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Short Communication

Inhibitory Effect of Zacopride on Cisplatin-Induced Delayed Emesis in Ferrets

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Abstract. We evaluated the antiemetic effect of zacopride, a potent 5-HT_3-receptor antagonist with 5-HT_4-receptor agonist properties, on delayed emesis caused by cisplatin (5 mg/kg, i.p.) in ferrets, compared with granisetron, a selective 5-HT_3-receptor antagonist. Multiple intravenous injections of zacopride at 1 mg/kg, a dose that completely inhibited acute emesis caused by cisplatin (10 mg/kg, i.v.), significantly reduced delayed emesis. Granisetron (3.2 mg/kg) also reduced delayed emesis but this failed to reach statistical significance. The present study suggests that a combined 5-HT_3-receptor antagonist / 5-HT_4-receptor agonist, like zacopride, may be useful against both acute and delayed emesis induced by cancer chemotherapy.

Keywords: 5-HT_4 receptor, 5-HT_3 receptor, delayed emesis

Nausea and vomiting are the most common distressing side effects of cancer chemotherapy (1). Acute emesis, occurring within the first 24 h of chemotherapy, can be markedly reduced by the use of 5-HT_3-receptor antagonists. In contrast, the pathogenesis of delayed emesis, which occurs 24 h or later after chemotherapy, remains poorly understood. A recent study demonstrated that blocking 5-HT_4 receptors, in addition to 5-HT_3 receptors, did not have an additional effect on the control of cisplatin-induced delayed emesis in ferrets (2). Zacopride is a potent 5-HT_3-receptor antagonist with 5-HT_4-receptor agonistic properties (3). Zacopride is highly effective against acute emesis evoked by anticancer drugs in ferrets and dogs (4, 5), but zacopride given orally induces an emetic response in ferrets (6). The present study was designed to evaluate the antiemetic efficacy of zacopride against cisplatin-induced delayed emesis in ferrets, as well as to investigate the role of the agonistic action for the 5-HT_4 receptors in this model, compared with granisetron, a selective 5-HT_3-receptor antagonist.

Adult male ferrets weighing 1.0 – 2.3 kg (Marshall Farms, North Rose, NY, USA) were used. Animals were individually housed in an animal room at 23 ± 1°C with lights on between 07:00 and 19:00 and routinely fed dry pellets (Ferret Diet; PMI Feeds, St. Louis, MO, USA), with free access to water. In all experiments, animals were removed from their home cages and transferred to observation cages in a quiet room. All animal experimental procedures were performed under the guidelines of the Animal Experiment Committee of Fujisawa Pharmaceutical Co., Ltd.

In the acute emesis experiment, animals were observed continuously for 4 h following administration of cisplatin (10 mg/kg, i.v.), and the incidence of emetic responses, consisting of retches and vomits, was counted. Zacopride (1 mg/kg) or vehicle (1 ml/kg) was intravenously administered at 8 h before the injection of cisplatin. Zacopride at 1 mg/kg, a dose considered to be in the high therapeutic range for controlling emesis, was selected on the basis of previous studies (4, 7).

In the experiment of delayed emesis, ferrets were intraperitoneally injected with cisplatin (5 mg/kg) at 08:30. Animal behavior was recorded using a video

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camera with an automatic night photograph system for 72 h and analyzed at the end of the experiment. Zacopride (1 mg/kg) or vehicle (1 ml/kg) was intravenously administered at 36, 48, and 60 h; and granisetron (3.2 mg/kg) or vehicle was administered at 32, 40, 48, 56, and 64 h after cisplatin-treatment. Granisetron at 3.2 mg/kg was selected on the basis of a previous study (2). A 12-h artificial light cycle (lights on between 07:00 to 19:00) was used throughout the study. Ferrets were given food (70 g/day) and water ad libitum for 3 days.

Cisplatin (Sigma-Aldrich, Inc., St. Louis, MO, USA) was prepared in normal saline at 70°C followed by gradual cooling to 40°C and administered immediately

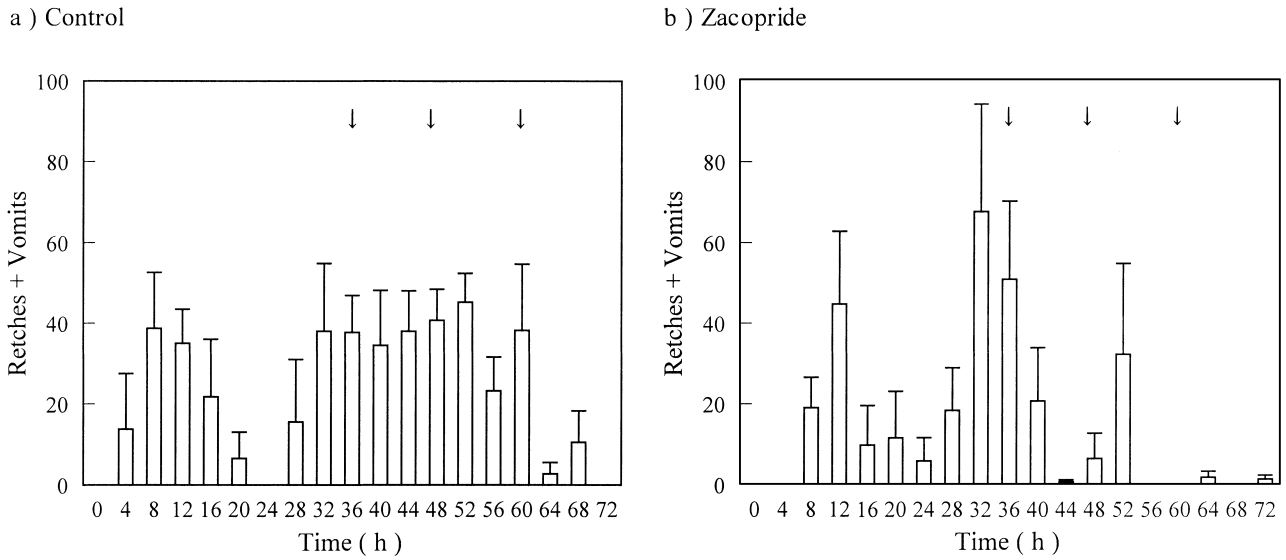

Fig. 1. Inhibitory effect of zacopride on cisplatin-induced emesis in ferrets. Ferrets received cisplatin (5 mg/kg, i.p.), followed by the intravenous injection of a) 1 ml/kg vehicle (Control) or b) 1 mg/kg zacopride. Administration point of vehicle or drug is indicated by the arrow. Results represent the mean ± S.E.M. of the total numbers of retches + vomits occurring at 4-h time intervals after cisplatin injection at 0 h.

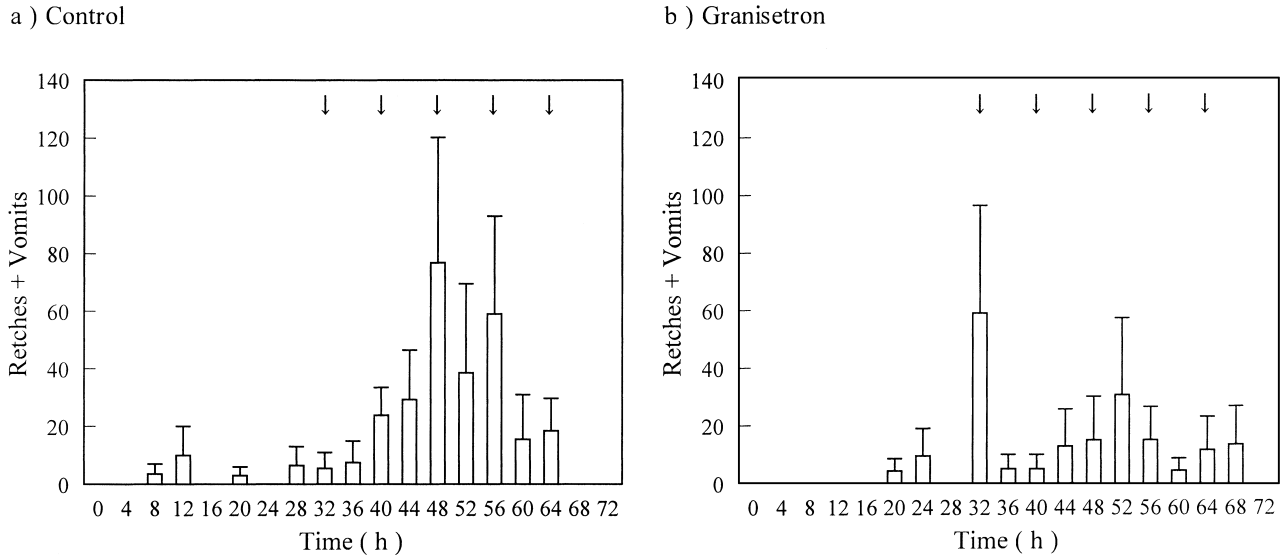

Fig. 2. Inhibitory effect of granisetron on cisplatin-induced emesis in ferrets. Ferrets received cisplatin (5 mg/kg, i.p.), followed by the intravenous injection of a) 1 ml/kg vehicle (Control) or b) 3.2 mg/kg granisetron. Administration point of vehicle or drug is indicated by the arrow. Results represent the mean ± S.E.M. of the total numbers of retches + vomits occurring at 4-h time intervals after cisplatin injection at 0 h.

Table 1. Effects of zacopride and granisetron on cisplatin-induced delayed emesis in ferrets

Treatment[a]	Animals (vomits/total)	Retches + Vomits		
		Time after cisplatin (h)		
		0 – 24	24 – 36	36 – 72
Control	4/4	116 ± 32	91 ± 34	233 ± 39
Zacopride	5/5	91 ± 9	137 ± 52	62 ± 33**
		0 – 24	24 – 32	32 – 72
Control	4/4	13 ± 10	12 ± 7	267 ± 92
Granisetron	4/4	17 ± 12	59 ± 37	109 ± 70

[a]Zacopride (1 mg/kg) was intravenously administered at 36, 48, and 60 h after the injection of cisplatin. Granisetron (3.2 mg/kg) was intravenously administered at 32, 40, 48, 56, and 64 h after the injection of cisplatin. **Compared with the control, $P<0.01$.

in a volume of 5 ml/kg for ferrets. Zacopride and granisetron were synthesized in the Medicinal Chemistry Laboratories of Fujisawa Pharmaceutical Co., Ltd. (Osaka), and they were freshly dissolved in 5% glucose solution. Group results are expressed as the mean ± S.E.M. Student's *t*-test was used as a measure of significance. Values of $P<0.05$ were regarded as statistically significant.

Cisplatin (10 mg/kg, i.v.) induced emesis within 82 ± 3 min and there were 183 ± 58 retches ± vomits in three animals. Pretreatment with zacopride (1 mg/kg, i.v.) 8 h before the injection of cisplatin completely antagonized cisplatin-induced acute emesis in three animals. Because zacopride (1 mg/kg) and granisetron (3.2 mg/kg) significantly prevented cisplatin-induced acute emesis in ferrets in the present and previous study (2), respectively, we examined whether zacopride and granisetron could inhibit delayed emesis caused by cisplatin. The pattern of emesis induced by cisplatin at 5 mg/kg in ferrets is shown in Figs. 1a and 2a. Until 32 or 36 h after the injection of cisplatin, there was no difference in the mean number of retches and vomits during 0 – 24 h (acute phase) and 24 – 32 h or 24 – 36 h (delayed phase) in the drug groups compared with their controls (Figs. 1 and 2, Table 1). As shown in Fig. 1 and Table 1, zacopride (1 mg/kg, i.v.) at 36, 48, and 60 h significantly reduced delayed emesis (36 – 72 h) by 74%, compared with the vehicle-treated control. In addition, granisetron (3.2 mg/kg) at 32, 40, 48, 56, and 64 h inhibited, but not significantly, delayed emesis by 59%, compared with the control during 32 – 72 h (Fig. 2 and Table 1).

In the present study, we first examined the effect of zacopride on cisplatin (10 mg/kg, i.v.)-induced acute emesis in ferrets, in order to determine appropriate intervals of treatment for antiemetics in the experiments on delayed emesis by cisplatin (5 mg/kg, i.p.). Since zacopride (1 mg/kg, i.v.) administered 8 h prior to cisplatin completely inhibited acute emesis in ferrets, this suggests that the duration of action for zacopride is at least 12 h. A previous study showed that the duration of action for granisetron at 3.2 mg/kg was at least 8 h (2). We also confirmed the previously reported finding that zacopride (1 mg/kg, i.v.) apparently abolished the emetic response in ferrets (4, 7).

Cisplatin (5 mg/kg, i.p.)-induced delayed emesis in ferrets is often used because the profiles of vomiting are similar to that of the delayed emesis observed in humans (8). Although a previous study showed that this delayed emesis in ferrets was significantly prevented by selective 5-HT_3-receptor antagonists, the amelioration of vomiting was incomplete (9), suggesting that there is still room for therapeutical improvement. In the present study, multiple intravenous injections of zacopride (1 mg/kg) significantly reduced cisplatin-induced delayed phase (36 – 72 h) emesis by 74%, compared with the control. Multiple injections of granisetron (3.2 mg/kg) also inhibited the delayed phase but this failed to reach statistical significance. Nakayama et al. (2) reported that the antiemetic efficacy of FK1052, a 5-HT_3- and 5-HT_4-receptor antagonist, against cisplatin-induced delayed emesis in ferrets was similar to that of granisetron. These results suggest that the 5-HT_3-receptor antagonist with 5-HT_4-receptor agonistic activity may be more effective than 5-HT3-receptor antagonist alone against cisplatin-induced delayed emesis in ferrets. However, the statistical comparison was performed in a different condition: five animals for zacopride but only four for granisetron. We cannot deny the possibility that the data simply indicate that statistical significance for granisetron was not detected with smaller sample size.

A previous study showed that the antiemetic activity of BIMU 1, a compound with mixed 5-HT_4-receptor

agonist / 5-HT_3-receptor antagonist action like zacopride, on cisplatin-induced delayed emesis in piglets was the same as granisetron (10). Although the involvement of 5-HT_4 receptors in the induction of delayed emesis caused by cisplatin in piglets and ferrets is unclear, it is suggested that agonistic action of 5-HT_4 receptors failed to influence the control of cisplatin-induced delayed emesis by the 5-HT_3-receptor antagonists. In dogs, methotrexate-induced delayed emesis was significantly inhibited by FK1052, a 5-HT_3- and 5-HT_4-receptor antagonist, but not ondansetron, a selective 5-HT_3-receptor antagonist (11). As the involvement of 5-HT_4 receptors in the induction of emesis in piglets and dogs is controversial, further studies are required to elucidate the role of the 5-HT_4 receptor in the two models.

The present study suggests that a combined 5-HT_3-receptor antagonist / 5-HT_4-receptor agonist, like zacopride, may be useful against both acute and delayed emesis induced by cancer chemotherapy.

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