Selective Blockade of Cytotoxic Drug-Induced Emesis by 5-HT₃ Receptor Antagonists in *Suncus Murinus*

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ABSTRACT—Antiemetic effects of serotonin 5-HT₃ receptor antagonists (ICS205-930, zacopride, BRL43694, GR38032F) were investigated in *Suncus murinus*. Veratrine, nicotine, copper sulfate, cisplatin, cyclophosphamide and motion sickness were used as emetic stimuli. Serotonin 5-HT₃ receptor antagonists did not inhibit emetic responses to veratrine, nicotine, copper sulfate and motion sickness. However, cisplatin- and cyclophosphamide-induced emesis was strongly blocked by them. Both subcutaneous and intravenous injections of 5-HT₃ antagonists were effective. Serotonin 5-HT₁ and 5-HT₂ receptor antagonists were less effective. These results clearly indicate that a 5-HT₃ receptor-mediated mechanism(s) is involved in the emesis caused by cancer chemotherapeutic agents and that 5-HT₃ receptor antagonists are very effective as prophylactic drugs.

Nausea and vomiting are the major subjective side-effects of cancer chemotherapy, causing serious discomfort to the patients (1). It has been reported that several 5-HT₃ receptor antagonists such as MDL7222, ICS205-930, and zacopride inhibit emesis induced by cancer chemotherapeutic agents in the dog and ferret (2–4). However, the precise mechanisms of the emesis caused by antitumor drugs is still not clear. *Suncus murinus* (a house musk shrew), used in this study, is a species of insectivora, which is considered to be closer to primates than rodents, lagomorphs and carnivores in the phylogenetic system (5). We have shown previously that the *Suncus murinus* vomits in response to various drugs including cancer chemotherapeutics and motion stimulus and that the animal is suitable for research on antiemetic drugs (6–8).

In the present study, the antiemetic effects of four 5-HT₃ receptor antagonists (ICS205-

930, zacopride, BRL43694, GR38032F) against several emetic stimuli (veratrine, nicotine, copper sulfate, cisplatin and cyclophosphamide) were studied and compared with those of 5-HT₁ and 5-HT₂ antagonists.

MATERIALS AND METHODS

Experiments were performed on healthy adult *Suncus murinus* of either sex weighing 50–70 g (male) and 30–50 g (female). The animals, originally introduced from the Central Institute for Experimental Animals (Kanagawa, Japan), were bred in a temperature-controlled room at 24 ± 1°C at the The Animal Care Institute of The University of Tokyo. They were allowed free access to pellet chow, supplied by the Central Institute for Experimental Animals, and tap water.

Experimental conditions were similar to those reported from our laboratory (6, 7). Emetic stimuli employed were subcutaneous injection of copper sulfate (CuSO₄: 40 mg/kg),

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nicotine (10 mg/kg), and veratrine (0.5 mg/kg); intravenous injection of cisplatin (40 mg/kg) and cyclophosphamide (200 mg/kg); and motion. An indwelling catheter was used for intravenous injection (see below). When the motion stimulus (frequency: 1 Hz, amplitude: 40 mm, duration: 5 min) was applied, each animal was placed in a transparent cage ($5_w \times 15_L \times 12_H$ cm) fixed on a reciprocal shaker (TAITEC R-30 mini, Taiyo Scientific Industrial Co., Ltd., Japan). After a 5-min equilibration period, the motion stimulus was started. The number of vomiting episodes and the latency to the first vomiting were recorded for 5 min (motion), 30 min (copper sulfate, nicotine, veratrine), or 90 min (cisplatin, cyclophosphamide).

Cisplatin and cyclophosphamide were toxic, and the suncus died within a week. Therefore, the animals injected with chemotherapeutics were killed after the experiment. Both male and female animals were used because there was no apparent sex difference. However, a more detailed experiment is currently ongoing.

The four 5-HT₃ receptor antagonists (ICS205-930, zacopride, BRL43694, GR38032F) were subcutaneously injected to the animal 30 min prior to the emetic stimuli except cancer chemotherapeutic agents. In the case of cisplatin and cyclophosphamide, 5-HT₃ receptor antagonists were injected intravenously. We also studied the effects of subcutaneous injection of ICS205-930 against intraperitoneally injected cisplatin-induced emesis. The dose of 5-HT₃ receptor antagonists was started from 1 mg/kg, and changed with a factor of 2. The ID₅₀ value of the antiemetic effect was determined by Brownlee's up-and-down method. Antiemetic effects of pindolol (5-HT_{1A} antagonist), mianserin (5-HT_{1C} and 5-HT₂ antagonist) and ketanserin (5-HT₂ antagonist) against cisplatin were also studied.

A chronic jugular catheter was indwelt for the intravenous administration of 5-HT₃ antagonists according to the method of Florczyk and Schurig (9) with a modification. The suncus was anesthetized with ether, and the fur on the ventral and dorsal area of the neck was

clipped and swabbed with 70% alcohol. A single incision of about 1 cm was made with scissors on the ventral portion of the neck, and the left jugular vein was identified. A piece of 7-cm long polyethylene tubing (0.28 mm, I.D. by 0.61 mm, O.D., PE 10, Clay Adams, Parsippany, NJ, U.S.A) was heat flared at 1 cm from one end and heat bent at 2 cm from the same end. A small incision of about 5 mm was made on dorsal portion of the neck. Using a trochar, the catheter was guided from the ventral incision to the nape under the skin. The catheter was filled with 200 U/ml of sodium heparin, and a small stainless bar was used to seal the nape-side end. Then the catheter was inserted into the jugular vein and ligated together with the flare. The incision was closed using surgical glue which can set instantaneously. When the drugs were administered, 15 cm of polyethylene tubing was connected to the catheter, and the drug solution was injected into the animals without restraint. In some experiments, the emetic effects of copper sulfate (40 mg/kg, p.o.), nicotine (10 mg/kg, s.c.) or veratrine (0.5 mg/kg, s.c.) were studied using the catheter-indwelt animals, and we confirmed that the chronic implantation did not significantly affect vomiting responses of the animal.

The compounds except for cisplatin were dissolved in saline. The volume of drug solution injected was adjusted to 0.1 ml/50 g body weight except for cisplatin (i.v., 1.0 ml/50 g, i.p., 0.5 ml/50 g) and cyclophosphamide (0.5 ml/50 g). In the case of cisplatin, at first, the pH of the saline was adjusted to 4.0 using 0.1 N HCl, and the saline was warmed to about 50°C. Cisplatin was dissolved with this saline in the final concentration of 2 mg/ml, and the solution was cooled to 37°C just before the administration.

RESULTS

Copper sulfate, nicotine, and veratrine caused vomiting within 15 min and motion did within 2 min. However, cisplatin and cyclo-

phosphamide caused emesis with a longer latency. Figure 1 shows the relationship between the time after the injection of cisplatin and cyclophosphamide and the averaged number of vomiting episodes for every 5 min. There is a striking difference in the time course of the number of vomiting episodes between the two drugs. Cisplatin caused emesis with a longer latency, and the emesis occurred periodically or not continuously. However, the emesis caused by cyclophosphamide was transient and completed within 30 min.

Table 1 shows the effect of 5-HT₃ receptor antagonists against several emetic stimuli. The drugs up to 64 mg/kg had no effect against the

emesis induced by copper sulfate, nicotine, veratrine and motion. However, 5-HT₃ antagonists strongly inhibited the cancer chemotherapeutics-induced emesis. Intravenous injection of ICS205-930 prevented the emesis induced by cisplatin and cyclophosphamide. Zacopride, BRL43694 and GR38032F also blocked cisplatin-induced emesis. When ID₅₀ values against cisplatin were compared on the molar basis, ICS205-930 (0.359) and zacopride (0.379) were stronger than BRL43694 (3.21) and GR38032F (3.41 μ mol/kg).

At a dose of 64 mg/kg, ICS205-930, zacopride and BRL43694 caused emesis themselves (Table 2). We could not study the emetogenic

Fig. 1. Relationship between the time after the intravenous injection of cisplatin (upper, 40 mg/kg) and cyclophosphamide (lower, 200 mg/kg) and the number of vomiting episodes. The ordinate indicates the averaged number of vomiting episodes observed during every 5 min. Vertical bars represent the S.E.M. The number of animals was 5.

effect of GR38032F because of its poor solubility. The maximum dose of GR38032F tested, 4 mg/kg, did not cause emesis. The severity of emesis of the drugs was apparently parallel to the potency of the antiemetic effects. At doses of less than 16 mg/kg, 5-HT₃ receptor antagonists induced no extraordinary behavior in the animal.

Since 5-HT₃ receptor antagonists were injected intravenously when antiemetic effects against cancer chemotherapeutics were studied, it is possible that the preventive effects depend on the route of administration. Therefore, effects of subcutaneously injected 5-HT₃ antagonists were also studied. Emetic effects of cisplatin did not depend on the route of administration (M. Mutoh et al., submitted); The latency and number of vomiting episodes

were virtually the same between intravenous and intraperitoneal injection. Therefore, intraperitoneal injection of cancer chemotherapeutics was employed in the following experiment. Subcutaneous injection of ICS205-930 also inhibited the emesis induced by intraperitoneal administration of 20 mg/kg cisplatin and 200 mg/kg cyclophosphamide with the ID₅₀ value of 25 and 73 µg/kg, respectively.

Table 3 depicts the effects of 5-HT₁ and 5-HT₂ receptor antagonists. Subcutaneous injection of pindolol, mianserin and ketanserin inhibited the emesis caused by intraperitoneal injection of cisplatin with ID₅₀ values of 11.3, 6.9 and 4.0 mg/kg, respectively. However, pindolol induced excitation in the suncus, and spontaneous movement was increased when a dose higher than 16 mg/kg was administered.

Table 1. Antiemetic effects of 5-HT₃ receptor antagonists against several emetic stimuli

Emetic stimuli	Dose (mg/kg)	Route	ID ₅₀ values (µg/kg)			
			ICS205-930	Zacopride	BRL43694	GR38032F
Nicotine	10	s.c.	—	—	—	—
Veratrine	0.5	s.c.	—	—	—	—
Copper sulfate	40	p.o.	—	—	—	—
Motion			—	—	—	—
Cisplatin	40	i.v.	102	117	1000	1000
Cyclophosphamide	200	i.v.	685	NT	NT	NT
Cisplatin	20	i.p.	25	NT	NT	NT
Cyclophosphamide	200	i.p.	73	NT	NT	NT

ID₅₀ values were determined using the up-and-down method. —: no prevention of vomiting at doses up to 64 mg/kg, except for GR38032F which was up to 4 mg/kg. NT: not tested. Motion stimulus: reciprocal shaking on the horizontal plane (frequency 1 Hz, duration 5 min, amplitude 40 mm). Serotonin 5-HT₃ antagonists were injected intravenously except that ICS205-930 was injected subcutaneously against intraperitoneal injection of cisplatin and cyclophosphamide.

Table 2. Emetogenic effects of 5-HT₃ antagonists in suncus

Antagonists	Dose (mg/kg)	No. of suncus vomited/tested	No. of vomiting episodes	Latency (min)
ICS205-930	64	3/3	22.0 ± 12.0	6.6 ± 1.7
Zacopride	64	2/4	19, 22	1, 1
BRL43694	64	3/3	1.7 ± 0.5	21.0 ± 9.0

Values are the mean ± S.E.M., except for zacopride in which actual values are indicated. The drugs were injected subcutaneously. At a dose of 32 mg/kg, no emetic response was observed.

Table 3. Effects of 5-HT₁ and 5-HT₂ receptor antagonist against cisplatin-induced emesis

Antagonists	Dose (mg/kg)	No. of suncus vomited/tested	No. of vomiting episodes	Latency (min)	ID ₅₀ (mg/kg)
Pindolol	32	0/1	—	—	11.3
	16	1/3	1	42	
	8	4/5	9.0 ± 5.0	45.3 ± 8.4	
	4	2/2	2, 8	53, 61	
	2	1/1	7	40	
	1	1/1	8	56	
Mianserin	32	0/1	—	—	6.9
	16	0/3	—	—	
	8	3/6	6.0 ± 5.0	60.3 ± 6.5	
	4	5/6	12.0 ± 8.6	45.2 ± 24.5	
	2	2/2	7, 18	33, 46	
	1	1/1	8	57	
Ketanserin	8	1/5	8	55	4.0
	4	4/4	12.3 ± 6.3	59.0 ± 4.1	
	2	3/4	16.0 ± 9.4	51.6 ± 6.3	
	1	5/5	11.0 ± 4.4	56.0 ± 17.6	

Each antagonist was injected subcutaneously 30 min prior to the intraperitoneal injection of 20 mg/kg cisplatin. Values for the number of vomiting episodes and the latency are the mean ± S.E.M., but actual values are indicated if the number of vomiting animals was less than three.

Mianserin (> 8 mg/kg) and ketanserin (> 4 mg/kg) caused sedative effects.

DISCUSSION

The existence of several subtypes of 5-HT receptors in a variety of tissue is well known, and recent introduction of highly selective 5-HT₃ antagonists made it possible to study the role of 5-HT₃ receptors more precisely (see 10). Serotonin 5-HT₃ receptors were initially thought to exist solely in the peripheral nervous system, but recent studies suggest their existence in central nervous system (11).

In the present study 5-HT₃ receptor antagonists selectively inhibited the emesis induced by cancer chemotherapeutic agents in *Suncus murinus*. The results are consistent with those using other animals, i.e., dog, ferret and cat (2–4, 12). A clinical study also showed the effectiveness of 5-HT₃ antagonists (13). Antagonists for 5-HT₁ and 5-HT₂ receptor blocked the emesis induced by cisplatin (Table 2), but about 40 to 100 times higher dose was

necessary to show the antiemetic effect compared to 5-HT₃ antagonists. Side effects were significant with these high doses. Therefore, it is clear that a 5-HT₃ receptor-mediated mechanism(s) is involved in cancer chemotherapeutics-induced emesis. Although antiemetic effects of 5-HT₃ receptor antagonists were reported from several laboratories, this is the first report to compare several antagonists using the same experimental procedure. Furthermore, our data showed that subcutaneous injection of 5-HT₃ antagonists was as effective as intravenous injection.

High doses of ICS205-930, zacopride and BRL43694 were emetogenic themselves. Sancilio et al. (14) reported that orally administered zacopride induced emesis in ferrets. These data indicate that the 5-HT₃ receptor antagonists currently available are partial agonists.

At present, we do not know the exact mechanism of the antiemetic effects of 5-HT₃ receptor antagonists. The existence of 5-HT₃ receptors in peripheral sensory nervous system

is reported (15). Cisplatin-induced emesis was completely inhibited by abdominal vagotomy, indicating that the drug stimulates vagus afferent (M. Mutoh et al., submitted). The 5-HT₃ antagonists blocked only cisplatin- and cyclophosphamide-induced emesis. These results may indicate that the site of action for 5-HT₃ antagonists is peripheral. However, it is suggested that 5-HT₃ antagonists interact with mesolimbic dopaminergic systems (16). Matsuki et al. (17) showed that SCH23390, a selective D₁-antagonist, and YM-09151-2, a selective D₂-antagonist, inhibited cisplatin-induced emesis but that dopamine antagonists also blocked the emesis induced by copper sulfate or motion. Therefore, interaction with the central dopaminergic nervous system is also plausible. Further experiments are necessary to clarify the emetic effects of cisplatin and the site of action for 5-HT₃ antagonists.

There was a considerable difference between the emetic profiles induced by cisplatin and cyclophosphamide. Cyclophosphamide caused transient emesis, while cisplatin induced long-lasting emesis with long latency. Since ICS205-930 effectively blocked both types of emesis, a common mechanism seems to be exist. Injection of serotonin itself caused instantaneous emesis in the suncus (Y. Torii et al., unpublished observation). Therefore, the different patterns of emesis may reflect a difference in releasing serotonin rather than a different mechanism of emesis.

In conclusion, the present study indicates that 1) a 5-HT₃ receptor-mediated mechanism(s) is involved in the emesis caused by cytotoxic drugs, 2) 5-HT₃ antagonists are very effective as prophylactic drugs and 3) the suncus is a useful experimental animal for the development of antiemetic drugs.

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