J Neural Transm (1998) 105: 1143–1154

Journal of Neural Transmission

Serotonin 5-HT_3 receptor antagonists prevent cisplatin-induced emesis in Cryptotis parva: a new experimental model of emesis

N. A. Darmani

Department of Pharmacology, Kirksville College of Osteopathic Medicine, Kirksville, MO, U.S.A.

Received March 2, 1998; accepted May 7, 1998

Summary. The aim of this manuscript is to introduce Cryptotis parva (the least shrew) as a new experimental emesis model. The chemotherapeutic agent, cisplatin, caused a dose-dependent increase in the number of animals exhibiting vomiting and retching behaviours with ED_{50} values of 6.43 ± 1 and 7.9 ± 1.2 mg/kg, respectively. The frequencies of these parameters were also dose-dependent. Intraperitoneal administration of 5-HT_3 receptor antagonists (tropisetron or MDL 72222) prevented cisplatin-induced emesis and retching behaviours in the least shrew by a dose-dependent mechanism with respective ID_{50} values of 4.28 ± 2.8 and 2.05 ± 2 for emesis, and 2.71 ± 4.5 and 2.52 ± 2.59 for retching. Intraperitoneal injection of selective and nonselective 5-HT_3 receptor agonists potently, and in a dose-dependent fashion, induced emesis in the least shrew with the following ED_{50} potency order: 2-methyl 5-HT ≈ 5-HT ($p > 0.05$) <5-HTQ ($p < 0.01$) <mCPBG ($p < 0.001$). As with other established experimental animal emesis models, the present data indicate that cisplatin causes emesis by activating 5-HT_3 receptors indirectly via release of 5-HT.

Keywords: Cryptotis parva, least shrew, emesis, cisplatin, ICS 205 930, MDL 72222, 5-HT, 2-methyl 5-HT, 5-HTQ, mCPBG, 5-HT_3 receptor.

Introduction

Nausea and vomiting are the most distressing side effects of cancer chemotherapy. Cancer patients may also experience clinical anticipatory nausea and vomiting (Coates et al., 1983) which can ultimately result in the patients' refusal to continue effective antitumor therapy (Trizzi and Laszlo, 1987). Serotonin 5-HT_3 receptor antagonists are highly effective in reducing the acute emesis induced by such agents in both humans (Reviews: de Bruijin, 1992; Milne and Heel, 1991; Plosker and Goa, 1991) and animals (Review: Andrews et al., 1990).

Although cisplatin is clinically effective against a variety of tumors (Rosenberg, 1985), it is also one of the most emotogenic of the available anticancer drugs (Laszlo, 1982). Thus, to investigate the potential of antiemetic drugs, many preclinical investigators have used cisplatin-induced vomiting in animal models of emesis. Serotonergic 5-HT_3 receptor antagonists have been shown to prevent cisplatin-induced emesis in dogs (Fukui et al., 1992; Gidda et al., 1995; Smith et al., 1989), cats (Lucot, 1989; Smith et al., 1988) and ferrets (Costall et al., 1987; Stables et al., 1987; Kamoto et al., 1993). The exact site of antiemetic action of 5-HT_3 receptor antagonists is still under debate. Current literature suggests that such antagonists exert their antiemetic effects via an action in the periphery by antagonizing the effect of serotonin at 5-HT_3 receptors on vagal afferent neurons that innervate the gastrointestinal tract (Gidda et al., 1995). Indeed, vagotomy can abolish cisplatin-induced emesis in dog (Fukui et al., 1992), ferret (Hawthorn et al., 1988), and suncus murinus (Mutoh et al., 1992). Furthermore, large amounts of 5-HT are contained in the enterochromaffin cells located close to vagal afferent terminals in the gastrointestinal mucosa and cisplatin has been shown to release 5-HT from these cells in both guinea-pigs (Schwörer et al., 1991) and ferrets (Gunning et al., 1987). Clinical studies also provide support of 5-HT release following cisplatin therapy (Cubeddu et al., 1992; Barnes et al., 1990). However, not all studies indicate a peripheral site for the action of 5-HT_3 receptor antagonists. Indeed several studies point to a central site of action since direct microinjections of 5-HT_3 antagonists into the CNS can prevent cisplatin-induced emesis in ferrets (Higgins et all., 1989; Yoshida et al., 1992), dogs (Gidda et al., 1995) or cats (Smith et al., 1988). In addition, complete inhibition of cisplatin-induced emesis was observed following ablation of the area of postrema in cats (McCarthy and Borison, 1984) and dogs (Bhandari et al., 1989).

Most animal emesis studies are confined to large animals such as cats, dogs or ferrets. However, more recently Japanese investigators have introduced a smaller animal (adult being 50–100 g in weight), the house musk shrew (Suncus murinus), as an experimental emetic model (Matsuki et al., 1988; Torii et al., 1991a,b). Suncus murinus is endogenous to Asia and Africa. The family Soricidae, to which shrews belong, constitutes some 266 species (Churchfield, 1990). Shrews are placed in the order of insectivora and are among the most ancient animals. Shrews are considered to be closer to primates than rodents, lagomorphs and carnivores in the phylogenetic system (Colbert, 1958). Unlike suncus murinus, the least shrew (Cryptotis parva) is relatively smaller (adult weighing 4–6 g) and is found in Central and North America.

According to receptor recognitory, transductional and structural characteristics, there are at least 7 major classes of 5-HT receptors (5-HT_{1-7}) and many of these receptors possess subtypes (Hoyer and Martin, 1997). The least shrew can produce serotonergically-mediated head-twitches in response to the 5-HT_{2A} agonist, DOI (Darmani et al., 1994), and the serotonin syndrome (a 5-HT_{1A}-mediated response) following the injection of selective 5-HT_{1A} agonist 8-OH DPAT (Darmani and Zaoh, 1998). Preliminary studies in this

laboratory indicate that the least shrew can also vomit. The aims of the present study were: 1) to determine the dose-response emetic effects of cisplatin in the least shrew; 2) to investigate whether 5-HT_3 receptor antagonist pretreatment (tropisetron and MDL 72222) can prevent cisplatin-induced emesis; and 3) to explore whether serotonin and selective 5-HT_3 receptor agonists, 2-methyl 5-HT (Richardson et al., 1985) and 1-(m-chlorophenyl)-biguanide (mCPBG) (Kilpatrick et al., 1990) can induce emesis in this new experimental model of emesis. The effect of the peripherally acting selective 5-HT_3 receptor agonist, 5-HTQ (N,N,N-trimethylserotonin iodide) (Dukat et al., 1991), was also investigated.

Materials and methods

Animals

Shrews (Cryptotis parva) were bred and maintained in the animal facilities of the Kirksville College of Osteopathic Medicine. Both male and female (4–5 g, 35–70 days old) shrews were used throughout the study. The animals were kept on a 14:10-h light-dark cycle at a room temperature of 21±°C in open-top clear polycarbonate cages (20 × 18 × 21 cm) lined with heated dry loam soil. Depending upon the size of the litter, 3–6 litter mates were housed per cage. A wooden nest box (5.5 × 5.5 × 9 cm) containing dry grass, a food bowl, and a lick tube water bottle were placed in each cage. Animals were fed twice daily. In the morning, 5–6 mealworms (Tenebrio sp) were given per animal, and in the evening each shrew was offered a 6-g mixture consisting of two-thirds dry cat food (PMI Nutrition Cat formula) and one third canned cat food (Kozy Kitten) in sufficient water to give the mixture a paste-like consistency. All experiments were performed between 0900 and 1,700 h.

Drugs

The following drugs were purchased from Research Biochemical Inc. (Natick, MA): 1-(m-chlorophenyl-biguanide HCl (mCPBG); 2-methylserotonin maleate (2-methyl 5-HT); ICS-205 930 methiodide (tropisetron); MDL 72222 and trimethylserotonin iodide (5-HTQ). 5-Hydroxytryptamine creatinine sulphate complex and cis-platinum (II) diamine dichloride ($Pt(NH_3)_2\ Cl_2$) were bought from Sigma Chemical Co (St. Louis, MO). MDL 72222 was dissolved in 1/3 concentrated HCl acid and was then back titrated to pH 5.0 by Na OH. All other drugs were dissolved in distilled water and were given i.p. at a volume of 10 ml/kg. Doses of drugs are expressed as their stated salts.

Experimental protocols

In preliminary experiments, animals were observed for 3 h and the lowest tested cisplatin dose in responsive shrews produced the first vomit within 90 min following its injection. Thus, a 90-min observation period was taken for the cisplatin dose-response emesis studies. To habituate the shrews to the test environment, each animal was randomly selected and transferred to a 20 × 18 × 21 cm clean clear plastic holding cage and was offered 4 mealworms 30 min prior to experimentation. Then, different groups of shrews were injected intraperitoneally with either vehicle (n = 8) or varying doses of cisplatin (5, 10 and 20; n = 8–13 per dose). Immediately following injection, each shrew was placed in the observation cage and the onset latency to first vomit and the frequencies of vomiting and retching were recorded separately for each individual shrew for the next 90 min. To determine whether 5-HT_3 receptor antagonist pretreatment can abolish cisplatin-induced emesis, varying groups of shrews were injected intraperitoneally with either vehicle (n = 12) or different doses (5, 10 and 20 mg/kg, n = 8–12 per dose) of tropisetron or

MDL 72222. Immediately after the injection, each animal was offered 4 mealworms and 30 min later was injected with a 10 mg/kg dose of cisplatin. The emesis parameters were recorded for the next 90 min as was described above.

In the third series of experiments, the dose-response emetic effects of serotonin (2.5, 5 and 10 mg/kg, n = 6 per group) and selective 5-HT_3 receptor agonists [2-methyl 5-HT (1.25, 2.5 and 5 mg/kg, n = 8–9 per group); 5-HTQ (5, 10, and 20 mg/kg, n = 6–7 per group); and mCPBG (5, 10 and 20 mg/kg, n = 8–9 per group)] were determined in a manner similar to cisplatin-induced dose-response study. However, the emesis observation period for these drugs were 30 min following their intraperitoneal injection since preliminary studies had indicated a short latency to vomiting following their administration. Such agonists also induce emesis in the house musk shrew within a couple of minutes following their i.p. administration (Torii et al., 1991b).

Statistical analysis

The data were analyzed by the Kruskal-Wallis nonparametric one-way analysis of variance (ANOVA) and posthoc analysis by Dunn's multiple comparisons test. A p-value of <0.05 was necessary to achieve statistical significance. The ED_{50} (the effective dose that produced emesis or retching in 50% of animals) and ID_{50} (the inhibitory dose that attenuated the vomiting parameters in 50% of animals) were calculated by the use of a computerized program (Graph Pad InPlot, San Diego, CA).

Results

Kruskal-Wallis nonparametric ANOVA test indicated that intraperitoneal administration of cisplatin induced both emesis and retches in the least shrew in a dose-dependent manner (Table 1) [($kw_{3,37} = 18.45$, $p < 0.0003$) and ($kw_{3,37} = 13.75$, $p < 0.003$) respectively]. At 5 mg/kg, only 22% of animals exhibited emesis, whereas the 10 and 20 mg/kg doses of cisplatin caused emesis in 77 to 88% of tested shrews. Thus, not all animals exhibited vomiting or retching behaviours in response to cisplatin. Dunn's multiple comparisons test indicated that relative to the vehicle-injected control group, significant enhancements in the number of animals exhibiting emesis occurred in the groups injected with 10 ($p < 0.001$) and 20 mg/kg ($p < 0.001$) doses of cisplatin. Depending upon the dose of cisplatin used, the mean latency to onset of first emesis varied from 19 ± 2 to 49 ± 32 min. The number of animals exhibiting retching approximately mirrored the vomiting data and significant enhancement occurred at the 10 ($p < 0.05$) and 20 mg/kg ($p < 0.01$) doses. The 20 mg/kg cisplatin dose was very toxic to shrews and they generally tended to die within 24 h following its injection. Thus, these animals were sacrificed following the termination of observation. Cisplatin-induced vomiting and retching respective ED_{50} values, 6.43 ± 1 and 7.9 ± 1.2 mg/kg, are not significantly different from each other. The vomiting and retching frequencies were also increased in a dose-dependent manner in responsive animals (Table 1).

The Kruskal-Wallis nonparametric ANOVA test showed that the tested 5-HT_3 receptor antagonists (tropisetron and MDL 72222) inhibited cisplatin (10 mg/kg)-induced emesis in the least shrew in a dose-dependent fashion (Table 2) [($kw_{3,40} = 18.76$, $p < 0.0003$) and ($kw_{3,38} = 14.14$, $p < 0.003$) respectively]. Dunn's multiple comparisons test indicated that significant reductions occurred at the 10 ($p < 0.005$) and 20 mg/kg ($p < 0.01$) doses for both 5-HT_3 receptor antagonists. Their respective ID_{50} values are 4.28 ± 2.8 and 2.05 ± 2,

Table 1. Dose-dependent emetogenic effects of cisplatin in Cryptotis parva

Cisplatin mg/kg	Numbers of animals vomiting/tested	Vomiting frequency (90 minutes)	Latency to first vomit (minutes)	Number of animals retching/tested	Retching frequency
0	0/8	–	–	0/8	–
5	2/9	2.5 ± 1.5	49 ± 32	3/9	2.7 ± 1.2
10	10/13**	4.2 ± 0.8	37 ± 10	8/13*	9.9 ± 4.3
20	7/8**	9.0 ± 1.5	19 ± 2	7/8**	8 ± 2.2

Values for vomiting frequency, latency to first vomit and retching frequency are mean ± SEM of those animals which exhibited either vomiting or retching behaviours. Animals which failed to vomit or retch were not included in these mean values. The above parameters were recorded for 90 min following intraperitoneal administration of the cited doses of cisplatin. *$p < 0.05$ and **$p < 0.01$ indicate significant differences relative to vehicle control by Dunn's multiple comparisons test

Table 2. Dose-dependent antiemetic effects of intraperitoneally administered 5-HT_3 receptor antagonists against cisplatin-induced vomiting

	Dose (mg/kg)	Number of animals vomiting/tested	Number of animals retching/tested
Control	0	9/12	7/12
Tropisetron	5	7/10	5/10
	10	2/12*	0/12*
	20	0/10**	0/10*
MDL 72222	5	4/10	4/10
	10	2/12*	1/12*
	20	0/8**	0/8*

Different animals received intraperitoneally either vehicle or the cited doses of tropisotron or MDL 72222 30 min prior to administration of cisplatin (10 mg/kg, i.p.). Emesis and retching behaviours were recorded for the next 90 min immediately following cisplatin injection. *$p < 0.05$ and **$p < 0.01$ indicate significant differences relative to vehicle control by Dunn's multiple comparisons test

which are not significantly different from each other. Both tropisetron and MDL 72222 also attenuated the appearance of retches in the shrews in a dose-dependent manner ($kw_{3,40} = 16.31$, $p < 0.001$ and $kw_{3,38} = 11.18$, $p < 0.01$ respectively). Dunn's multiple comparisons test showed that significant attenuations in cisplatin-induced retching occurred at the 10 ($p < 0.01$ and $p < 0.05$ respectively) and 20 mg/kg doses ($p < 0.05$ for both antagonists). Their respective ID_{50} values were computed as 2.71 ± 4.5 and 2.52 ± 2.59 mg/kg, which are not significantly different from each other.

Intraperitoneal administration of serotonin induced emesis in the least shrew in a dose-dependent manner ($kw_{3,22} = 19.84$, $p < 0.0002$) (Table 3). Dunn's multiple comparisons test showed that significant enhancements in the number of shrews exhibiting vomiting occured at the 5 ($p < 0.01$) and

Table 3. Dose-dependent emetic effects of some selective and nonselective 5-HT_3 agonists

	Dose (mg/kg)	Number of animals vomiting/tested	Vomiting frequency (90 minutes)	Latency to first vomit (minutes)
Control	0	0/8	–	–
2-methyl-5-HT	1.25	2/9	2 ± 0	1.05 ± 0.13
	2.5	6/8*	2.3 ± 0.7	1.2 ± 0.17
	5	8/8***	2.9 ± 0.5	1.2 ± 0.17
5-HT	2.5	2/6	2 ± 0	0.72 ± 0.22
	5	6/6**	1.8 ± 0.4	0.63 ± 0.27
	10	6/6**	2.7 ± 0.6	0.65 ± 0.1
5-HTQ	5	3/7	2.3 ± 0.3	0.75 ± 0.32
	10	5/7*	2 ± 0.5	2.47 ± 0.53
	20	6/6*	4.2 ± 1	1.68 ± 0.7
mCPBG	5	1/8	1 ± 0	10
	10	3/9	1.3 ± 0.3	14 ± 9.2
	20	6/9*	1.7 ± 0.5	6.1 ± 1.2

Emetic parameters were recorded for 30 minutes immediately following the intraperitoneal administration of the cited doses of selective and nonselective 5-HT_3 receptor agonists. Values for vomiting frequency and latency to first vomit are mean ± SEM of those animals which exhibited emesis. Animals which failed to vomit were not included in the mean values. *$p < 0.05$; **$p < 0.01$ and ***$p < 0.001$ indicate significant differences relative to vehicle-injected control by Dunn's multiple comparisons test

10 mg/kg ($p < 0.01$) doses of 5-HT. Its ED_{50} is equal to 2.6 ± 1 mg/kg. The selective 5-HT_3 receptor agonist, 2-methyl-5-HT, also induced emesis ($kw_{3,29} = 20.13$, $p < 0.0002$) in a dose-dependent fashion with an ED_{50} value of 1.9 ± 1.14 mg/kg (Table 3). Posthoc analysis indicated that significant enhancements occurred at the 2.5 ($p < 0.05$) and 5 mg/kg ($p < 0.001$) doses. In addition, another selective 5-HT_3 receptor agonist, mCPBG, caused emesis in a dose-dependent manner ($kw_{3,30} = 10.21$, $p < 0.017$) with an ED_{50} of 16.12 ± 1.18 mg/kg (Table 3). Significant enhancement in vomiting was only seen at its 20 mg/kg dose ($p < 0.05$). The peripherally acting 5-HT_3 receptor agonist, 5-HTQ, also produced emesis in the least shrew ($kw_{3,24} = 14.88$, $p < 0.002$) (Table 3). Its ED_{50} value was computed to be 7.35 ± 1.31 mg/kg. Dunn's multiple comparisons test indicated that significant potentiations in the number of shrews vomiting occurred at 10 ($p < 0.05$) and 20 mg/kg ($p < 0.01$) doses of 5-HTQ. Depending upon the dose administered, mCPBG required 6–14 min to induce emesis in the shrews, whereas other 5-HT agonists generally caused vomiting within 2 min of their administration. The latency to onset of emesis was not dependent on the dose of particular 5-HT_3 agonist administered.

Discussion

This study introduces the least shrew (Cryptotis parva) as a new experimental vomiting model. Outside the family of shrews (Soricidae), the smallest avail-

able mammal model (more than 1 kg) for investigating emetic and antiemetic properties of different agents is the ferret (Costall et al., 1987; Florczyk et al., 1982). The adult house musk shrew (Suncus murinus) is similar in size to a young adult rat and has been characterized as an experimental animal model for the induction of emesis (Matsuki et al., 1988; Mutoh et al., 1992; Okada et al., 1994; Torii et al., 1991a,b, 1993, 1994). It appears that in the house musk shrew cisplatin induces emesis via its active metabolite, cis-diaquodiammine platinum II (Mutoh et al., 1992).

The least shrew is at least five times smaller than a young adult mouse. It is probably easier and cheaper to test vomiting and anti-vomiting effects of various drugs in this animal. As in the house musk shrew, the present study demonstrated that intraperitoneal administration of cisplatin in the least shrew caused a dose-dependent increase in the number of animals exhibiting vomiting. In addition, the frequency of emetic episodes in animals exhibiting vomiting increased in a dose-dependent manner following cisplatin administration. Cisplatin appears to be a more potent emotogenic drug in the least shrew (ED_{50} = 6.43 ± 1) than in the house musk shrew (ED_{50} = 10 mg/kg) (Mutoh et al., 1992). Furthermore, dose for dose, the latency to onset of first cisplatin-induced emetic response is shorter in the least shrew (i.e. 19–37 min versus 47–88 min). The least shrew and the house musk shrew belong to two different subfamilies of Soricidae, namely the Soricinae and the Crocidurinae respectively (Churchfield, 1990). The Soricidae have very high metabolic rates relative to Crocidurinae genus (Churchfield, 1990) and therefore exhibit vomiting more quickly in response to cisplatin since they probably convert cisplatin to its active metabolite(s) more rapidly. So far, published studies have not discussed the effects of cisplatin on the induction of retching in the house musk shrew. In the least shrew intraperitoneal injection of cisplatin produced retching frequencies similar to the number of vomiting episodes. However, retching frequency in a given animal appears to be more variable than vomiting episodes. As in the case of vomiting, retching behaviour was not observed in all animals which received up to 20 mg/kg intraperitoneal injection of cisplatin. Although cisplatin (i.p.) produced similar mean vomiting frequencies in the least shrew relative to the house musk shrew (Mutoh et al., 1992; Torii et al., 1993) or the ferret (Costall et al., 1987; Higgins et al., 1989), however, the drug's ability to induce retching behaviour in the least shrew is much weaker than in the ferret.

As discussed in the introduction, prior treatment with 5-HT_3 receptor antagonists prevent cisplatin-induced emesis not only in man but also in dog, cat, ferret and house musk shrew. Likewise, in the present study, two tested 5-HT_3 receptor antagonists (tropisetron and MDL 72222) prevented cisplatin-induced emesis and retching in the least shrew in a dose-dependent manner with similar ID_{50} values (2–4 mg/kg). The largest dose tested (20 mg/kg) completely prevented retching and vomiting. In comparison with the least shrew, intraperitoneal or subcutaneous pretreatment with these antagonists has been shown to more potently prevent intraperitoneally administered, cisplatin-induced, emesis, both in the house musk shrew (Mutoh et al., 1992) and in the ferret (Higgins et al., 1989). These results seem to suggest that either 5-HT_3

receptor antagonists are generally less potent in the least shrew, or more likely, as discussed earlier, the least shrew probably very rapidly metabolizes these drugs. Some 5-HT_3 receptor antagonists such as zacopride can induce emesis by itself in ferrets following oral or i.v. administration (King, 1990; Sancilio et al., 1991). In preliminary studies in the present investigation neither tropisetron nor MDL 72222 caused emesis by themselves at doses up to 20 mg/kg. However, at 40 mg/kg (i.p.) MDL 72222 caused emesis in 8 out of 9 animals and two of these died within 30 min of injection. The effect of a large dose of tropisetron was not investigated. Intravenous injection of a large dose (64 mg/kg) of ICS 205 930, zacopride or BRL 43694 can also induce emesis in the house musk shrew (Torii et al., 1991a). These and other published data (Gale and Bunce, 1995) suggest that some of the 5-HT_3 receptor antagonists may act as partial agonists at large doses.

Intraperitoneal administration of 5-HT in the house musk shrew has been shown to potently induce emesis with an ED_{50} of 2.7 mg/kg (Torii et al., 1991b). 5-HT administration via the i.v. or s.c. routes were twice less active than its i.p. injection. In the present study, i.p. injection of 5-HT in the least shrew also caused emesis in a dose-dependent manner with a similar ED_{50} value (2.6 ± 1 mg/kg). Likewise, the 5-HT precursor, 5-hydroxytryptophan (5-HTP), induced vomiting in the least shrew (data not given). On the other hand, 5-HT appears not to be emotogenic in the ferret (Kamoto et al., 1993; Ravenscroft et al., 1992). Since 5-HT and 5-HTP (following its conversion to serotonin) are nonselective 5-HT receptor agonists, the emetic effects of some more selective 5-HT_3 receptor agonists were investigated in the least shrew. 2-Methyl 5-HT appeared to be the most potent emotogenic 5-HT_3 receptor agonist with the following ED_{50} potency order: 2-methyl 5-HT ≈ 5-HT ($p > 0.05$) <5-HTQ ($p < 0.01$) < mCPBG ($p < 0.001$). 2-Methyl 5-HT also seems to be the most potent emotogenic serotonergic agent in the house musk shrew (Torii et al., 1991b) and it can cause emesis in the ferret (Sancilio et al., 1991). 5-HT, 5-HTP, 2-methyl 5-HT and 5-HTQ generally produced emesis in the least shrew within 2-min of their i.p. administration. Both 5-HT and 2-methyl 5-HT have similar rapid onset vomiting latency in the house musk shrew (Torii et al., 1991b; Ito et al., 1995). Similar to 5-HT, 2-methyl 5-HT appears not to induce emesis, but can cause retching in ferrets (Higgins et al., 1989; Kamoto et al., 1993). 1-m-Chlorophenylbiguanide (mCPBG) appeared to be the weakest emotogenic 5-HT_3 receptor agonist in the least shrew and only at the highest dose tested (20 mg/kg) produced a significant increase in the number of animals vomiting. In addition, its vomiting onset latency was relatively much longer (6–14 min). Likewise, mCPBG has been shown not to be consistently emotogenic in the house musk shrew following its i.v. or i.p. injection, however, those animals which exhibited emesis produced the behaviour within 1 min of its injection (Ito et al., 1995). Furthermore, in the ferret mCPBG fails to induce consistent vomiting at lower doses, but it can cause both retching and vomiting behaviours at large doses (Kamoto et al., 1993; Sancilio et al., 1991). Although the cited studies on the action of 5-HT_3 receptor agonists have not discussed retching behaviour in the house musk shrew, in the present study the tested 5-HT_3 receptor agonists

failed to induce retching with the exception of 5-HTQ. Unlike selective 5-HT_3 receptor agonists, both selective 5-HT_{1A} agonist (8-OH DPAT) and a selective 5-$HT_{2A/C}$ agonist (DOI) failed to induce emesis in the least shrew (data not given).

The present and discussed studies clearly show significant inter- and intraspecies differences in the action of various 5-HT_3 receptor agonists in producing emesis. Furthermore, 5-HT_3 receptor antagonists show differences in their antiemetic effects against cisplatin-induced vomiting. Indeed, the antiemetic potency of ondansetron is greater than granisetron in the house musk shrew (Ito et al., 1995; Torii et al., 1991a). However, most studies with dog and ferret exhibit opposite results (Haga et al., 1993; Kamoto et al., 1991). Such differences are also apparent in radioligand binding studies. Indeed, mCPBG was shown to exhibit a 25-, 93- and 2,000-fold higher affinity for 5-HT_3 receptors in the rat cortex than in NG 108–15 cells, rabbit ileum and guinea-pig ileum, respectively (Wong et al., 1993a). Moreover, 5-HT_3 receptor number significantly varies when labeled by different radioligands, even in the same tissue (Wong et al., 1993b). In addition, 5-HT release from enterochromaffin cells of guinea-pig small intestine is sensitive to inhibitory action of granisetron but not ondansetron (Gebauer et al., 1993). The present and discussed studies are highly suggestive of variants in the 5-HT_3 receptor class. However, a rigorous classification has yet to be achieved (Reviews: Hoyer and Martin, 1997; Jackson and Yakel, 1995).

As mentioned in the introduction section, it is well established that 5-HT_3 receptor antagonists can prevent cytotoxic drug-induced emesis in both man and animals. However, the precise site of their inhibitory action is unclear. In general, much of published literature favors a peripheral mechanism, since vagotomy can abolish emesis produced by chemotherapeutic agents in several animal species (see introduction). However, direct injection of 5-HT_3 receptor antagonists into the CNS can abolish peripherally administered cisplatin-induced emesis (Gidda et al., 1995; Higgins et al., 1989; Smith et al., 1988; Yoshida et al., 1992). Of particular interest is the novel finding that unlike zatosteron (a 5-HT_3 receptor antagonist), i.v. injection of zatosteron-quat (a quaternary analogue of zatesteron with a similar binding affinity for 5-HT_3 site), which cannot penetrate the blood brain-barrier, failed to antagonize cisplatin-induced vomiting in dogs (Gidda et al., 1995). But, like zatosteron, its quaternary analogue potently inhibited the induced vomiting following its intracerebroventricular injection. This would suggest a peripheral site for the action of 5-HT_3 receptor antagonists. However in the ferret, unlike in the dog, peripheral administration of quaternary forms of either zatosteron or tropisetron can prevent cisplatin induced emesis (Gidda et al., 1991). In the present study, intraperitoneal administration of the "peripherally acting" quaternary analogue of serotonin, 5-HTQ [a selective 5-HT_3 receptor agonist (Dukat et al., 1990)] induced emesis in the least shrew within 2-min of its administration. Although, relative to 5-HT, 5-HTQ has 10 times greater affinity for the 5-HT_3 site, it was nearly 3 times less potent than 5-HT in causing emesis. The question, does 5-HTQ produce emesis via a peripheral mechanism in the least shrew, cannot yet be answered since radiolabeled 5-HTQ is

unavailable. This laboratory is currently in the process of investigating the antiemetic effects of direct CNS injection of this agent.

In summary, the results of this investigation indicate that the least shrew is a valid experimental emesis model. As with other animals, pretreatment with 5-HT_3 receptor antagonists prevent cisplatin-induced emesis in the least shrew. Furthermore, 5-HT, its precursor 5-HTP, or analogues of 5-HT, which can directly stimulate 5-HT_3 receptors induce emesis rapidly in this new animal emesis model.

Acknowledgements

The author thanks C. F. Gerdes for technical assistance and R. Chronister for typing the manuscript. This work was supported by NIDA grant DA 07627 and EPA grant CR 823734010.

References

Andrews PLR, Davis CJ, Maskell L (1990) The abdominal visceral innervation and the emetic reflex: pathways, pharmacology, and plasticity. Can J Physiol Pharmacol 68: 325–345

Barnes NM, Ge J, Jones WG, Naylor RJ, Rudd JA (1990) Cisplatin induced emesis: preliminary results indicative of changes in plasma levels of 5-hydroxytryptamine. Br J Pharmacol 62: 862–864

Bhandari P, Gupta YK, Seth SD, Chugh A (1989) Cisplatin-induced emesis: effect of chemoreceptor trigger zone ablation in dogs. Asia Pacific J Pharmacol 4: 209–211

Churchfield S (1990) The natural history of shrews. Cornell University Press, Comstock Publishing Associates, Ithaca NY

Coates A, Abraham S, Kaye SB, Sowerbutts T, Frewin C, Fox RM, Tattersal MHN (1983) On the receiving end – patient perception of the side-effects of cancer chemotherapy. Eur J Cancer Clin Oncol 19: 203–208

Colbert EH (1958) Evolution of the vertibrates. John Wiley and Sons, New York, pp 249–261

Costall B, Domeney AM, Naylor RJ, Tattersall FD (1987) Emesis induced by cisplatin in the ferret as a model for the detection of antiemetic drugs. Neuropharmacology 26: 1321–1326

Cubeddu LX, Hoffman IS, Fuenmayor NT, Malave JJ (1992) Changes in serotonin metabolism in cancer patients: its relationship to nausea and vomiting induced by chemotherapeutic drugs. Br J Cancer 66: 198–203

de Bruijin KM (1992) Tropisetron: a review of the clinical experience. Drugs 43: 11–22

Darmani NA, Zhao W (1998) Production of serotonin syndrome by 8-OH DPAT in Cryptotis parva. Physiol Behav (in press)

Darmani NA, Mock OB, Towns LC, Gerdes CF (1994) The head-twitch response in the least shrew (Cryptotis parva) is a 5-HT_2- and not a 5-HT_{1C}-mediated phenomenon. Biochem Pharmacol Behav 48: 383–396

Dukat M, Miller K, Titler M, Glennon RA (1990) Binding of amine substituted and quaternary amine analogs of serotonin at 5-HT_3 serotonin receptors. Med Chem Res 1: 271–276

Florczyk AP, Schurig JE, Bradner WT (1982) Cisplatin-induced emesis in the ferret: a new animal model. Cancer Treatment Rep 66: 187–189

Fukui H, Yamamoto M, Sato S (1992) Vagal afferent fibers and peripheral 5-HT receptors mediate cisplatin-induced emesis in dogs. Jpn J Pharmacol 59: 221–226

Gale JD, Bunce KT (1995) Pharmacological characterization of 5-hydroxytryptamine receptors in the gastrointestinal tract. In: Gaginella TS, Galligan JJ (eds) Serotonin and gastrointestinal function. CRC Press, Bocca Raton, Fl, pp 33–52

Gebauer A, Nerger M, Kilbinger H (1993) Modulation by 5-HT_3 and 5-HT_4 receptors of the release of 5-hydroxytryptamine from guinea-pig small intestine. Naunyn Schmiedebergs Arch Pharmacol 347: 137–140

Gidda JS, Evans DC, Krushinsk JH, Robertson DW (1991) Differential effects of quaternized 5-HT_3 receptor antagonists in dogs and ferrets. In: Bradley PB (ed) Serotonin 1991: 5-HT-CNS receptors and brain function. University of Birmingham Press, Birmingham, pp 165–172

Gidda JS, Evans DC, Cohen ML, Wong DT, Robertson DW, Parli CJ (1995) Antagonism of serotonin$_3$ (5-HT_3) receptors within the blood-brain barrier prevents cisplatin-induced emesis in dogs. J Pharmacol Exp Ther 273: 695–701

Gunning SJ, Hagan RM, Tyers MB (1987) Cisplatin induces biochemical and histological changes in the small intestine of the ferret. Br J Pharmacol 90: 135P

Haga K, Inaba K, Shoji H, Morimoto Y, Fukuda T, Setoguchi M (1993) The effects of orally administered Y-25130, a selective 5-HT_3 receptor antagonist, on chemotherapeutic-agent induced emesis. Jpn J Pharmacol 63: 377–383

Hawthorn J, Ostler KJ, Andrews PLR (1988) The role of the abdominal visceral innervation and 5-hydroxytryptamine M-receptors in vomiting induced by the cytotoxic drugs cyclophosphamide and cisplatin in the ferret. Quat J Exp Physiol 73: 7–21

Higgins GA, Kilpatrick GJ, Bunce KT, Jones BJ, Tyers MB (1989) 5-HT_3 receptor antagonists injected into the area postrema inhibit cisplatin-induced emesis in the ferret. Br J Pharmacol 97: 247–255

Hoyer D, Martin G (1997) 5-HT receptor classification and nomenclature: towards a hormonization with the human genome. Neuropharmacology 36: 419–428

Ito C, Isobe Y, Kijima H, Kiuchi Y, Ohtsuki H, Kawamura R, Tsuchida K, Higuchi S (1995) The antiemetic activity of GK-128 in Suncus murinus. Eur J Pharmacol 285: 37–43

Jackson MB, Yakel JL (1995) The 5-HT_3 receptor channel. Ann Rev Physiol 57: 447–468

Kamato T, Miyata K, Ito H, Yuki H, Yamano M, Honda K (1991) Antiemetic effects of YM060, a potent and selective serotonin (5-HT)$_3$-receptor antagonist, in ferrets and dogs. Jpn J Pharmacol 57: 387–395

Kamato T, Ito H, Nagakura Y, Nashida A, Yuki H, Yamano M, Miyata K (1993) Mechanisms of cisplatin- and m-chlorophenylbiguanide-induced emesis in ferrets. Eur J Pharmacol 196: 299–305

Kilpatrick GJ, Butler A, Burridge J, Oxford AW (1990) 1-(m-Chlorophenyl)-biguanide, a potent high affinity 5-HT_3 receptor agonist. Eur J Pharmacol 182: 193–197

King GL (1990) Emesis and defications induced by the 5-hydroxytryptamine (5-HT_3) receptor antagonist zacopride in the ferret. J Pharmacol Exp Ther 253: 1034–1041

Laszlo J (1982) Treatment of nausea and vomiting caused by cancer chemotherapy. Cancer Treat 9: 3–9.

Lucot JB (1989) Blockade of 5-hydroxytryptamine 3 receptors prevents cisplatin-induced but not motion- or xylazine-induced emesis in the cat. Pharmacol Biochem Behav 32: 207–210

Matsuki N, Ueno S, Kaji T, Ishihara A, Wang C-H, Saito H (1988) Emesis induced by cancer chemotherapeutic agents in the Suncus murinus: a new experimental model. Jpn J Pharmacol 48: 303–306

McCarthy LE, Boyison HL (1984) Cisplatin-induced vomiting eliminated by ablation of the area postrema in cats. Cancer Treatment Rep 68: 401–404

Milne RJ, Heel RC (1991) Ondansetron: therapeutic use as an antiemetic. Drugs 41: 574–595

Mutoh M, Imanishi H, Torii Y, Tamura M, Saito H, Matsuki N (1992) Cisplatin-induced emesis in Suncus murinus. Jpn J Pharmacol 58: 321–324

Okada F, Torii Y, Saito H, Matsuki N (1994) Antiemetic effects of serotonergic 5-HT_{1A}-receptor agonists in Suncus murinus. Jpn J Pharmacol 64: 109–114

Plosker GL, Goa KL (1991) Granisetron: a review of its pharmacological properties and therapeutic use as an antiemetic. Drugs 42: 805–824

Ravenscroft M, Wells U, Bhandari P, Andrews PLR (1992) Agonist evidence for the involvement of 5-HT_3 receptors in emesis in the ferret. In: Bianchi AL, Grelot L, Miller AD, King GL (eds) Mechanisms and control of emesis. J Libby Eurotext, Paris, pp 251–263

Richardson BP, Engel G, Donatsch P, Stadler PA (1985) Identification of serotonin M-receptor subtypes and their specific blockade by a new class of drugs. Nature 316: 126–131

Rosenberg B (1985) Fundamental studies with cisplatin. Cancer 55: 2303–2316

Rudd JA, Naylor RJ (1994) Effects of 5-HT_3 receptor antagonists on models of acute and delayed emesis induced by cisplatin in the ferret. Neuropharmacology 33: 1606–1608

Sancilio LF, Pinkus LM, Jackson CB, Munson HR (1991) Studies on the emetic and antiemetic properties of zacopride and its enantiomers. Eur J Pharmacol 192: 365–369

Schwörer H, Racké K, Kilbinger H (1991) Cisplatin increases the release of 5-hydroxytryptamine (5-HT) from the isolated vascularly perfused small intestine of the guinea-pig. Involvement of 5-HT_3 receptors. Naunyn Schmiedebergs Arch Pharmacol 344: 143–149

Smith WL, Callaham EM, Alphen RS (1988) The emetic activity of centrally administered cisplatin in cats and its antagonism by zacopride. J Pharm Pharmacol 40: 142–143

Smith WL, Alphen RS, Jackson CB, Sincilio LF (1989) The antiemetic profile of zacopride. J Pharm Pharmacol 41: 101–105

Stables R, Andrews PLR, Bailey HE, Costall B, Gunning SJ, Hawthorn J, Naylor RJ, Tyers MB (1987) Antiemetic properties of the 5-HT_3-receptor antagonist, CR38032F. Cancer Treat Rev 14: 333–336

Torii Y, Saito H, Matsuki N (1991a) Selective blockade of cytotoxic drug-induced emesis by 5-HT_3 receptor antagonists in Suncus murinus. Jpn J Pharmacol 55: 107–113

Torii Y, Saito H, Matsuki N (1991b) 5-Hydroxytryptamine is emotogenic in the house musk shrew, Suncus murinus. Naunyn Schmiedebergs Arch Pharmacol 344: 564–567

Torii Y, Mutoh M, Saito H, Matsuki N (1993) Involvement of free radicals in cisplatin-induced emesis in Suncus murinus. Eur J Pharmacol 248: 131–135

Torii Y, Saito H, Matsuki N (1994) Induction of emesis in Suncus murinus by pyrogallol, a generator of free radicals. Br J Pharmacol 111: 431–434

Trizzi PL, Laszlo J (1987) Optimum management of nausea and vomiting in cancer chemotherapy. Drugs 34: 136–149

Wong EHF, Bonhaus DW, Wu I, Stefanich E, Eglen RM (1993a) Labelling of 5-hydroxytryptamine$_3$ receptors with a novel 5-HT_3 receptor ligand, ^{3}[H]RS-42358-197. J Neurochem 60: 921–930

Wong EHF, Bonhaus DW, Lee AJ, Wu I, Loury DN, Eglen RM (1993b) Different densities of 5-HT_3 receptors are labeled by [^{3}H] quipazine, [^{3}H] GR65630 and [^{3}H] granisetron. Neuropharmacology 32: 869–875

Yoshida N, Omoya H, Ito T (1992) DAT-582, a novel 5-HT_3 receptor antagonist, is a potent and long lasting antiemetic agent in the ferret and dog. J Pharmacol Exp Ther 260: 1159–1165

Author's address: Assoc. Prof. Dr. N. A. Darmani, Department of Pharmacology, Kirksville College of Osteopathic Medicine, Kirksville, MO 63501, U.S.A.