

Possible Involvement of 5-HT₄ Receptors, in Addition to 5-HT₃ Receptors, in the Emesis Induced by High-Dose Cisplatin in *Suncus murinus*

Kaori Horikoshi*, Toshihide Yokoyama, Nobuyuki Kishibayashi,
Kenji Ohmori, Akio Ishii and Akira Karasawa

Drug Development Research Laboratories, Pharmaceutical Research Institute, Kyowa Hakko Kogyo Co., Ltd.,
1188 Shimotogari, Nagaizumi-cho, Sunto-gun, Shizuoka 411-8731, Japan

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ABSTRACT—To clarify the mechanism for the severe emesis concomitant with intensive chemotherapy, we investigated the effects of 5-HT₃- and 5-HT₄-receptor antagonists on the emesis induced by the high-dose of cisplatin in *Suncus murinus*. The emesis induced by 50 mg/kg of cisplatin was reduced by the oral pretreatment with tropisetron, which is known as a 5-HT₃- and 5-HT₄-receptor dual antagonist in vitro, with the ID₅₀ value of 0.52 mg/kg. On the contrary, granisetron, a selective 5-HT₃-receptor antagonist, did not markedly inhibit the emesis at up to 30 mg/kg. Moreover, GR125487, a selective 5-HT₄-receptor antagonist, did not inhibit the emesis. However, co-administration of GR125487 and granisetron significantly reduced the number of emetic episodes. The study of the co-administration of GR125487 with tropisetron showed that GR125487 did not further enhance the inhibitory effect of tropisetron alone, suggesting that the anti-emetic effect of tropisetron is mediated via the blockade of both 5-HT₃ and 5-HT₄ receptors. These results suggest that both the 5-HT₃ and 5-HT₄ receptors are involved in the emesis induced by the high-dose of cisplatin in *Suncus murinus*.

Keywords: Emesis, Cisplatin, 5-HT₃ receptor, 5-HT₄ receptor, *Suncus murinus*

Although cisplatin is an effective anti-cancer drug, it produces emesis and nausea as its side-effects (1). 5-HT₃-receptor antagonists have been shown to exhibit potent anti-emetic activities against cisplatin-induced acute emesis in various models of dog, ferret and *Suncus murinus*, where the doses of cisplatin were close to the minimal dose to evoke emesis (2–7). Therefore, it is certain that the acute emesis that is induced by the low or medium doses of cisplatin is mostly mediated by activation of 5-HT₃ receptors. In clinical practice, a wide range of doses for cisplatin are prescribed, and the frequency and severity of emesis are dependent on the dose of cisplatin (8). Thus far, however, there has been no report investigating the anti-emetic activities of 5-HT₃-receptor antagonists against the high-dose of cisplatin-induced emesis in animal models. In a study examining the effects of various 5-HT₃-receptor antagonists on the high-dose of cisplatin in *Suncus murinus*, we found that the 5-HT₃-receptor antagonists having affinity for other receptors were superior to the pure 5-HT₃-receptor antago-

nist in attenuating emesis.

Previous studies suggest that 5-HT₄ receptors are involved in some types of emesis in animal models, including copper sulfate- and the methotrexate-induced emesis (9, 10). Additionally, Bhandari and Andrews (11) showed that zacopride, a 5-HT₄-receptor agonist, provoked emetic responses in ferrets. It is, however, not fully understood whether 5-HT₄ receptors mediate the acute emesis induced by intensive chemotherapy. These observations led us to the hypothesis that 5-HT₄ receptors in addition to 5-HT₃ receptors are involved in the high-dose of cisplatin-induced emesis. To address this hypothesis, we investigated the possible involvement of the 5-HT₃ and 5-HT₄ receptors in the emesis induced by the high-dose of cisplatin in *Suncus murinus* in the present study.

MATERIALS AND METHODS

Animals

One- to 10-month-old male *Suncus murinus* Jic:SUN (Clea Japan, Inc., Tokyo), weighing 50 to 80 g, were used. The animals were housed at 22 ± 3°C with 30–70% rela-

*Corresponding author. FAX: +81-559-86-7430
E-mail: akira.karasawa@kyowa.co.jp

tive humidity and the room was lit from 7 a.m. to 7 p.m. They had free access to tap water and pelleted chow (CIEA305; Clea Japan, Inc.). Each animal was used for only one experiment. All animals received human care in compliance with the Guiding Principles for the Care and Use of Laboratory Animals formulated by the Japanese Pharmacological Society. The protocol was approved by the Bioethical Committee of Pharmaceutical Research Institute, Kyowa Hakko Kogyo Co., Ltd.

Drugs

Cisplatin was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Tropisetron hydrochloride (tropisetron) was kindly supplied by Novartis Pharma AG (Basel, Switzerland). Ondansetron hydrochloride (ondansetron), granisetron hydrochloride (granisetron) and GR125487 (1-[2-[(methylsulphonyl)amino]ethyl]-4-piperidinyl-methyl 5-fluoro-2-methoxy-1*H*-indole-3-carboxylate) were synthesized in our laboratories. Cisplatin was prepared in saline adjusted to pH 4 at 50°C followed by gradual cooling to 37°C immediately before use. GR125487 was dissolved in saline and other drugs were dissolved in distilled water just before use.

Experimental procedure

For the assessment of emetic behavior, animals were transferred to individual cages. Number of emetic episodes and latency to the first emetic episodes (latency period) were recorded following the pretreatment with cisplatin. The emesis was characterized by rhythmic abdominal contractions associated with (vomiting) or without (retching) the oral expulsion of materials from the gastrointestinal tract. If an animal did not show emesis, the latency period was regarded as equal to the observation period (2 or 4 h).

To determine the high-dose of cisplatin used in the following experiments, we estimated the emetogenic activity of cisplatin at various doses. Cisplatin (5, 10, 20, 30 and 50 mg/kg) or the vehicle (25 ml/kg) was intraperitoneally administered to animals.

Anti-emetic activities of three 5-HT₃-receptor antagonists on the high-dose of cisplatin-induced emesis were investigated as follows: either tropisetron (0.3, 0.4, 0.6, 0.8, 1 and 2 mg/kg), ondansetron (2, 3, 5, 10 and 20 mg/kg), granisetron (3, 10 and 30 mg/kg) or distilled water (10 ml/kg) was orally administered 1 h before the injection of cisplatin (50 mg/kg, i.p.).

To clarify the possible involvement of 5-HT₄ receptors in the high-dose of cisplatin-induced emesis, the effect of a 5-HT₄-receptor antagonist with or without a 5-HT₃-receptor antagonist was investigated. The specific 5-HT₄-receptor-antagonist GR125487 (12) (0.3 mg/kg) or saline (10 ml/kg) was intraperitoneally administered to the animal 30 min after oral administration of tropisetron (2 mg/kg), grani-

setron (30 mg/kg) or distilled water (10 ml/kg). Thirty minutes thereafter, cisplatin (50 mg/kg) was intraperitoneally injected to the animal.

In the preliminary experiment, most of the emetic responses induced by 50 mg/kg of cisplatin were observed within 100 min after the administration of cisplatin, and more than 90% of the emesis were detected within 4 h in all the animals. Accordingly, the observation period was determined 2 and 4 h after the administration of cisplatin in the experiments of the dose-response study for cisplatin and the drug-effect study for 5-HT antagonists, respectively.

Statistical analyses

Values for the number of emetic episodes and the latency period were expressed as means \pm S.E.M. of 5 animals. The Wilcoxon rank sum test was used to confirm the statistical significance of differences between any two groups (SAS Release 6.12.; SAS institute, Inc., Cary, NC, USA). The Kruskal-Wallis test followed by the Steel test was performed for multiple comparisons (SAS Release 6.12.; SAS institute, Inc.). *P* values of less than 0.05 were considered to be statistically significant. Inhibition rate (%) was assessed from the ratio of the number of emetic episodes in the treatment groups to that in the control group. ID₅₀ values were calculated from the inhibition rate using the Probit logistic model method (SAS Release 6.12.; SAS institute, Inc.).

RESULTS

Emetogenic activity of cisplatin

Administration of cisplatin provoked emesis in all the animals at the doses of 20 mg/kg and above (Table 1). The number of emetic episodes increased and the latency period decreased with the increase of doses for cisplatin from 20 mg/kg to 50 mg/kg. At a dose of 50 mg/kg, cisplatin consistently provoked emetic episodes 15 times or more, starting within 28.2–34.5 min in all the animals. Accordingly, the dose of 50 mg/kg, which was 2.5 times higher than the minimum dose inducing emesis in all animals, was selected for the following studies.

Effects of tropisetron, granisetron or ondansetron on the emesis

Pretreatment with tropisetron (0.3–2 mg/kg, p.o.) reduced the number of emetic episodes induced by 50 mg/kg (i.p.) of cisplatin in an apparently dose-related manner (Table 2, Fig. 1). The ID₅₀ value was calculated as 0.52 mg/kg (p.o.). The latency period was significantly prolonged at all the doses studied as compared with that in the control group (Table 2). Doses of 1 and 2 mg/kg of tropisetron completely abolished the emesis in one of 5 animals during 4 h after the cisplatin injection. Pretreatment with ondansetron (2–20 mg/kg, p.o.) reduced the number

Table 1. Emetogenic responses to the doses of cisplatin in *Suncus murinus*

Dose of cisplatin (mg/kg, i.p.)	Number of animals with emesis/tested	Number of emetic episodes	Latency period (min)
0	0/5	0 ± 0	120 ± 0.0
10	0/5	0 ± 0	120 ± 0.0
20	5/5	8 ± 2	63.5 ± 7.0
30	5/5	12 ± 2	42.6 ± 1.5
50	5/5	23 ± 2	31.3 ± 1.0

Values for the number of emetic episodes and latency period are expressed as means ± S.E.M. of 5 animals. If an animal did not vomit, the latency period was regarded as equal to the observation period (2 h).

of emetic episodes with the ID₅₀ value of 6.75 mg/kg (p.o.) (Table 2, Fig. 1). Ondansetron also significantly extended the latency period at all the doses studied (Table 2). One of 5 animals did not show the emesis for 4 h in the group of animals treated with 20 mg/kg of ondansetron. Although pretreatment with granisetron (3–30 mg/kg, p.o.) reduced

Table 2. Effects of tropisetron, ondansetron and granisetron on the high-dose of cisplatin-induced emesis in *Suncus murinus*

Drug	Dose (mg/kg)	Number of animals with emesis/tested	Number of emetic episodes	Latency period (min)
Tropisetron	0	5/5	39 ± 6	25.1 ± 1.5
	0.3	5/5	22 ± 4	71.4 ± 5.8*
	0.4	5/5	26 ± 5	75.5 ± 11.0*
	0.6	5/5	18 ± 5	111.7 ± 27.6*
	0.8	5/5	19 ± 5	71.3 ± 9.9*
	1	4/5	9 ± 6	146.5 ± 33.8*
	2	4/5	8 ± 3*	117.5 ± 32.5*
Ondansetron	0	5/5	51 ± 9	24.8 ± 1.2
	2	5/5	45 ± 9	53.5 ± 3.8*
	3	5/5	28 ± 6	48.6 ± 4.3*
	5	5/5	20 ± 4	63.5 ± 7.3*
	10	5/5	25 ± 3	63.5 ± 4.1*
	20	4/5	18 ± 6	97.3 ± 36.2*
Granisetron	0	5/5	52 ± 8	26.8 ± 1.4
	3	5/5	30 ± 6	37.3 ± 3.4
	10	5/5	29 ± 4	45.8 ± 4.5*
	30	5/5	33 ± 7	48.7 ± 5.9*

Either tropisetron, ondansetron, granisetron or distilled water was orally administered 1 h before the administration of cisplatin (50 mg/kg, i.p.). Animals were observed for 4 h after the administration of cisplatin. Values for the number of emetic episodes and latency period are expressed as means ± S.E.M. of 5 animals. If an animal did not vomit, the latency period was regarded as equal to the observation period (4 h). * $P < 0.05$, compared with the value in the control (0 mg/kg, p.o.) group (Steel test).

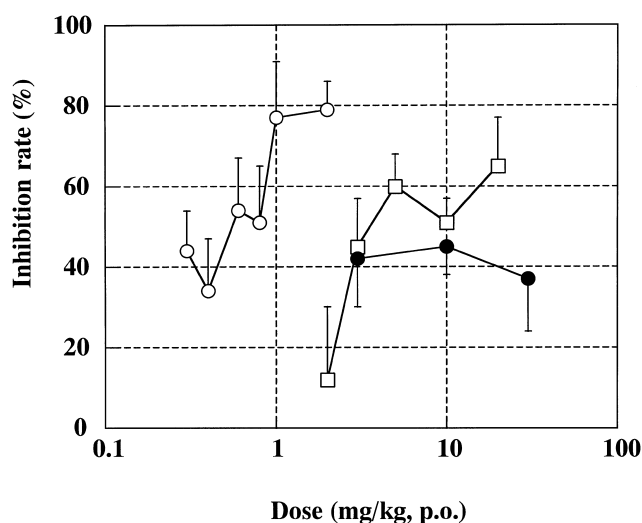


Fig. 1. Effects of tropisetron (○), ondansetron (□) and granisetron (●) on cisplatin-induced emesis in *Suncus murinus*. Either tropisetron (0.3–2 mg/kg, p.o.), ondansetron (2–20 mg/kg, p.o.) or granisetron (3–30 mg/kg, p.o.) was administered 1 h before the administration of cisplatin (50 mg/kg, i.p.). The number of emetic episodes was recorded continuously for 4 h after the administration of cisplatin. Each point with vertical bar represents the mean ± S.E.M. of the reduction rate of emetic episodes in the tested animals compared to that in control animals. The number of animals was 5.

the number of emetic episodes, the maximum inhibition rate was lower than 50% (Table 2, Fig. 1). The prolongation of the latency period was slight but reached statistically significant values at doses of 10 and 30 mg/kg (Table 2).

Effects of co-administration of tropisetron or granisetron with GR125487 on emesis

GR125487 (0.3 mg/kg, i.p.) alone did not reduce, but slightly increased, the number of emetic episodes induced by cisplatin (50 mg/kg, i.p.) (Fig. 2). Similarly, granisetron (30 mg/kg, p.o.) alone did not reduce the number (Fig. 2). However, the emesis was significantly inhibited, with the rate of 72%, by the co-administration of GR125487 (0.3 mg/kg, i.p.) and granisetron (30 mg/kg, p.o.). On the other hand, tropisetron (2 mg/kg, p.o.) alone significantly reduced the number of emetic episodes with the inhibition rate of 73%. Co-administration of GR125487 (0.3 mg/kg, i.p.) with tropisetron (2 mg/kg, p.o.) did not enhance the inhibitory effect of tropisetron alone.

DISCUSSION

It has been well established that the acute emesis induced by the usual doses of cisplatin is mediated by the activation of 5-HT₃ receptors in various animal models (2–7). In this study, we investigated the mechanisms underlying the emesis induced by cisplatin at a higher dose than that used in

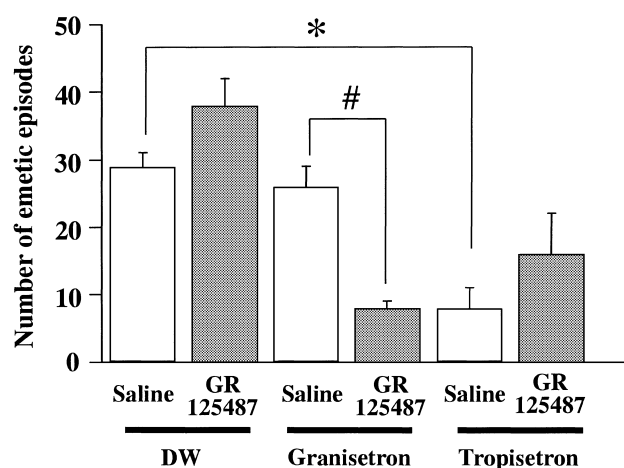


Fig. 2. Effects of co-administration of GR125487 and 5-HT₃-receptor antagonists on cisplatin-induced emesis in *Suncus murinus*. Tropisetron (2 mg/kg, p.o.), granisetron (30 mg/kg, p.o.) or distilled water (DW, 10 ml/kg, p.o.) was administered 1 h before the administration of cisplatin (50 mg/kg, i.p.). GR125487 (0.3 mg/kg, i.p.) and saline were administered 30 min before the administration of cisplatin. The number of emetic episodes was recorded continuously for 4 h after the administration of cisplatin. Each column with vertical bar represents the mean \pm S.E.M. of 5 animals. * P <0.05, compared with the value of the DW + saline group (Wilcoxon rank sum test). # P <0.05, compared with the value of the granisetron + saline group (Wilcoxon rank sum test).

previous studies (6, 7) in *Suncus murinus*. We employed a dose of cisplatin as much as 2.5 times higher than the minimum dose inducing emesis in all animals. Tropisetron, which is known as a 5-HT₃-receptor antagonist with 5-HT₄-receptor antagonistic activity in vitro (12, 13), strongly inhibited the emesis induced by the high-dose of cisplatin. On the other hand, granisetron, a selective 5-HT₃-receptor antagonist (13, 14), exhibited only minimal anti-emetic activity on the emesis induced by the high-dose of cisplatin, in contrast to the previous reports examining the cisplatin-induced emesis in *Suncus murinus* (6, 7). These results suggest that the mechanisms for the emesis induced by the high-dose of cisplatin is different from those induced by the low or medium doses of cisplatin.

In previous studies, involvement of 5-HT₄ receptors has been supposed in several animal models of emesis. Copper sulfate- and methotrexate-induced emesis in the dog are inhibited by the dual antagonist for 5-HT₃ and 5-HT₄ receptors, but not by the selective 5-HT₃-receptor antagonists (9, 10), indicating that 5-HT₄ receptors are at least partly involved in these emesis models. Moreover, zacopride and 5-methoxytryptamine, both of which are 5-HT₄-receptor agonists, are reported to induce emesis in the ferret and the dog, respectively (9, 11). Therefore, we supposed that the high-dose of cisplatin-induced emesis in *Suncus murinus* also involved emetogenic 5-HT₄ receptors, in contrast to the emesis induced by the low or medium doses of cisplatin, which

were mostly mediated by 5-HT₃ receptors.

To clarify the possible involvement of 5-HT₄ receptors in the emesis induced by the high-dose of cisplatin, we investigated the effect of GR125487, a selective 5-HT₄-receptor antagonist (12), on the emesis. Although the pretreatment with GR125487 alone did not inhibit the emesis, the co-administration of GR125487 with granisetron significantly enhanced the effect of granisetron alone. Our results thus suggest that 5-HT₄ receptor is involved in the emesis induced by the high-dose of cisplatin under the condition of 5-HT₃-receptor blockade. On the other hand, GR125487 did not enhance the anti-emetic activity of tropisetron alone in this study. Tropisetron is known to block 5-HT₄ receptors at approximately 100 times higher concentrations than those inhibiting 5-HT₃ receptors (12, 13). These observations suggest that the anti-emetic effect of tropisetron involves inhibition of both the 5-HT₃ and 5-HT₄ receptors and that the strong inhibition of 5-HT₄ receptors is not required to inhibit the emesis.

It is assumed that chemotherapy induces the 5-HT release from enterochromaffin (EC) cells of the small intestine, resulting in the induction of the emesis (15). Activation of the 5-HT₃ receptors on EC cells induces the positive-feedback mechanism causing an increase of 5-HT release (15). Accordingly, 5-HT₃-receptor antagonists inhibit the chemotherapy-induced emesis by the blockade of 5-HT₃ receptors on EC cells as well as those on the vagus nerve endings (16). On the other hand, activation of 5-HT₄ receptors is reported to either simulate or inhibit the 5-HT release in several animal species including ferret, rat, guinea pig, pig and human (17–19). Thus, there is no consensus as to whether the blockade of 5-HT₄ receptors either inhibits or stimulates the emesis. In the present experiments, GR125487 when combined with granisetron, showed anti-emetic activity, whereas this drug tended to increase the number of the emetic episodes when administered alone or in combination with tropisetron. The role of 5-HT₄ receptors in emesis is rather complex and is likely to depend on the experimental conditions and the animal species examined. Further studies, including the possible emetogenic effect of the higher dose of GR125487, are required to clarify the exact role of 5-HT₄ receptors in the emesis induced by the high-dose of cisplatin.

The maximum anti-emetic activity of ondansetron was less than that of tropisetron but higher than that of granisetron. It is unclear why ondansetron, having no affinity for 5-HT₄ receptors (12, 13), showed a relatively prominent inhibitory effect on emesis. Ondansetron was found to have affinities for emetogenic receptors such as 5-HT_{1B} and α_1 -adrenergic receptors (14, 20, 21), as well as that for 5-HT₃ receptors. Modulation of these receptors may be involved in the anti-emetic activity of ondansetron in the present experiment.

In conclusion, the present study indicates that 5-HT₄ receptors, as well as 5-HT₃ receptors, are involved in the emesis induced by the high dose of cisplatin in *Suncus murinus*. The blockade of 5-HT₄ receptors in addition to the 5-HT₃ receptors may be necessary to inhibit the emesis. Moreover, it is suggested that tropisetron blocks 5-HT₄ receptors as well as 5-HT₃ receptors in vivo, resulting in the potent inhibitory effect on emesis.

REFERENCES

- 1 Joss RA, Brand BC, Buser KS and Cerny T: The symptomatic control of cytostatic drug-induced emesis. A recent history and review. *Eur J Cancer* **26** Suppl **1**, S2 – S8 (1990)
- 2 Costall B, Domeney AM, Naylor RJ and Tattersall FD: 5-Hydroxytryptamine M-receptor antagonism to prevent cisplatin-induced emesis. *Neuropharmacology* **25**, 959 – 961 (1986)
- 3 Miner WD and Sanger GJ: Inhibition of cisplatin-induced vomiting by selective 5-hydroxytryptamine M-receptor antagonism. *Br J Pharmacol* **88**, 497 – 499 (1986)
- 4 Hawthorn J, Ostler KJ and Andrews PL: The role of the abdominal visceral innervation and 5-hydroxytryptamine M-receptors in vomiting induced by the cytotoxic drugs cyclophosphamide and cis-platin in the ferret. *Q J Exp Physiol* **73**, 7 – 21 (1988)
- 5 Turconi M, Donetti A, Schiavone A, Sagrada A, Montagna E, Nicola M, Cesana R, Rizzi C and Micheletti R: Pharmacological properties of a novel class of 5-HT₃ receptor antagonists. *Eur J Pharmacol* **203**, 203 – 211 (1991)
- 6 Torii Y, Saito H and Matsuki N: Selective blockade of cytotoxic drug-induced emesis by 5-HT₃ receptor antagonists in *Suncus murinus*. *Jpn J Pharmacol* **55**, 107 – 113 (1991)
- 7 Ito C, Isobe Y, Kijima H, Kiuchi Y, Ohtsuki H, Kawamura R, Tsuchida K and Higuchi S: The anti-emetic activity of GK-128 in *Suncus murinus*. *Eur J Pharmacol* **285**, 37 – 43 (1995)
- 8 Gregory RE and Ettinger DS: 5-HT₃ receptor antagonists for the prevention of chemotherapy-induced nausea and vomiting. *Drugs* **55**, 173 – 189 (1998)
- 9 Fukui H, Yamamoyo M, Sasaki S and Sato S: Possible involvement of peripheral 5-HT₄ receptors in copper sulfate-induced vomiting in dogs. *Eur J Pharmacol* **257**, 47 – 52 (1994)
- 10 Yamakuni H, Sawai H, Maeda Y, Imazumi K, Sakuma H, Matsuo M, Mutoh S and Seki J: Probable involvement of the 5-hydroxytryptamine₄ receptor in methotrexate-induced delayed emesis in dogs. *J Pharmacol Exp Ther* **292**, 1002 – 1007 (2000)
- 11 Bhandari P and Andrews PLR: Preliminary evidence for the involvement of the putative 5-HT₄ receptor in zacopride- and copper sulfate-induced vomiting in the ferret. *Eur J Pharmacol* **204**, 273 – 280 (1991)
- 12 Schiavi GB, Brunet S, Rizzi CA and Ladinsky H: Identification of serotonin 5-HT₄ recognition sites in the porcine caudate nucleus by radioligand binding. *Neuropharmacology* **33**, 543 – 549 (1994)
- 13 Grossman CJ, Kilpatrick GJ and Bunce KT: Development of a radioligand binding assay for 5-HT₄ receptors in guinea-pig and rat brain. *Br J Pharmacol* **109**, 618 – 624 (1993)
- 14 Van Wijngaarden I, Tulp MTM and Soudijn W: The concept of selectivity in 5-HT receptor research. *Eur J Pharmacol* **188**, 301 – 312 (1990)
- 15 Minami M, Saito H and Yoshioka M: Toxicological aspects of cisplatin-induced emesis with emphasis on serotonin release. *J Toxicol Sci* **20**, 77 – 85 (1995)
- 16 Tyers MB and Freeman AJ: Mechanism of the anti-emetic activity of 5-HT₃ receptor antagonists. *Oncology* **49**, 263 – 268 (1992)
- 17 Minami M, Tamakai H, Ogawa T, Endo T, Hamaue N, Hirafuji M, Yoshioka M and Blower PR: Chemical modulation of 5-HT₃ and 5-HT₄ receptors affects the release of 5-hydroxytryptamine from the ferret and rat intestine. *Res Commun Mol Pathol Pharmacol* **89**, 131 – 142 (1995)
- 18 Gebauer A, Merger M and Kilbinger H: Modulation by 5-HT₃ and 5-HT₄ receptors of the release of 5-hydroxytryptamine from the guinea pig small intestine. *Naunyn Schmiedeberg's Arch Pharmacol* **347**, 137 – 140 (1993)
- 19 Schwörer H and Ramadori G: Autoreceptors can modulate 5-hydroxytryptamine release from porcine and human small intestine in vitro. *Naunyn Schmiedeberg's Arch Pharmacol* **357**, 548 – 552 (1998)
- 20 Perry CM and Markham A: Sumatriptan. An updated review of its use in migraine. *Drugs* **55**, 889 – 922 (1998)
- 21 Hikasa Y, Ogasawara S and Takase K: Alpha adrenoceptor subtypes involved in the emetic action in dogs. *J Pharmacol Exp Ther* **261**, 746 – 754 (1992)