Vagal Afferent Fibers and Peripheral 5-HT3 Receptors Mediate Cisplatin-Induced Emesis in Dogs

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ABSTRACT — The involvement of visceral afferent fibers and 5-HT3 receptors in the emesis induced by cisplatin was studied in beagle dogs. The emesis induced by cisplatin (3 mg/kg, i.v.) was inhibited by the intravenous administration of ICS205930 (2 × 0.01 or 2 × 0.1 mg/kg) and MDL72222 (2 × 0.5 mg/kg), 5-HT3 receptor antagonists, but not by the intravenous administration of metoclopramide (2 × 0.5 mg/kg), a dopamine D2 receptor antagonist. The cisplatin-induced emesis was also suppressed by the intravenous administration of para-chlorophenylalanine (300 mg/kg/day for 3 days), an inhibitor of 5-HT synthesis. On the other hand, the administration of ICS205930 into the IVth ventricle (2 × 0.01 mg/animal) had no effects on the cisplatin-induced emesis. The cisplatin-induced emesis was completely inhibited by abdominal vagotomy and splanchnicectomy, but not by splanchnicectomy alone. On the contrary, the emesis induced by apomorphine was suppressed by the intravenous (0.1 mg/kg) or intracerebroventricular (0.05 mg/animal) administration of metoclopramide, but not by visceral nerve section. These results strongly suggest that cisplatin evokes emesis mainly by acting on the vagal afferent terminals through the release of 5-HT and that peripheral 5-HT3 receptors are involved in this action.

Keywords: Cisplatin-induced emesis, 5-HT3 receptor antagonist, Vagal afferent

A number of studies on the emetic response induced by cisplatin have been carried out in ferrets (1–5), cats (6–8), Suncus murinus (9, 10) and dogs (11–13). It seems to be clear that 5-HT3 receptors play an important role in cisplatin-induced emesis, since it is strongly inhibited by 5-HT3 receptor antagonists such as ICS205930 (14) and MDL72222 (15) in all the species mentioned above (2–4, 7, 10–12). However, there is conflicting evidence about the site of action, and whether a peripheral site or central site, if either, is implicated in cisplatin-induced emesis is still unclear. The experiments using ferrets have demonstrated that cisplatin-induced emesis is inhibited by vagotomy (16–18). Milano et al. (19) have shown that the vomiting induced by electrical stimulation of the vagal nerves is not suppressed by 5-HT3 antagonists in cats. These results suggest that cisplatin acts mainly on peripheral sites. On the other hand, complete inhibition of cisplatin-induced emesis is observed following ablation of the area postrema, which is recognized as the chemoreceptor trigger zone (CTZ) in cats and dogs (8, 13), or injection of 5-HT3 receptor antagonists into the IVth ventricle in ferrets and cats (5, 7), suggesting that the site of action may be central.

In the present study, therefore, 5-HT3 receptor antagonists or an inhibitor of 5-HT synthesis were administered intravenously or intracerebroventricularly to dogs, and the effects on cisplatin-induced emesis were investigated. The effects of vagotomy and splanchnicectomy were also examined.

MATERIALS AND METHODS

Animals

Beagle dogs of either sex weighing 6.4–12.5 kg were used. These dogs were housed in an animal room under the following conditions: room temperature, 23 ± 3°C; relative humidity, 55 ± 15%; and air exchange, 10–20 times/hr. Each animal was fed dog food (CD-5, Clea Japan, Inc.) once daily and allowed tap water ad libitum.

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Surgical procedure

Animals were anesthetized with ketamine hydrochloride (50 mg/kg, i.m.) after pretreatment with atropine sulfate (0.05 mg/kg, s.c.) and xylazine hydrochloride (2 mg/kg, s.c.). Operations were carried out under aseptic conditions.

Catheterization for intracerebroventricular administration: A 18-gauge catheter (Epineed, Terumo) was implanted in the IVth ventricle through the spinal dura mater, and the catheter was passed subcutaneously to an exit on the animal’s back. The implantation site was confirmed by injecting Evans Blue (Wako Pure Chemical Ind., Ltd.) via the catheter after exanguination at the end of the experiments.

Vagotomy and splanchnicectomy: The dorsal and ventral vagal trunks coursing by the supra-diaphragmatic esophagus and/or the left and right greater splanchnic nerves were ligated and sectioned. For a few days after the operation, all animals received penicillin intramuscularly (0.2 million units/animal), and gentamicin sulfate ointment was applied to the sutural sites to protect against infection. All animals were allowed at least one week to recover from surgery prior to use in experiments.

Drugs used

Cisplatin (Wako Pure Chemical Ind., Ltd.) was dissolved by sonication in physiological saline at 60°C. ICS205930 and MDL72222 (Research Biochemical Inc.) were dissolved in physiological saline adjusted to pH 7 with 1N HCl. Metoclopramide monohydrochloride and apomorphine hydrochloride (Sigma Chemical Co.) and dl(±)-para-chlorophenylalanine ethyl ester hydrochloride (PCPA; Wako Pure Chemical Ind., Ltd.) were dissolved in physiological saline. Each drug was administered immediately after formulation.

Effects of drugs and visceral nerve section on cisplatin-induced emesis

Cisplatin was administered intravenously at a dosage of 3.0 mg/kg. ICS205930 (0.1 mg/kg), MDL72222 (0.5 mg/kg) or metoclopramide (0.1 or 2.0 mg/kg) was administered intravenously 30 min before and 2 hr after cisplatin dosing according to the method of Gyllys et al. (11). PCPA (300 mg/kg) was administered intravenously once daily for 3 days, and cisplatin was injected 24 hr after the last dose. For intracerebroventricular administration, ICS205930 (0.01 mg/0.1 ml/animal) was injected into the IVth ventricle via the indwelling catheter 30 min before and 2 hr after cisplatin dosing. Cisplatin was also administered intravenously to the dogs subjected to splanchnicectomy alone or splanchnicectomy and vagotomy. The number of emetic episodes was counted for 5 hr after the administration of cisplatin. Episodes occurring within a 1 min-interval were recorded as a single episode.

Effects of metocloplamid and visceral nerve section on apomorphine-induced emesis

Apomorphine was administered subcutaneously at a dosage of 0.1 mg/kg. Metoclopramide was administered intravenously at a dosage of 0.1 mg/kg or intracerebroventricularly at a dosage of 0.05 mg/0.1 ml/animal 30 min before apomorphine dosing. Apomorphine was also administered intravenously to the dogs subjected to vagotomy and splanchnicectomy. Emetic episodes were counted for 1 hr after apomorphine dosing in the same way as they were following cisplatin administration.

Statistics

The data in the tables are expressed as the mean ± S.E. The data on the numbers of emetic episodes were analyzed for difference from the control using Student’s t-test, and the data on the latency period were analyzed by Student’s t-test after rank transformation.

RESULTS

Effects of drugs and visceral nerve section on cisplatin-induced emesis

The results are summarized in Table 1, and the patterns of the emetic response in the animals receiving cisplatin alone (control), ICS205930 (2 × 0.1 mg/kg, i.v.) or PCPA (3 × 300 mg/kg, i.v.) are shown in Fig. 1. In the control animals, emetic episodes began 1.91 ± 0.11 hr (mean ± S.E.) after cisplatin dosing, and the most frequent emetic episodes were observed from 2 to 3 hr after dosing. Thereafter, the number of emetic episodes decreased gradually. The number of emetic episodes in the 5-hr observation period was 11.4 ± 1.3. Both ICS205930 (2 × 0.1 mg/kg, i.v.) and MDL72222 (2 × 0.5 mg/kg, i.v.) reduced the mean number of emetic episodes induced by cisplatin by 97.4%, and the latency period was markedly prolonged. A lower dosage of ICS205930 (2 × 0.01 mg/kg, i.v.) reduced the number of emetic episodes induced by cisplatin by 64.9%. Metoclopramide reduced the number of emetic episodes induced by cisplatin by 71.1% at a high dosage (2 × 2.0 mg/kg, i.v.), whereas a low dosage (2 × 0.1 mg/kg, i.v.) showed no significant effects. Pretreatment with PCPA at a dosage of 300 mg/kg/day for 3 days decreased the number of emetic episodes evoked by cisplatin by 64.9%. On the other hand, injection of ICS205930 into the IVth ventricle (2 × 0.01 mg/animal) had no effects on cisplatin-induced emesis. Cisplatin-induced emesis was completely inhibited by vagotomy.
### Table 1. Effects of drugs and visceral nerve section on cisplatin-induced emesis in dogs

| Treatment          | Route  | Dose           | No. of dogs | No. of emetic episodes (mean ± S.E.) | Latency period (hr)(mean ± S.E.) |
|--------------------|--------|----------------|-------------|-------------------------------------|----------------------------------|
| Control           | —      | —              | 7/7         | 11.4 ± 1.3                          | 1.91 ± 0.11                      |
| ICS205930<sup>a</sup> | i.v.   | 2 × 0.01 mg/kg | 3/3         | 4.0 ± 0.0*                          | 2.46 ± 0.04*                     |
| ICS205930<sup>b</sup> | i.v.   | 2 × 0.1 mg/kg  | 1/3         | 0.3 ± 0.3**                         | 4.32 ± 0.68**                    |
| ICS205930<sup>b</sup> | i.v.   | 2 × 0.01 mg/dog| 3/3         | 11.7 ± 2.7                          | 1.85 ± 0.09                      |
| MDL72222<sup>b</sup>  | i.v.   | 2 × 0.5 mg/kg  | 1/3         | 0.3 ± 0.3**                         | 4.17 ± 0.83**                    |
| Metoclopramide<sup>b</sup> | i.v.   | 2 × 0.1 mg/kg  | 3/3         | 14.0 ± 4.0                          | 1.95 ± 0.25                      |
| Metoclopramide<sup>b</sup> | i.v.   | 2 × 2.0 mg/kg  | 3/3         | 3.3 ± 1.9**                         | 2.58 ± 0.48                      |
| PCPA<sup>c</sup>      | i.v.   | 3 × 300 mg/kg  | 2/3         | 4.0 ± 2.0*                          | 2.87 ± 1.07                      |
| Vagotomy +          | —      | —              | 0/3         | 0**                                 | > 5**                            |
| Splanchnicectomy    | —      | —              | 4/4         | 11.8 ± 2.9                          | 1.66 ± 0.26                      |

<sup>a</sup>Control animals were treated with cisplatin (3 mg/kg, i.v.) alone.  
<sup>b</sup>Each drug was administered 30 min before and 2 hr after cisplatin dosing.  
<sup>c</sup>Cisplatin was administered 24 hr after the last dose of PCPA. Compared with the control, **P < 0.01, *P < 0.05. If a dog did not vomit, the latency period was taken as equal to the observation period (5 hr).

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**Fig. 1.** The pattern of the emetic response in dogs following cisplatin administration and the effect of ICS205930 or PCPA on the emesis.  
(A) Control animals were given cisplatin (3 mg/kg, i.v.).  
(B) ICS205930 (0.1 mg/kg, i.v.) was administered 30 min before and 2 hr after cisplatin dosing.  
(C) PCPA (300 mg/kg, i.v.) was administered once daily for 3 days, and cisplatin was injected 24 hr after the last dose. The results are the mean ± S.E. for the number of emetic episodes in each 10 min of the total observation period.
and splanchnicectomy, but not by splanchnicectomy alone.

Effects of metoclopramide and visceral nerve section on apomorphine-induced emesis

As shown in Table 2, the number of emetic episodes that occurred in the first hr after the administration of apomorphine was 5.3 ± 0.7 (mean ± S.E.). The intravenous administration of metoclopramide (0.1 mg/kg) reduced the number of emetic episodes induced by apomorphine by 81.1% and prolonged the latency period. Apomorphine-induced emesis was reduced by 75.5% by the intracerebroventricular administration of metoclopramide (0.05 mg/animal). On the other hand, the emetic response to apomorphine was unaffected by vagotomy and splanchnicectomy.

DISCUSSION

In the present study, 5-HT3 receptor antagonists, ICS205930 (2 X 0.1 mg/kg) and MDL72222 (2 X 0.5 mg/kg), administered i.v. almost completely inhibited cisplatin-induced emesis. On the other hand, metoclopramide had no effects on cisplatin-induced emesis at a dosage (2 X 0.1 mg/kg, i.v.) which inhibited apomorphine-induced emesis. It has been shown that metoclopramide blocks dopamine D2 receptors at conventional doses (0.1–0.4 mg/kg, i.v.) (20), while the drug also blocks 5-HT3 receptors at higher dosages (2.0–4.0 mg, i.v.) (2, 4, 20, 21). In fact, the present study demonstrated that cisplatin-induced emesis was suppressed by injection of a high dose of metoclopramide (2 X 2.0 mg/kg, i.v.). These results are in good agreement with the results of previous studies (1-4, 6, 9-12), confirming that 5-HT3 receptors, but not dopamine D2 receptors, play an important role in cisplatin-induced emesis.

Recently, it has been reported that 5-HT3 receptors are located in the area postrema as well as the gastrointestinal tract (22–24). However, whether central sites or peripheral sites are mainly implicated in cisplatin-induced emesis is unclear. In cats and dogs, cisplatin-induced emesis is inhibited by ablation of the area postrema, one of the circumventricular organs which is outside of the blood-brain barrier (8, 13). The emesis is also inhibited by the injection of 5-HT3 antagonists into the IVth ventricle in ferrets and cats (5, 7). These results suggest that 5-HT released following cisplatin administration may cause emesis via 5-HT3 receptors in the area postrema. On the contrary, there is a line of evidence suggesting that cisplatin acts on the peripheral sites. Emesis induced by cisplatin is prevented by abdominal vagotomy in ferrets (16–18). In cats, vomiting induced by electrical stimulation of the vagal nerve is not suppressed by the intravenous administration of 5-HT3 antagonists (19), and vomiting induced by the intraperitoneal administration of 5-HT3 agonist is not abolished by ablation of the area postrema (25). The present study demonstrated that the injection of ICS205930 (2 X 0.01 mg/animal) into the IVth ventricle had no effects on cisplatin-induced emesis, although ICS205930 administered i.v. significantly inhibited the emesis at a low dose (2 X 0.01 mg/kg). Furthermore, vagotomy and splanchnicectomy, but not splanchnicectomy alone, completely abolished cisplatin-induced emesis. These results strongly suggest that cisplatin induces emesis by activating mainly abdominal vagal afferent fibers, even though involvement of the area postrema cannot be completely ruled out. It is well known that apomorphine evokes emesis by stimulating dopamine D2 receptors in the CTZ (5, 26). In the present study, apomorphine-induced emesis was inhibited by an intravenous or intracerebroventricular injection of metoclopramide, but not by vagotomy and splanchnicectomy. These results indicate that the i.c.v.-administration and visceral nerve section techniques used in the present study are valid.

Cisplatin has been shown to induce an increase in 5-HT levels in the ileal mucosa, but has been shown not

| Treatment                      | Route     | Dose            | No. of dogs Emesis/Tested | No. of emetic episodes (mean ± S.E.) | Latency period (hr)(mean ± S.E.) |
|-------------------------------|-----------|-----------------|---------------------------|-------------------------------------|----------------------------------|
| Control                       |           | 0.1 mg/kg       | 3/3                       | 5.3 ± 0.7                           | 0.05 ± 0.02                      |
| Metoclopramide                | i.v.      | 0.1 mg/kg       | 2/3                       | 1.0 ± 0.6**                         | 0.44 ± 0.28                      |
| Metoclopramide                | i.c.v.    | 0.05 mg/dog     | 2/3                       | 1.3 ± 0.7**                         | 0.41 ± 0.29                      |
| Vagotomy                      | +         |                 | 3/3                       | 5.3 ± 0.3                           | 0.05 ± 0.02                      |
| Splanchnicectomy             |           |                 |                           |                                     |                                  |

*a*Control animals were treated with apomorphine (0.1 mg/kg, s.c.) alone. *b*Metoclopramide was administered 30 min before apomorphine dosing. Compared with the control, **P < 0.01. If a dog did not vomit, the latency period was taken as equal to the observation period (1 hr).
to affect the levels in the gastric mucosa or area postrema (27–29). In the present study, we have demonstrated that pretreatment with PCPA, an inhibitor of 5-HT synthesis, inhibits cisplatin-induced emesis in dogs. These facts suggest cisplatin-induced emesis is mediated by increased 5-HT levels in the small intestine, which in turn stimulate vagal afferent fibers through 5-HT3 receptors.

In conclusion, cisplatin-induced emesis was inhibited by intravenous, but not intracerebroventricular, administration of 5-HT3 receptor antagonists. Furthermore, the emesis induced by cisplatin was completely inhibited by vagotomy and splanchnicectomy but not splanchnicectomy alone. These results strongly suggest that cisplatin evokes emesis mainly by acting on the vagal afferent terminals through the release of 5-HT and that peripheral 5-HT3 receptors are involved in this action.

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