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The broad-spectrum anti-emetic activity of AS-8112, a novel dopamine D_2, D_3 and 5-HT_3 receptors antagonist

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1 The anti-emetic and pharmacological profile of AS-8112 ((*R*)-5-bromo-*N*-(1-ethyl-4-methylhexahydro-1*H*-1,4-diazepin-6-yl)-2-methoxy-6-methylamino-3-pyridinecarboxamide·2 fumarate), a novel and potent dopamine D_2, D_3 and 5-hydroxytryptamine-3 (5-HT_3) receptors ligand, was investigated in the present study.

2 In guinea-pig isolated colon, AS-8112 produced a rightward shift of the concentration-response curves of 2-methyl-5HT, a 5-HT_3 receptor agonist (pA_2 value of 7.04). Other 5-HT_3 receptor antagonists also produced such a shift in the following antagonistic-potency order: granisetron > ondansetron = AS-8112 > > metoclopramide.

3 In mice, AS-8112 (1.0–3.0 mg kg^{-1} s.c.) potently inhibited hypothermia induced by the dopamine D_3 receptor agonist; *R*(+)-7-OH-DPAT (*R*(+)-7-hydroxy-2-(*N*,*N*-di-*n*-propylamino)tetraline) (0.3 mg kg^{-1} s.c.). Domperidone and haloperidol, which have affinity for dopamine D_3 receptor, also inhibited *R*(+)-7-OH-DPAT-induced hypothermia.

4 In ferrets or dogs, AS-8112 dose-dependently inhibited emesis induced by *R*(+)-7-OH-DPAT, apomorphine, morphine or cisplatin with ID_{50} values of 2.22 μg kg^{-1} s.c., 10.5 μg kg^{-1} s.c., 14.2 μg kg^{-1} i.v. and 17.6 μg kg^{-1} i.v., respectively. Moreover, oral administration of AS-8112 significantly inhibited emesis induced by these emetogens. AS-8112 (0.3 mg kg^{-1} i.v.) significantly inhibited emesis induced by cyclophosphamide and doxorubicin.

5 In conclusion, AS-8112 is a potent dopamine D_2, D_3 and 5-HT_3 receptors antagonist, and a novel anti-emetic agent with a broad-spectrum of anti-emetic activity. These results suggest that this compound is worthy of clinical investigation.

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Keywords: AS-8112; dopamine D_2 receptor; dopamine D_3 receptor; 5-HT_3 receptor; antiemetic

Abbreviations: AS-8112, (*R*)-5-bromo-*N*-(1-ethyl-4-methylhexahydro-1*H*-1,4-diazepin-6-yl)-2-methoxy-6-methylamino-3-pyridinecarboxamide·2 fumarate; 5-HT, 5-hydroxytryptamine; *R*(+)-7-OH-DPAT, *R*(+)-7-hydroxy-2-(*N*,*N*-di-*n*-propylamino)tetraline

Introduction

Nausea and vomiting are distressing symptoms associated with a variety of conditions such as motion sickness, pain following surgery, release of endogenous pro-emetic agents, disturbance of the vestibular system and a number of gastrointestinal disorders. Moreover, cancer chemotherapy elicits an immediate emetic response on the day of therapy (acute emesis) and protracts vomiting for up to 5 days thereafter (delayed emesis). Emesis following chemo- or radio-therapy of cancer may be particularly severe, greatly reducing the quality of life and affecting compliance with such therapy in some patients.

The discovery of the 5-HT_3 receptor antagonists has dramatically improved the treatment of emesis induced by anti-cancer therapy (Aapro, 1991; Morrow *et al*., 1995). Their major beneficial effects were observed in the acute phase of chemotherapy-induced emesis in animal models (Bermudez *et al*., 1988; Higgins *et al*., 1989; Rudd & Naylor, 1996) and in humans (Morrow *et al*., 1995). However, 5-HT_3 receptor antagonists such as ondansetron and granisetron failed to control the delayed nausea and vomiting associated with chemotherapy (De Mulder *et al*., 1990; Butcher, 1993). In addition, as 5-HT_3 receptor antagonists do not block all components of the emesis induced by cytotoxic drugs or by centrally acting emetic stimuli; e.g. motion, loperamide, morphine or apomorphine, transmitters other than 5-HT are believed to be implicated.

It is well known that dopamine D_2 receptors in the area postrema play an important role in the regulation of emetic responses in ferrets, dogs and humans (Andrews *et al*., 1990; Harding *et al*., 1987). Recently, we reported that not only dopamine D_2 receptor but also D_3 receptor in the area postrema plays an important role in the regulation of emesis, as dopamine D_3 receptor agonist, *R*(+)-7-OH-DPAT elicited nausea and vomiting in ferrets and dogs (Yoshida *et al*., 1995; Yoshikawa *et al*., 1996). From a clinical point of view, dopamine receptor antagonists such as phenothiazines, butyrophenones and benzamides, which has affinity for dopamine D_2 and D_3 receptors, are used as antiemetic agents. They are very useful against emesis associated with administration of anti-parkinsonian drugs, loperamide,

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morphine and apomorphine. However, these dopamine receptor antagonists have little or weak effects on emesis induced by anti-cancer therapy and postoperative nausea.

This evidence suggests that dual dopamine D_2 and 5-HT_3 receptor antagonists may be more useful as anti-emetics with a broad-spectrum of activity than classical anti-emetics which act on each receptor separately. Recently, clinical and experimental studies demonstrated that the combination of a dopamine D_2 and a 5-HT_3 receptor antagonist was more effective against postoperative nausea and vomiting than the monotherapy (McKenzie *et al*., 1996; Wynn *et al*., 1993; Wu *et al*., 2000). Therefore, there is a clear need for the development of dual dopamine D_2 and 5-HT_3 receptor antagonists which exert a broad-spectrum of anti-emetic activity. We have previously reported that AS-8112, a novel pyridinecarboxamide compound (Figure 1), has high and selective affinity for dopamine D_2, D_3 and 5-HT_3 receptors in radioligand binding study (Yoshikawa *et al*., 1998). In the present study, we investigated the anti-emetic profile of AS-8112 and compared it to those of existing 5-HT_3, or dopamine D_2 and D_3 antagonists. To evaluate the anti-emetic activities mediated *via* dopamine D_2, D_3 or 5-HT_3 receptors, the emetic models induced by apomorphine in dogs, *R*(+)-7-OH-DPAT in ferrets or cancer-chemotherapeutic agents (cisplatin, cyclophosphamide or doxorubicin) in ferrets were used. Moreover, morphine-induced emesis model in dogs was used to evaluate the anti-emetic activity against postoperative nausea, because morphine is a well known emetogenic agent in man in the postoperative setting (Palazzo & Strunin, 1984).

Methods

Animals

Male beagle dogs (Nihon Nohsan Kohgyo Inc., Yokohama, Japan) weighing 9–14 kg, male Marshall ferrets (Nihon Charles River Inc., Yokohama, Japan) weighing 1.0–1.5 kg, male 7 week-old mice of the ddY strain (Nihon SLC Inc., Shizuoka, Japan) and male guinea-pigs of the Hartley strain (Nihon SLC Inc., Shizuoka, Japan) weighing 200–500 g were used.

All animals were housed in a room kept at 22–25°C under a 12-h light/dark cycle with free access to water. Dogs were individually housed in experimental cages and given dog food (20 g dry weight kg^{-1} of body weight, Oriental Yeast, Tokyo, Japan) at 1500 h. daily. Ferrets were given a standard cat diet (70–80 g $animal^{-1}$, Purina®). Mice and guinea-pigs had free access to food.

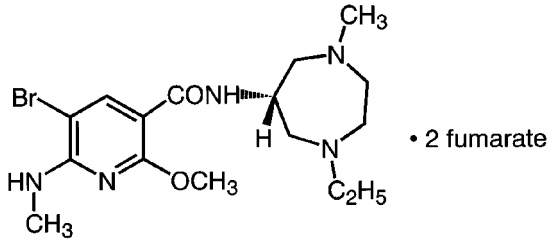

Figure 1 Structural formula of AS-8112.

The ferrets and dogs were fasted overnight prior to all experiments.

Drugs and solutions

The drugs used in the experiments were; AS-8112, domperidone, ondansetron hydrochloride, granisetron hydrochloride, *R*(+)-7-OH-DPAT hydrobromide and 2-methyl-5-HT (2Me5HT) (synthesized at Dainippon Pharmaceutical Co.), metoclopramide hydrochloride, apomorphine hydrochloride and cisplatin (Sigma Chemical Co., USA), haloperidol (Wako Pure Chemicals Industries, Ltd., Japan), morphine hydrochloride (Takeda Chemical Industries, Ltd., Japan), cyclophosphamide (Shionogi & Co., Japan), doxorubicin hydrochloride (Kyowa Hakko Kogyo Co., Japan). The enantiomeric purities of AS-8112 and *R*(+)-7-OH-DPAT were determined to be >99% enantiomeric excess on the basis of high-performance liquid chromatograms.

For *in vivo* experiments, AS-8112, metoclopramide, ondansetron and granisetron were dissolved in saline, and haloperidol and domperidone were first dissolved in 1% lactic acid and their doses were calculated as the salts. All drugs were diluted with saline. The solvent final concentration did not exceed 0.1% lactic acid, a concentration that did not have any effect on all emetogens-induced emetic responses. *R*(+)-7-OH-DPAT, morphine, cisplatin and doxorubicin were freshly prepared as solution in saline and apomorphine and cyclophosphamide were freshly prepared as solution in water for injection. For the experiments conducted to determine the effects of AS-8112 given orally, AS-8112 was suspended in 0.5% tragacanth solution for ferrets or put into gelatin capsules (#0, Matuya Co. Ltd., Japan) for dogs.

For contractile experiments in guinea-pigs, the drugs were dissolved in saline (pH 3.5–5.5), and then diluted with saline.

2-methyl-5-HT-induced contractions in isolated guinea-pig colon

Guinea-pigs were killed by a blow on the head. The distal portion of the colon was removed, cleaned in fresh Krebs-Henseleit solution (pH 7.3–7.5) at room temperature and divided into approximately 20 mm segments. The ionic composition of the Krebs-Henseleit solution (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25, $MgSO_4$ 1.2 and glucose 10. The preparations were suspended longitudinally under an initial tension of approximately 1 g in Krebs-Henseleit solution at 37°C saturated with 95% O_2 and 5% CO_2, and allowed to equilibrate for over 60 min. Submaximal contractions were first elicited by repeated application of 10^{-5} M 2-methyl-5-HT (2Me5HT) until constant responses were obtained. The cumulative concentration-response curves of 2Me5HT were then constructed by increasing the bath concentration of 2Me5HT approximately 3 fold (Van Rossum, 1963). Antagonists of the 5-HT_3 receptors (AS-8112, metoclopramide, ondansetron or granisetron) were added to the bath after the concentration-response curves of 2Me5HT had been obtained. Tissue was exposed to each antagonist for 30 min before rechallenge with 2Me5HT (control). Under these conditions, the cumulative concentration curves of 2Me5HT could be constructed three times using the same preparation without significantly changing E_{max} and EC_{50} values (data not shown).

R(+)-7-OH-DPAT-induced hypothermia in mice

Mice were habituated to the insertion of a rectal probe (Takara Thermistor Co. Ltd., Yokohama, Japan) before the experiment was started. Body temperature was recorded with the rectal probe (inserted 1.5 cm) while the mouse was loosely restrained by the tail. For the measurement of antagonistic activity, mice subcutaneously received saline or test drugs (AS-8112, domperidone, metoclopramide, haloperidol or granisetron) followed, 30 min later, by R(+)-7-OH-DPAT (1.0 mg kg^{-1} s.c.). Rectal temperature was recorded before administration of all agents including *R*(+)-7-OH-DPAT and 30 min after administration of *R*(+)-7-OH-DPAT. Changes in rectal temperature were evaluated before and after administration of *R*(+)-7-OH-DPAT.

Emesis in ferrets and dogs

Ferrets were used to investigate the anti-emetic effects of AS-8112 against emesis induced by *R*(+)-7-OH-DPAT, cisplatin, cyclophosphamide and doxorubicin, and dogs were used in apomorphine- or morphine-induced emetic responses. Although ferrets and dogs exhibit emetic effects induced by all emetogens described above, an emetic response to apomorphine or morphine in dogs is more sensitive than that in ferrets (King, 1990). Each animal received either test drugs (AS-8112, domperidone, metoclopramide, haloperidol or ondansetron) or saline subcutaneously 30 min before administration of emetogens (*R*(+)-7-OH-DPAT and apomorphine) or intravenously 15 min before morphine injections. In the case of cisplatin-induced emetic responses, each ferret simultaneously received test drugs (i.v.) (AS-8112, domperidone, metoclopramide, haloperidol, ondansetron or granisetron) and emetogens. In the cases of cyclophosphamide- and doxorubicin-induced emetic responses, AS-8112 (i.v.) and emetogens were simultaneously administered. To evaluate the activity of AS-8112 given orally, each animal received AS-8112 or vehicle 60 min before administration of emetogens (*R*(+)-7-OH-DPAT, apomorphine and morphine) or 30 min before cisplatin administration. The latency to first retch and vomit and the number of vomits were recorded for each animal. Vomiting was scored as oral expulsion of liquid or solid stomach contents. The doses of emetogens, routes of administration and observation periods are shown in Table 1. When animals did not show an emetic response to emetogens, the latency was determined by the observation periods.

Statistical analysis

Significant differences were evaluated using non-parametric Dunnett's multiple comparison test or the Wilcoxon rank sum test. The significance level was set at $P<0.05$. The ID_{50} values of test drugs (dose causing 50% inhibition of the number of emetic episodes elicited by various emetogens) were determined by the method of logit analysis. The pA_2 value and slope were calculated by the method of Arunlakshana & Schild (1959).

Result

2-methyl-5-HT-induced contractions in isolated guinea-pig colon

5-HT_3 receptor-blocking potency of the antagonists studied was evaluated in the guinea-pig colon using 2-methyl-5-HT (2Me5HT) as a 5-HT_3 receptor agonist. 2Me5HT (10^{-6}–10^{-4} M) caused concentration-dependent contractions, with an EC_{50} value of $(6.94 \pm 0.30) \times 10^{-6}$ M. AS-8112 (10^{-7}–10^{-6} M), ondansetron (10^{-7}–10^{-6} M), granisetron (10^{-8}–10^{-7} M) and metoclopramide (3×10^{-6}–3×10^{-5} M) produced parallel and concentration-dependent shifts to the right of the 2Me5HT-concentration-response curves (Figure 2). Based on the pA_2 values, the potency of AS-8112 was less than that of granisetron, equal to that of ondansetron, and more potent than that of metoclopramide (Table 2). Schild regression analysis of the data yielded a Schild plot for AS-8112 with a slope of 1.24 (0.745–1.73), which was not significantly different from 1.

R(+)-7-OH-DPAT-induced hypothermia in mice

Dopamine D_3 receptor-blocking potency of the antagonists studied was evaluated in the *R*(+)-7-OH-DPAT-induced hypothermia in mice. A selective dopamine D_3 receptor agonist, *R*(+)-7-OH-DPAT (0.1, 0.3, 1.0 mg kg^{-1} s.c.), dose-dependently induced hypothermia in mice (data not shown). Figure 3 shows that AS-8112 (1–10 mg kg^{-1} s.c.) dose-dependently reduced the hypothermia evoked by *R*(+)-7-OH-DPAT. This inhibitory effect of AS-8112 was as potent as that of domperidone or haloperidol and more potent than that of metoclopramide. Granisetron, even at a high dose of 10 mg kg^{-1} s.c., had no effect on *R*(+)-7-OH-DPAT-induced hypothermia in mice. All compounds used in this study did

Table 1 Range of emetogens administered to dogs or ferrets

Emetogen	*Dose*	*Dose volume*	*Route*	*Observation period* (h)
Dogs				
Apomorphine	0.3 mg kg^{-1}	0.3 ml kg^{-1}	s.c.	0.5
Morphine	1 mg kg^{-1}	0.5 ml kg^{-1}	s.c.	0.5
Ferrets				
R(+)-7-OH-DPAT	0.3 mg kg^{-1}	2.0 ml kg^{-1}	s.c.	0.5
Cisplatin	10 mg kg^{-1}	3.0 ml kg^{-1}	i.v.	4
Cyclophosphamide	150 mg kg^{-1}	7.5 ml kg^{-1}	i.p.	4
Doxsorubicin	15 mg kg^{-1}	3.0 ml kg^{-1}	i.p.	4

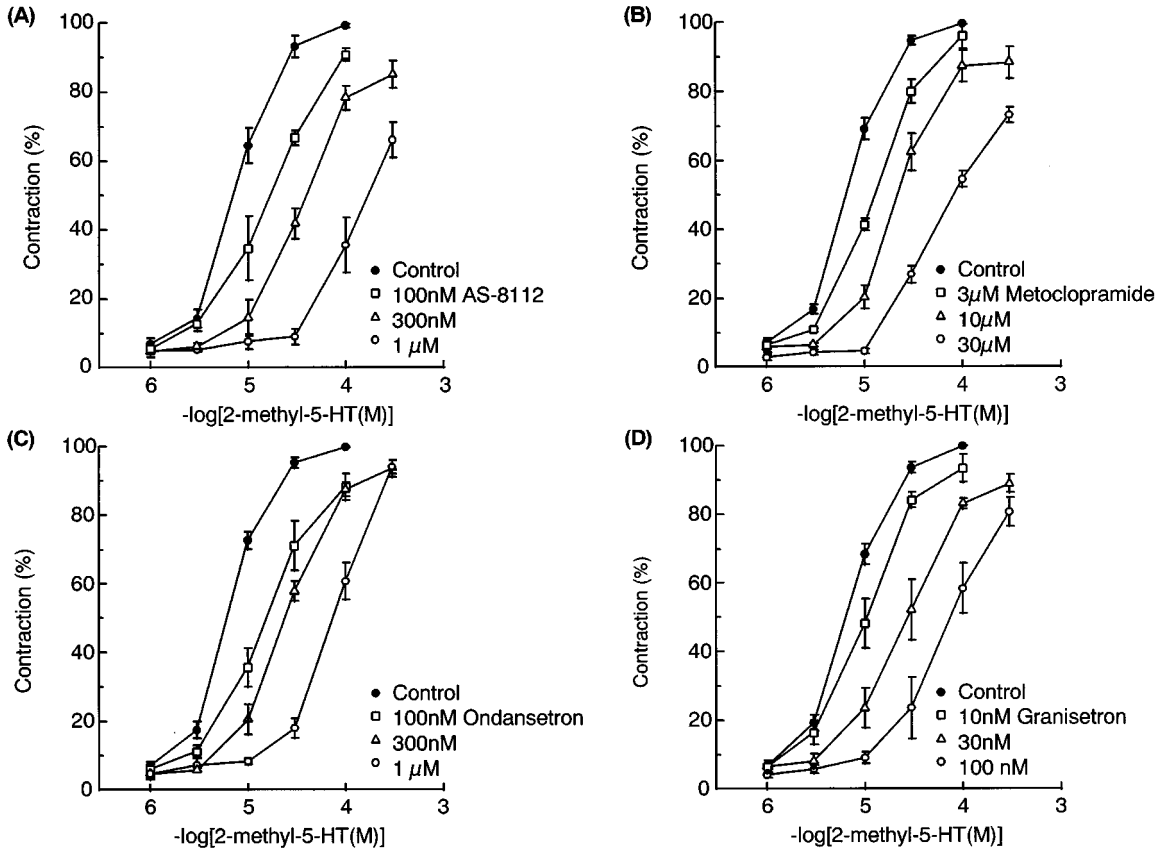

Figure 2 Antagonism by AS-8112 (A), metoclopramide (B), ondansetron (C) and granisetron (D) of contractile effects of 2-methyl-5-HT in the isolated guinea-pig colon. The results are the mean ± s.e.mean of four experiments.

Table 2 pA_2 values and Schild slopes of AS-8112 and various compounds at 5-HT_3 receptors in guinea-pig colon

Compounds	*N*	*pA_2 value*	*Slope*
AS-8112	4	7.04 ± 0.12	1.24 (0.745 – 1.73)
Metoclopramide	4	5.46 ± 0.05	1.11 (0.895 – 1.33)
Ondansetron	4	7.10 ± 0.06	0.939 (0.772 – 1.11)
Granisetron	4	7.88 ± 0.05	1.16 (0.926 – 1.38)

Data are the mean ± s.e.mean (pA_2) and the 95% C.L. slope of Schild plot. *N* shows the number of experiments.

not modify the rectal temperature of mice when used alone (data not shown).

Anti-emetic effects of AS-8112

The anti-emetic effects of AS-8112, administered s.c. or i.v. were compared to those of typical dopamine D_2 or 5-HT_3 receptor antagonists. The effects of oral treatment with AS-8112 were also examined. All emetogens administered to the ferrets or to the dogs in this study evoked marked emetic responses (Tables 3 and 4, Figure 4). The latency to the first emetic response and the number of emetic episodes observed after administration of each emetogen in combination with saline (vehicle control) are shown in each table (Tables 3 and 4) and Figure 4.

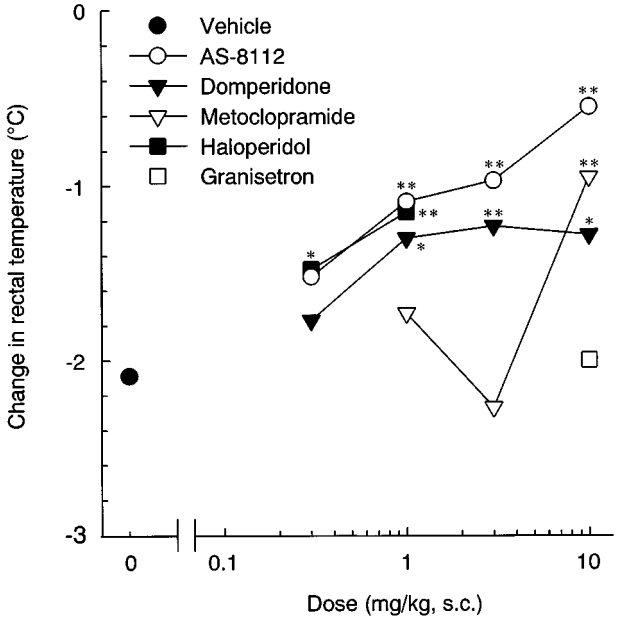

Figure 3 Influence of AS-8112 or various compounds upon the change in rectal temperature by *R*(+)-7-H-DPAT (1 mg kg^{-1} s.c., 30 min). Saline or test compound was administered s.c. 30 min before treatment with *R*(+)-7-OH-DPAT (1 mg kg^{-1} s.c.). Statistically significant difference from the saline treated group is indicated by $*P<0.05$, $**P<0.01$, Dunnett's multiple comparison test. Data are the means, $n=5-14$. s.e.'s did not exceed ±0.29°C of mean values.

Table 3 Effects of AS-8112 on emesis induced by *R*(+)-7-OH-DPAT in ferrets and apomorphine in dogs

Emetogens	*AS-8112* *mg kg^{-1} s.c.*	*Latency* (min)	*No. of emetic episodes*	*No. of animal vomiting/tested*
R(+)-7-OH-DPAT	Saline	5.00±0.61	5.50±0.45	12/12
	0.001	4.72±0.95	3.83±1.14	5/6
	0.003	10.85±3.92	2.00±0.52	5/6
	0.01	12.43±3.66	1.50±0.34*	5/6
	0.03	20.84±4.52*	0.83±0.48**	3/6
	0.1	30.00±0.00***	0.0±0.0***	0/6
Apomorphine	Saline	3.22±0.20	7.83±0.60	12/12
	0.003	3.65±0.43	8.00±0.71	5/5
	0.01	5.81±0.32	3.80±0.97	5/5
	0.03	14.27±3.96**	1.20±0.37**	4/5
	0.1	30.00±0.0***	0.00±0.00	0/5

Each value represents the mean±s.e.mean. Saline or AS-8112 was injected 30 min before *R*(+)-7-OH-DPAT (0.3 mg kg^{-1} s.c.) or apomorphine (0.3 mg kg^{-1} s.c.) administration. Animals were observed for 30 min after *R*(+)-7-OH-DPAT or apomorphine treatment. Statistically significant difference from the saline treated group is indicated by *$P<0.05$, **$P<0.01$, ***$P<0.001$, non-parametric Dunnett's multiple comparison test.

Table 4 Effects of AS-8112 on cancer chemotherapeutic agents-induced emesis in ferrets

Emetogens	*AS-8112* *mg kg^{-1} i.v.*	*Latency* (min)	*No. of emetic episodes*	*No. of animal vomiting/tested*
Cisplatin	Saline	71.5±1.5	11.92±0.62	12/12
	0.01	84.0±7.1	9.80±2.48	5/5
	0.03	159.9±32.7*	2.20±0.92**	3/5
	0.1	188.8±31.5**	1.20±0.80**	2/5
	0.3	240.0±0.0***	0.00±0.00***	0/5
Cyclophosphamide	Saline	25.0±2.4	10.50±0.99	6/6
	0.3	236.6±3.4**	0.20±0.20**	1/5
Doxorubicin	Saline	142.5±8.0	9.17±1.25	6/6
	0.3	240±0**	0±0**	0/5

Each value represents the mean±s.e.mean. AS-8112 and cisplatin (10 mg kg^{-1} i.v.), cyclophosphamide (150 mg kg^{-1} i.p.) or doxorubicin (15 mg kg^{-1} i.p.) were injected simultaneously. Animals were observed for 4 h after administration of emetogens. Statistically significant difference from the saline treated group is indicated by *$P<0.05$, **$P<0.01$, ***$P<0.001$, non-parametric Dunnett's multiple comparison test (cisplatin) and the Wilcoxon rank sum test (cyclophosphamide and doxorubicin).

Emesis induced by R(+)-7-OH-DPAT in ferrets and by apomorphine in dogs

For emesis induced by *R*(+)-7-OH-DPAT in ferrets and by apomorphine in dogs, AS-8112 dose-relatedly prolonged the latency and inhibited the number of emetic episodes (Table 3) with ID_{50} values of 2.22 (1.05–4.69) μg kg^{-1} s.c. and 10.5 (7.87–13.9) μg kg^{-1} s.c., respectively (Table 5). According to the ID_{50} values of other dopamine D_2 or 5-HT_3 receptor antagonists examined, the effect of AS-8112 on *R*(+)-7-OH-DPAT-induced emesis was almost equal to that of domperidone, about 5-times or 80-times more potent than that of haloperidol or metoclopramide. The potency of AS-8112 against apomorphine-induced emesis was about half that of domperidone, 2-times or 12-times more than that of haloperidol or metoclopramide. In contrast, ondansetron at 1 mg kg^{-1} s.c. failed to inhibit the emetic response induced by *R*(+)-7-OH-DPAT and apomorphine.

Emesis induced by cancer-chemotherapeutic agents in ferrets

AS-8112 dose-relatedly prolonged the latency to the first emetic response and reduced the number of emetic episodes induced by cisplatin in ferrets with an ID_{50} value of 17.6 (11.7–26.4) μg kg^{-1} i.v. (Tables 4 and 5). AS-8112 at 0.3 mg kg^{-1} i.v. completely inhibited cisplatin-induced emesis for 4 h. This antiemetic effect of AS-8112 was as potent as that of ondansetron or granisetron, and about 30-times and 50-times more potent than that of metoclopramide or haloperidol (Table 5). Domperidone (1 mg kg^{-1} i.v.), however, did not significantly reduce the emetic episodes induced by cisplatin in ferrets.

For the emesis induced by other cancer-chemotherapeutic agents; cyclophosphamide and doxorubicin, AS-8112 (0.3 mg kg^{-1} i.v.) significantly reduced the number of emetic episodes and prolonged the latency (Table 4).

Morphine-induced emesis

AS-8112 dose-relatedly prolonged the latency to the first emetic response and reduced the number of emetic episodes induced by morphine in dogs with an ID_{50} value of 14.2 (6.59–30.4) μg kg^{-1} i.v. (Figure 4, Table 5). This antiemetic effect of AS-8112 was as potent as that of haloperidol and about 20-times more potent than that of metoclopramide. However, even at a high dose of 1 mg kg^{-1}, i.v., ondansetron and domperidone did not cause 50% inhibition in the number of emetic episodes.

The effect of oral administration of AS-8112 in dogs and ferrets

Table 6 shows the effects of AS-8112, administered orally, on emesis induced by various emetogens in dogs and ferrets.

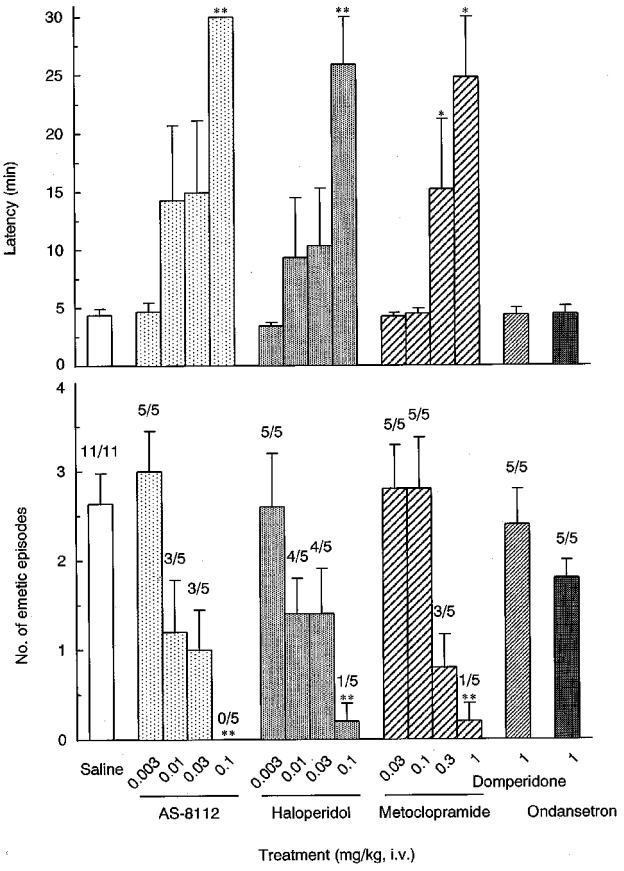

Figure 4 Effects of AS-8112, haloperidol, metoclopramide, domperidone and ondansetron against emesis induced by morphine in dogs. Each column represents the means ± s.e.mean, $n = 5-11$. Saline or test compound was administered 15 min before morphine (1 mg kg^{-1} s.c.) intravenously. Animals were observed for 30 min after morphine administration. The fractions represent the number of animals that showed emetic episodes over the number of animals tested in that group. Statistically significant difference from the saline treated group is indicated by $^{*}P<0.05$, $^{**}P<0.01$, Dunnett's multiple comparison test.

AS-8112 dose-dependently inhibited the emetic effects of *R*(+)-7-OH-DPAT and morphine with ID_{50} values of 55.8 (36.0–86.4) and 136 (57.8–318) μg kg^{-1} p.o., respectively. At a dose of 1 mg kg^{-1} p.o., AS-8112 completely inhibited the emetic responses induced by these two emetogens. Moreover, cisplatin-induced emesis was completely inhibited by AS-8112 (0.3 mg kg^{-1} p.o.) with an ID_{50} value of 27.1 (15.3–48.0) μg kg^{-1} p.o. AS-8112 also dose-relatedly inhibited emesis induced by apomorphine with an ID_{50} value of 117 (64.1–214) μg kg^{-1} p.o.

Discussion

This study demonstrates that a novel pyridinecarboxamide compound, AS-8112 acted as a potent antagonist for dopamine D_2, D_3 and 5-HT_3 receptors. We have previously reported that AS-8112 had high affinity for dopamine D_2, D_3 and 5-HT_3 receptors in radioligand binding assay (Yoshikawa *et al*., 1998). AS-8112 also showed low affinity for other serotonergic and dopaminergic receptor subtypes. Moreover, AS-8112 had negligible affinity for other neurotransmitter recognition sites tested. To confirm whether AS-8112 acted as an antagonist for 5-HT_3 receptors, we investigated the antagonism of AS-8112 on contractile responses in guinea-pig colon induced by 2-methyl-5-HT (2Me5HT), a 5-HT_3 receptor agonist. It has been demonstrated that the

Table 6 Antiemetic profile of oral administration of AS-8112 (ID_{50} values; μg kg^{-1} p.o.) in dogs and ferrets

Emetogens	*ID_{50} values; μg kg^{-1} p.o.*
Dogs	
Apomorphine	117 (64.1–214)
Morphine	136 (57.8–318)
Ferrets	
R(+)-7-OH-DPAT	55.8 (36.0–86.4)
Cisplatin	27.1 (15.3–48.0)

Each value represents the mean (95% c.l.), $n = 5-12$. AS-8112 was administered p.o. 60 min before treatment with *R*(+)-7-OH-DPAT, apomorphine or morphine, or 30 min before treatment with cisplatin. Dogs and ferrets were observed for 30 min (apomorphine, morphine and *R*(+)-7-OH-DPAT) or 4 h (cisplatin) after treatment with emetogens.

Table 5 Antiemetic profile of AS-8112 (ID_{50} values; μg kg^{-1})

Emetogens	*Route of test drugs*	*AS-8112*	*Ondansetron*	*Granisetron*	*Metoclopramide*	*Domperidone*	*Haloperidol*
Dogs							
Apomorphine	s.c.	10.5 (7.87–13.9)	>1000	ND	121 (87.3–167)	5.26 (3.77–13.9)	20.0 (15.4–25.9)
Morphine	i.v.	14.2 (6.59–30.4)	>1000	ND	283 (254–315)	>1000	20.2 (7.99–51.0)
Ferrets							
R(+)-7-OH-DPAT	s.c.	2.22 (1.05–4.69)	>1000	ND	181 (99.6–329)	5.03 (2.84–8.90)	11.7 (5.79–23.7)
Cisplatin	i.v.	17.6 (11.7–26.4)	16.0 (12.0–21.4)	10.7 (4.62–24.9)	605 (348–1050)	>1000	965 (449–2070)

Each value represents the mean (95% c.l.), $n = 5-12$. ND: not determined.

contractile responses to 2Me5HT in isolated guinea-pig colon are mediated through interactions involving a neuronal 5-HT receptor, which could be the 5-HT_3 receptor (Butler *et al*., 1990; Miyata *et al*., 1991). AS-8112 inhibited the contractions induced by 2Me5HT in a concentration-related manner with a pA_2 value of 7.0. Granisetron, ondansetron and metoclopramide also inhibited the contractions induced by 2Me5HT with a potency order of pA_2 values; granisetron > ondansetron = AS-8112 > metoclopramide. This order of potency was in accordance with that of their affinity for 5-HT_3 receptors in the rat frontal cortex (data not shown). It is well known that 5-HT_3 receptor antagonists block the bradycardia (Von Bezold-Jarisch reflex) induced by 2Me5HT, a receptor agonist that mediates the activation of 5-HT_3 receptors located on vagal afferent fibres in cardiac ventricles, and is widely used to assay 5-HT_3 receptor blocking activity *in vivo* (Yoshida *et al*., 1992; Kamato *et al*., 1993; Pires *et al*., 1998). AS-8112 dose-relatedly inhibited the 2Me5HT-induced bradycardia in rats with a potency equal to that of ondansetron (unpublished data). These findings suggest that AS-8112 may be classified as a potent 5-HT_3 receptor antagonist *in vitro* and *in vivo*.

To confirm whether AS-8112 acted as an antagonist for dopamine D_3 receptors, we investigated the inhibitory effect of AS-8112 on hypothermia in mice induced by *R*(+)-7-OH-DPAT, a selective dopamine D_3 receptor agonist. Based on correlational studies that compared the potency of a range of agonists with various levels of selectivity for dopamine D_3 receptor to induce changes in body temperature and their *in vitro* affinity, dopamine D_3 receptor has been considered to play an important role in regulating body temperature (Millan *et al*., 1995; Perrault *et al*., 1997; Varty & Higgins, 1998). Furthermore, the potency of dopamine D_3 receptor antagonists to reverse hypothermia induced by 7-OH-DPAT correlated better with their affinity for the D_3 receptors than in the case of D_2 receptors (Millan *et al*., 1995). In the present study, AS-8112 inhibited *R*(+)-7-OH-DPAT-induced hypothermia in mice with a potency equal to that of domperidone or haloperidol. For dopamine D_2 receptors, AS-8112 also acted as potent antagonist like domperidone or haloperidol, because AS-8112 potently inhibited apomorphine-induced emesis in dogs which is mediated *via* dopamine D_2 receptors. These findings indicate that AS-8112 is a dopamine D_2 and D_3 receptors antagonist *in vivo*.

The present study demonstrates that AS-8112 has a broad-spectrum anti-emetic activity in dogs and ferrets. AS-8112 blocked or significantly reduced vomiting and retching in ferrets given *R*(+)-7-OH-DPAT, cisplatin, cyclophosphamide and doxorubicin. AS-8112 also blocked emetic episodes induced by morphine and apomorphine in dogs. It is well known that 5-HT_3 receptor antagonists have a limited spectrum of antiemetic activity, being active mostly against emesis induced by chemotherapy (acute emesis) or radiotherapy of cancer and by ipecacuanha (Aaporo, 1991). Dopamine receptor antagonists also have a limited spectrum of antiemetic activity, being most active against emesis induced by apomorphine with much weaker inhibition of cisplatin-induced emesis (Costall *et al*., 1990). In the present study, we confirmed that 5-HT_3 receptor antagonists could block emesis triggered by cisplatin, cyclophosphamide and doxorubicin, but failed to block emesis triggered by *R*(+)-7-OH-DPAT, apomorphine and morphine. In contrast, dopamine receptor antagonists block emesis triggered by *R*+)-7-OH-DPAT, apomorphine and morphine, but are much weaker inhibitors of cisplatin-induced emesis. Metoclopramide, which has weak affinity for dopamine D_2 and 5-HT_3 receptors, considerably inhibited emesis triggered by *R*(+)-7-OH-DPAT, apomorphine, morphine, cisplatin, cyclophosphamide and doxorubicin. However, this inhibition was not complete except for emesis induced by *R*(+)-7-OH-DPAT and apomorphine. We previously reported that not only dopamine D_2 receptor but also D_3 receptor may be involved in the control of emesis, since a dopamine D_3 receptor agonist, *R*(+)7-OH-DPAT, caused emesis in dogs and ferrets (Yoshida *et al*., 1995; Yoshikawa *et al*., 1996). Therefore, the broad anti-emetic profile of AS-8112 can be explained by a combination of 5-HT_3 and dopamine D_2, D_3 receptors antagonist activity. Furthermore, following oral administration to both the ferrets and the dogs, AS-8112 significantly reduced emesis in a dose-dependent manner. Therefore, AS-8112 may be a potent and orally active anti-emetic agent.

Recent experimental studies have demonstrated that tachykinin NK_1 receptor antagonists also possess a broad-spectrum of anti-emetic activity in animal models. Clinical studies also showed that L-754,030, a selective NK_1 antagonist, prevented delayed cisplatin-induced emesis, which is not prevented by 5-HT_3 receptor antagonists (Navari *et al*., 1999). However, it is not clear whether a single dose of L-754,030 can be effective against acute emesis induced by chemotherapy in human, although the addition of L-754,030 to granisetron plus dexamethasone increased the number of patients who did not vomit during the acute-emesis phase. On the other hand, metoclopramide monotherapy significantly controlled cisplatin-induced delayed nausea better than ondansetron, although ondansetron was more effective than metoclopramide in preventing acute emesis induced by cisplatin (De Mulder *et al*., 1990). More recent studies showed that intranasal metoclopramide with or without dexamethasone was efficacious in the control of delayed emesis induced by moderate emetogenic chemotherapy (Ormrod & Goa, 1999). In the delayed phase, the anti-emetic effect of metoclopramide monotherapy may be mediated by a combined blockage of 5-HT_3, dopamine D_2 and D_3 receptors. Recent clinical and experimental studies also showed that postoperative nausea and vomiting are not completely inhibited by dopamine receptor antagonists or 5-HT_3 receptor antagonists, but a combination of these two antiemetics was more effective than the monotherapy (Wynn *et al*., 1993; McKenzie *et al*., 1996; Wu *et al*., 2000). In the present study, we demonstrate that AS-8112 is a potent dopamine D_2, D_3 and 5-HT_3 receptors antagonist and is a highly potent antiemetic against emesis induced by various emetogens. Metoclopramide also showed a broad-spectrum anti-emetic activity in this study. However, anti-emetic effects of metoclopramide were less potent than that of AS-8112. Furthermore, cisplatin- and morphine-induced emetic response was not completely reduced by metoclopramide at high doses (1 – 3 mg kg^{-1} s.c. or i.v.). Metoclopramide at high doses (3 – 10 mg kg^{-1} i.v.) also caused central nervous depression which was related to the blockade of dopamine D_2 receptors. On the other hand, AS-8112 at doses that perfectly reduced emetic responses did not cause central nervous depression, catalepsy and extrapyramidal syndrome in rats and monkeys (unpublished data). According

to these findings, AS-8112 may be a useful anti-emetic agent with strong activity against both acute and delayed emesis induced by cisplatin and against postoperative nausea and vomiting. Therefore, further experimental studies are needed to confirm the anti-emetic activity of AS-8112 against delayed emesis induced by cisplatin and against postoperative nausea and vomiting.

In conclusion, AS-8112 is a novel and potent dopamine D_2, D_3 and 5-HT_3 receptors antagonist with a broad-spectrum of anti-emetic activity associated with dopamine D_2, D_3 and 5-HT_3 receptors blockage. These results strongly suggest that AS-8112 is worthy of clinical investigation as an anti-emetic agent with strong activity against a range of emetic stimuli.

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