, i.v.) were examined in eight dogs. All animals treated with methotrexate (2.5 mg/kg, i.v.) were observed for survival once daily for at least 1 or 2 weeks after methotrexate administration, except for two animals that were killed 3 days after methotrexate administration. These two animals were anesthetized with sodium thiopental and killed by exsanguination from the common carotid artery. The gastrointestinal tract (stomach, duodenum, jejunum, ileum, cecum, colon and rectum) was examined visually.

2.4. Measurement of emesis

Vomiting episodes were recorded remotely by video recorders for 5 h after cisplatin administration and 72 h after methotrexate administration, and the number of episodes was counted for each respective observation pe-

Table 1
Effects of oral gavage or intravenous administration of ondansetron on cisplatin-induced acute vomiting in dogs

Pretreatment	Dose (mg/kg)	Route	No. of dogs vomiting/tested	No. of vomiting episodes (mean ± S.E.)	Inhibitory (%)	Latency period (h, mean ± S.E.)	Duration (h, mean ± S.E.)
None	–	–	6/6	16.0 ± 1.3	–	1.1 ± 0.2	2.0 ± 0.1
Ondansetron	3	p.o.	4/4	2.3 ± 0.3[b]	83	2.1 ± 0.0[b]	0.8 ± 0.1[b]
	1	i.v.	3/3	1.7 ± 0.3[b]	91	2.3 ± 0.1[b]	0.4 ± 0.3[b]

Control animals were treated intravenously with cisplatin at a dose of 3 mg/kg.
Ondansetron was administered orally or intravenously 30 min before administration of cisplatin.
Compared with the control, [b]$P < 0.01$.

riod. In the case of cisplatin, the presence of vomit or gastric juice on the pan under the housing cage was checked until 67 h after video recording was stopped (until 72 h after dosing). In dogs that had undergone abdominal visceral nerve section, the vomiting episodes were monitored for 96 h after methotrexate administration, and the number of vomiting episodes during the observation period was counted. The duration of emesis was calculated as the difference between the time at which the animal first vomited and that of the final vomiting episode during the observation period. Vomiting was defined as the expulsion of vomit or gastric juice from the stomach. Retching was not counted, because it was almost never observed in the present study. Vomiting episodes occurring less than 1 min apart were recorded as single episodes. No animal was used more than once.

2.5. Statistics

Data on the number, latency and duration of vomiting episodes induced by methotrexate or cisplatin were analyzed for differences from the control. The *t*-test was performed to compare the mean for the control group with that for one treatment group. Dunnett's test was conducted to compare the mean for the control group with those for the multiple treatment groups.

2.6. Drugs

Methotrexate (Methotrexate® parenteral 50 mg, Lederle, Osaka, Japan; 4-amino-10-methylfolic acid) was dissolved in physiological saline and injected intravenously at 2.5 mg/kg. The injection volume of methotrexate was 1 ml/kg. Cisplatin (Aldrich Chemical, St. Louis, MO) was dissolved by sonication in physiological saline at 60°C and injected intravenously at 3 mg/kg. The injection volume of cisplatin was 3 ml/kg. Ondansetron (Zofran® injection, Glaxo, Tokyo, Japan) was administered intravenously in a volume of 0.5 ml/kg or orally in a volume of 1.5 or 5 ml/kg. Dexamethasone (Corson® injection, Takeda Chemical Ind., Osaka, Japan) was administered intravenously in a volume of 0.76 ml/kg. Each drug was administered immediately after preparation.

3. Results

3.1. Effects of ondansetron on acute vomiting induced by cisplatin

No animals receiving 3 mg/kg of cisplatin died within 2 weeks or longer after drug administration. The results are summarized in Table 1, and the pattern of vomiting following intravenous administration of cisplatin (3 mg/kg, $n = 6$) is shown in Fig. 1. In the control animals, vomiting

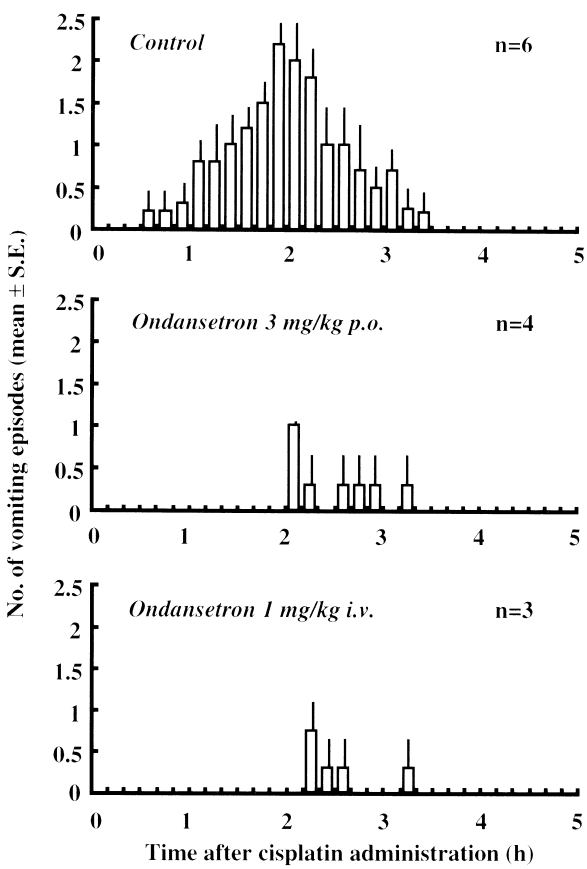

Fig. 1. The pattern of acute vomiting in dogs ($n = 3$) following the administration of cisplatin (3 mg/kg, i.v.), and the effects of ondansetron on the induced vomiting. Ondansetron was administered 30 min before cisplatin administration. Vertical bars represent the mean number of vomiting episodes, with S.E. for each 10-min period of the 5-h observation period.

episodes began 1.1 ± 0.2 h (mean ± S.E., $n = 6$) after cisplatin administration, and the most frequent vomiting episodes were observed from 1 to 3 h after dosing. No vomiting episodes were observed from 3.5 to 5 h after dosing, and no evidence suggesting the occurrence of vomiting, such as vomit and gastric juice, was found on the pan under the housing cage until 72 h after dosing. In the controls, the number of episodes in the 5-h observation period was 16.0 ± 1.3. Both oral (3 mg/kg, $n = 4$) and intravenous (1 mg/kg, $n = 3$) ondansetron reduced the number of vomiting episodes induced by cisplatin by 83% and 91%, respectively.

3.2. Effects of ondansetron, dexamethasone, a combination of the two drugs, and visceral nerve section on delayed vomiting induced by methotrexate

No deaths occurred in animals that received 2.5 mg/kg methotrexate for 1 week or more. The results are summarized in Table 2, and the pattern of vomiting following intravenous administration of methotrexate (2.5 mg/kg, $n = 3$) is shown in Fig. 2. In the control animals, the first vomiting episode induced by methotrexate started between 20 and 24 h after methotrexate administration, but no vomiting was observed between 24 and 28 h after administration. The second vomiting episode began between 28 and 32 h and lasted until 72 h after dosing. In the controls, the number of vomiting episodes from 24–72 h after methotrexate was 11.7 ± 0.9 (mean ± S.E., $n = 3$). Oral administration of ondansetron (10 mg/kg, $n = 3$) had no statistically significant effect on methotrexate-induced vomiting, although a tendency for inhibition was noted. Intravenous administration of ondansetron (1 mg/kg, $n = 3$) or dexamethasone (2.5 mg/kg, $n = 3$) significantly reduced the number of vomiting episodes induced by methotrexate by 55% or 66%, respectively. Furthermore, the combination of dexamethasone (2.5 mg/kg, i.v.) and ondansetron (1 mg/kg, i.v.) significantly reduced the number of vomiting episodes by 91% ($n = 3$). On the other hand, the emetic response to methotrexate was unaffected by bilateral abdominal vagotomy and bilateral greater splanchnic nerve section (Table 3). At necropsy,

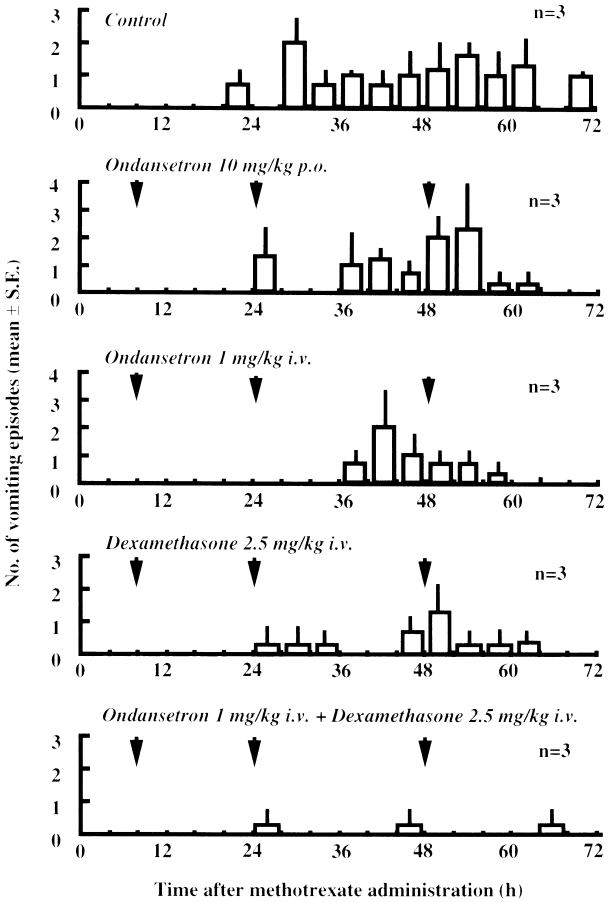

Fig. 2. The pattern of delayed vomiting in dogs ($n = 3$) following intravenous administration of methotrexate (2.5 mg/kg), and the effects of ondansetron, dexamethasone or a combination of the two on the induced vomiting. Each drug was administered 8, 24 and 48 h after methotrexate administration. Vertical bars represent the mean number of vomiting episodes, with S.E., for each 4-h period of the 72-h observation period.

Table 2
Effects of ondansetron, dexamethasone or a combination of the two on methotrexate-induced delayed vomiting in dogs

Treatment	Dose (mg/kg)	Route	No. of dogs vomiting/tested	24–72 h after methotrexate		Latency period (h, mean ± S.E.)	Duration (h, mean ± S.E.)
				No. of vomiting episodes (mean ± S.E.)	Inhibitory (%)		
None	–	–	3/3	11.7 ± 0.9	–	25.3 ± 1.5	44.0 ± 1.7
Ondansetron	10	p.o.	3/3	9.3 ± 2.3	21	28.6 ± 3.9	27.8 ± 2.7
Ondansetron	1	i.v.	3/3	5.3 ± 0.3[a]	55	39.4 ± 1.1	14.4 ± 2.2
Dexamethasone	2.5	i.v.	2/3	4.0 ± 2.1[a]	66	43.1 ± 14.5	18.6 ± 9.5
Ondansetron and dexamethasone	1 2.5	i.v. i.v.	2/3	1.0 ± 0.6[b]	91	49.0 ± 12.8	12.9 ± 12.9[a]

Control animals were treated intravenously with methotrexate at a dose of 2.5 mg/kg.
Ondansetron, dexamethasone or a combination of the two drugs were administered orally 8, 24 and 48 h after methotrexate administration (2.5 mg/kg, i.v.).
Compared with the control, [a]$P < 0.05$, [b]$P < 0.01$.
If a dog did not vomit, the latency period was taken to be equal to the observation period (72 h).

Table 3
Effects of visceral nerve section on methotrexate-induced delayed vomiting in dogs

Pretreatment	No. of dogs vomiting/tested	No. of vomiting episodes (mean ± S.E.)	Inhibitory (%)	Latency period (h, mean ± S.E.)	Duration (h, mean ± S.E.)
None	4/4	9.3 ± 1.1	–	27.6 ± 2.5	39.4 ± 8.2
Vagotomy and greater splanchnic nerve section	4/4	12.5 ± 2.5	0	23.6 ± 3.0	46.7 ± 11.5

Control animals and animals with visceral nerve section were treated intravenously with methotrexate at a dose of 2.5 mg/kg.

red or dark red discoloration in the stomach, duodenum or jejunum was observed in the two animals examined.

4. Discussion

In the present study, consistent with our previous reports (Fukui et al., 1992, 1993a,b; Fukui and Yamamoto, 1998), cisplatin at a non-lethal dose (3 mg/kg, i.v.) induced acute phase vomiting in dogs, up to around 3 h after dosing. Ondansetron administered orally (3 mg/kg) or intravenously (1 mg/kg) strongly inhibited the acute vomiting induced by cisplatin. In previous studies, we have demonstrated that, in dogs, other anticancer agents such as cyclophosphamide, nitrogen mustard-*N*-oxide and actinomycin D induce vomiting only in the acute phase, and that the acute vomiting induced by these agents is completely inhibited by 5-HT_3 receptor antagonists (Fukui et al., 1992, 1993b). These results are in good agreement with clinical data for humans, and suggest that this species would be useful for studies of chemotherapy-induced acute emesis. On the other hand, no delayed emetic response was observed after administration of cisplatin at 3 mg/kg. We had preliminarily examined the emetic effects of cisplatin using a lower or a higher dose, but no delayed vomiting was noted at any dose. Thus, cisplatin may not cause delayed emesis in dogs.

Methotrexate has been used as an anticarcinogenic agent for more than 40 years, but recently its clinical indication was extended to the treatment of rheumatoid arthritis and related diseases (Segal et al., 1990). Methotrexate at low (50–250 mg/m^2), intermediate (250–1000 mg/m^2) or high (more than 1000 mg/m^2) dosages produces a moderately low (10–30%), moderate (30–60%) or moderately high (60–90%) incidence of emesis in clinical practice (Hesketh et al., 1997). Cancer patients receiving methotrexate experience nausea or vomiting, or both, at acute (Chang et al., 1979) and after a delay (Chan et al., 1997). The present study using dogs demonstrated that methotrexate at a non-lethal dose caused vomiting that started 20–24 h after treatment and lasted until 72 h, although methotrexate did not evoke acute vomiting within 20 h after treatment. The delayed vomiting induced by methotrexate was partly inhibited by intravenous administration of ondansetron (1 mg/kg) or dexamethasone (2.5 mg/kg), even though oral administration of ondansetron (10 mg/kg) had no statistically significant effect, possibly because of the decreased amount of the drug absorbed due to vomiting shortly after administration. Moreover, dexamethasone (2.5 mg/kg, i.v.) in combination with ondansetron (1 mg/kg, i.v.) was more effective than either drug given alone in reducing the delayed emetic response induced by methotrexate. These results match those of studies with ferrets receiving cisplatin (Rudd and Naylor, 1996) and the clinical profiles of these agents in the treatment of cisplatin-induced emesis (Italian Group for Antiemetic Research, 1997), suggesting that the delayed emesis in dogs receiving methotrexate may occur through a mechanism similar to that observed in humans following cisplatin chemotherapy, and that methotrexate-induced delayed emesis in dogs would be a valuable model for studies on the mechanism of delayed emesis and for development of new antiemetic agents.

In the present study, cisplatin did not cause delayed emesis, and methotrexate did not produce acute emesis. These results do not agree with clinical findings in humans. The reasons are unknown, but the discrepancy does not necessarily mean that the dog is an inappropriate species for studies of emesis. As described above, acute emetic effects of anticancer agents and antiemetic effects of 5-HT_3 receptor antagonists have been detected in dogs, and canine methotrexate-induced delayed emesis has a profile similar to cisplatin-induced delayed emesis in humans. In addition, the emetic doses of cisplatin and methotrexate were non-lethal, and acute or delayed emesis occurred under relatively normal conditions. Therefore, it is considered that dogs, in addition to ferrets, would be useful for studies of both acute and delayed emesis. It should also be noted that the dog is a widely used non-rodent species in pharmacology and toxicity studies, and would be thus more relevant to humans.

The pathophysiology of methotrexate-induced delayed emesis still needs to be clarified. The major adverse effects of methotrexate are gastrointestinal irritation and hepatotoxicity (Kremer and Lee, 1986; Olsen et al., 1987; Rooney and Furst, 1993). Dogs given methotrexate (6 mg/kg, i.v.) show diffuse ulcerative fibronecrotic enteritis in the gastrointestinal tract macroscopically, and epithelial regeneration microscopically after having bloody diarrhea (Bortnowski and Rosenthal, 1991). In the present study, the animals killed 3 days after methotrexate administration had red or dark red discoloration in the gastrointestinal tract. These

findings suggest that peripheral sites may be involved in methotrexate-induced delayed emesis. However, ondansetron, whose antiemetic effects are considered to be due mainly to its antagonistic action on peripheral 5-HT_3 receptors (Andrews et al., 1990), produced only partial inhibition of methotrexate-induced emesis. Dexamethasone, which also partly inhibited the emesis, is known to have an action that reduces the permeability of the blood–brain barrier (Andrews et al., 1988). More directly, section of the abdominal visceral nerves had no effects on the emesis. Therefore, the central nervous system appears to be implicated in methotrexate-induced emesis, and cellular breakdown products produced by methotrexate, or neurotransmitters other than serotonin, may play a role. Recently it has been reported that a tachykinin NK_1 receptor antagonist, especially in combination with a 5-HT_3 receptor antagonist and dexamethasone, greatly improves the control of both acute and delayed emesis in cisplatin-treated patients (Navari et al., 1999). Thus, tachykinin NK_1 receptors in the central nervous system may be targets for the neurotransmitters involved in methotrexate-induced delayed emesis.

In conclusion, intravenous administration of methotrexate caused delayed vomiting at a non-lethal dose. The profile of vomiting was similar to that of the delayed emesis seen in humans. The delayed vomiting induced by methotrexate was significantly inhibited by intravenous administration of ondansetron. Furthermore, dexamethasone, especially in combination with intravenous ondansetron, was effective in reducing the delayed emetic response seen 24–72 h after methotrexate administration. These results suggest that this animal model would be useful for revealing the mechanisms of delayed emesis and for investigating new antiemetic agents for humans against chemotherapy-induced emesis that cannot be controlled with 5-HT_3 receptor antagonists.

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