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Action of 5-HT_3 receptor antagonists and dexamethasone to modify cisplatin-induced emesis in *Suncus murinus* (house musk shrew)

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Abstract

Ondansetron (1–3 mg/kg), granisetron (0.3–1 mg/kg) and dexamethasone (0.3–1 mg/kg), administered at 12-h intervals, were investigated for their potential to prevent cisplatin (30 mg/kg, i.p.)-induced emesis during a 72-h observation period. Ondansetron appeared more active than granisetron to antagonise the emetic response occurring in the first 4-h ($P<0.05$) period, but none of the regimens significantly antagonised emesis during the 0–24- and 24–72-h periods ($P>0.05$). However, ondansetron was more active to antagonise emesis on day 1 using a more frequent drug administration, whereas bilateral vagotomy only reduced emesis for 2 h, and 5-HT, 2-methyl-5-HT and 1-*m*-chloro-phenylbiguanide (up to 20–30 mg/kg, i.p.) were not emetic. The combination of ondansetron 1 mg/kg and dexamethasone 1 mg/kg, both administered every 12 h, significantly delayed the onset of emesis ($P<0.05$) but failed to reduce the total numbers of retches + vomits over the 3-day period ($P>0.05$). Results are discussed in relation to the clinical situation.

Keywords: Cisplatin; Anti-emetic; 5-HT_3 receptor; Glucocorticoid; Delayed emesis

1. Introduction

Nausea and emesis following treatment with chemotherapeutic drugs such as cisplatin is a well-established phenomenon. The nausea and emesis occurring in man can be classified into 'acute' (occurring in the first 24 h) or 'delayed' (occurring 24–120 h post the start of chemotherapy) phases (Kris et al., 1985; Martin, 1996). Generally, the acute phase is highly susceptible to antagonism by 5-hydroxytryptamine$_3$ (5-HT_3) receptor antagonists but the delayed phase is more resistant (Kris, 1998). However, it has been also proposed that delayed emesis can begin as early as 16 h, based on more detailed analysis of data from the use of 5-HT_3 receptor antagonists (Clark and Gralla, 1993), In general, glucocorticoids improve the control of both phases when used in combination with other anti-emetic drugs (Gralla et al., 1996; Saeki et al., 2001).

Suncus murinus (a house musk shrew) is a species of insectivore that has been used to study the emetic mechanism of action of cisplatin and other chemotherapeutic drugs (Matsuki et al., 1988). However, the majority of the previous studies only focused on the emesis occurring during the first 90–180 min after the administration of cisplatin. The studies revealed the mechanism of emesis could involve the generation of free radicals, a potential release of 5-HT, and the abdominal vagi (Matsuki et al., 1993; Mutoh et al., 1992). 5-HT_3 receptor antagonists and 5-HT_{1A} receptor agonists reduce cisplatin-induced emesis in *S. murinus* (Mutoh et al., 1992; Okada et al., 1994), as does morphine (Kakimoto et al., 1997) (possibly by activating μ-opioid receptors), (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (Okada et al., 1995) (possibly by activating 5-HT_2 receptors) and resiniferatoxin (Andrews et al., 2000) (possibly by activating vanilloid receptors). Some tachykinin NK_1 receptor antagonists are also active in this species to reduce cisplatin-induced emesis (Gardner et al., 1995).

Unfortunately, however, an assessment of a compounds' potential to reduce cisplatin-induced emesis over a 90–180 min period is not likely to predict the activity of the

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compound to prevent the entire acute or delayed phase of emesis in man (Naylor and Rudd, 1996). In the present studies, therefore, we have used *S. murinus* and longer observation times (up to 72 h) in an attempt to develop a new model of cisplatin-induced acute and delayed emesis. The selective 5-HT_3 receptor antagonists ondansetron (Butler et al., 1988) and granisetron (Sanger and Nelson, 1989), and the glucococorticoid dexamethasone (Swartz and Dluhy, 1978), were used as respective anti-emetic agents to characterize the profile of the cisplatin-induced emetic response. The effect of sectioning the abdominal vagi on the emetic action of cisplatin was also investigated and the emetic potential of 5-HT and 5-HT_3 receptor selective agonists was determined. The data are discussed in terms of the usefulness of the *S. murinus* cisplatin-induced model to anti-emetic research.

2. Methods

2.1. Animals

The experiments were performed on male or female *S. murinus* (30–85 g), bred at the Chinese University of Hong Kong. Prior to the experiments, they were housed in a temperature-controlled room at 24 ± 1 °C under artificial lighting, with lights on between 0700 and 1730 h. They were allowed free access to water and pelleted cat chow (Feline Diet 5003, PMI® Feeds, USA). Any animal experiencing a rapid loss of body weight (>20%), or impaired mobility, or labored breathing and cyanosis, was taken as evidence of the animals experiencing a moribund state, and the animals were excluded from the experiment. On exclusion, or at the end of the experiment, the animals were killed by an injection of sodium phenobarbitone (60 mg/kg, i.p.). All experiments and protocols were approved and conducted in accordance with the Animal Research Ethics Committee, The Chinese University of Hong Kong. Animals were not used more than once.

2.2. Lesion of abdominal vagi

The surgical techniques to lesion the vagi have been described previously (Mutoh et al., 1992). Briefly, the animals were anaesthetized with pentobarbital sodium (50 mg/kg, i.p.) and the ventral abdominal surface shaved from the costal margin to the inguinal ligament. The skin was subsequently sterilized with 0.5% chlorhexidine in 70% alcohol. A midline 1.5-cm laparotomy incision was then made and the ventral and dorsal trunks running along the oesphagus were located by blunt dissection and at least 0.5 cm of each nerve removed (the serosa of the oesophagus was slightly incised to facilitate the procedure). Braided silk suture (2/0, Mersilk, Ethicon, UK) was used to ligate the cut ends of the vagi. The abdominal contents were moistened with sterile saline and the peritoneum and skin layers closed separately with 2/0 braided silk sutures using interrupted stitches. Skin wounds were sterilized with 0.5% chlorhexidine in 70% alcohol and sprayed with antibiotic aerosol (Tribiotic Spray®, Riker Laboratories, UK) and then silicone wound dressing (Opsite®, Smith and Nephew, UK). Sham operation was performed using similar procedures except the nerves were not lesioned. All animals were allowed 7 days to recover from the operative procedures before further drug administration.

2.3. Induction and measurement of emesis

On the day of the experiment, *S. murinus* were transferred to clear Perspex observation chambers (21 × 14 × 13 cm). In preliminary experiments, cisplatin (10–80 mg/kg) or vehicle (saline, 0.9% w/v, adjusted to pH 4 with 0.1 N HCl) was injected intraperitoneally ($t = 0$) to determine the optimum dose producing emesis during a 72-h observation period. Animals that had received surgery were removed from their observation chambers and injected with cisplatin (30 mg/kg, i.p., at $t = 0$; dose determined from preliminary studies). In other experiments, animals were injected subcutaneously with granisetron (0.3–1 mg/kg), ondansetron (1–3 mg/kg) and/or dexamethasone (0.3–1 mg/kg), or their respective vehicles, 30 s post the administration of cisplatin (30 mg/kg, i.p., at $t = 0$; dose selected from preliminary studies). Generally, drug or vehicle treatment was continued at regular 12-h intervals for the duration of the experiment. However, in one experiment, ondansetron was administered at $t = 30$ s and $t = 6$ h. In other experiments, animals were injected intraperitoneally with 5-HT (10–30 mg/kg), 2-methyl-5-HT (5–20 mg/kg), 1-*m*-chloro-phenylbiguanide (5–20 mg/kg) or saline (0.9% w/v; 5 ml/kg) and observed for 30 min.

Behaviour of animals treated with cisplatin was recorded remotely for 24–72 h using a closed circuit video recording system. Emesis was characterized by rhythmic abdominal contractions that were either associated with the oral expulsion of solid or liquid material from the gastrointestinal tract (i.e. vomiting) or not associated with the passage of material (i.e. retching movements). An episode of retching and/or vomiting was considered separate when the animal changed its location in the observation cage, or when the interval between retches and/or vomits exceeded 2 s. At all stages of the experiments, the animals were allowed free access to water and pelleted cat chow (Feline Diet 5003, PMI® Feeds, USA).

2.4. Statistical analysis

The latency to retch or vomit and/or the total number of retches, vomits and episodes was calculated in each 1-h period and for the 0–4-, 0–24- and 24–72-h periods. The 0–4-h period was specifically analysed to provide information comparable with data obtained from other

experiments using other species. The significance of the difference between treatments was assessed by an unpaired Student's *t*-test, or one-way analysis of variance (ANOVA) followed by either a Dunnett's or Bonferroni's multiple comparison test, as appropriate (Graphpad Prism® version 3.0a, Graphpad Software, San Diego, USA). The significance of difference between the incidences of mortality was assessed by a Fisher's exact test (Statview®, Abacus Concepts, North Carolina, USA). Differences were considered significant when $P < 0.05$.

2.5. Drugs used

Cisplatin (Sigma-Aldrich, Saint Louis, USA) was formulated in saline (0.9% w/v, adjusted to pH 4 with 0.1 N HCl) by heating and sonication and administered in a volume of 10 ml/kg. Ondansetron hydrochloride dihydrate (GlaxoWellcome, Barnard Castle, UK), granisetron hydrochloride (SmithKline Beecham Pharmaceuticals, Brentford, UK) and dexamethasone 21-phosphase disodium salt (Sigma-Aldrich, Saint Louis, USA) were formulated in saline (0.9% w/v) and administered in a volume of 2 ml/kg. 5-Hydroxytryptamine creatinine sulphate (Sigma-Aldrich), 2-methyl-5-hydroxytryptamine maleate (Research Biochemicals International, Natric, MA, USA) and 1-*m*-chlorophenyl-biguanide hydrochloride (Research Biochmicals International) were prepared in distilled water and administered in a volume of 5 ml/kg. Doses are expressed as the free base.

3. Results

3.1. Emetic potential of cisplatin

Cisplatin at the dose of 10 mg/kg induced a retching and vomiting response in one out of five animals following a latency of 2.4 h. There were two episodes comprising three and four retches + vomits, respectively: the episodes were separated by about 2 h and no further episodes of retching or vomiting occurred (Fig. 1). Cisplatin at 20 mg/kg induced a retching and/or vomiting response in three out of five animals following a latency of 6.6 ± 5.8 h. The emesis that occurred comprised 24.2 ± 11.3 retches + vomits during the 0–24-h period and 22.4 ± 19.6 retches + vomits during the 24–72-h period. Cisplatin at 40 mg/kg was fatal in one out of six animals tested (the animal died unexpectedly at 58 h) and only data for the surviving animals was analysed. In these animals, cisplatin induced emesis following a latency of 0.6 ± 0.1 h, but only four out of five exhibited a response and comprised 70.2 ± 19.5 ($P < 0.01$, compared to vehicle controls) and 18.8 ± 10.9 retches + vomits ($P > 0.05$, compared to vehicle controls) during the 0–24- and 24–72-h periods, respectively (see Fig. 1). Cisplatin at 80 mg/kg was fatal in three out of four animals (animals died/or were terminated at 10, 32 and 38 h) and the surviving animal only exhibited 1 episode of 11 retches + vomits during the first 24-h period (latency: 0.6 h) and had 121 retches + vomits during the 24–72-h period (data not shown). Saline (0.9% w/v, adjusted to pH 4 with

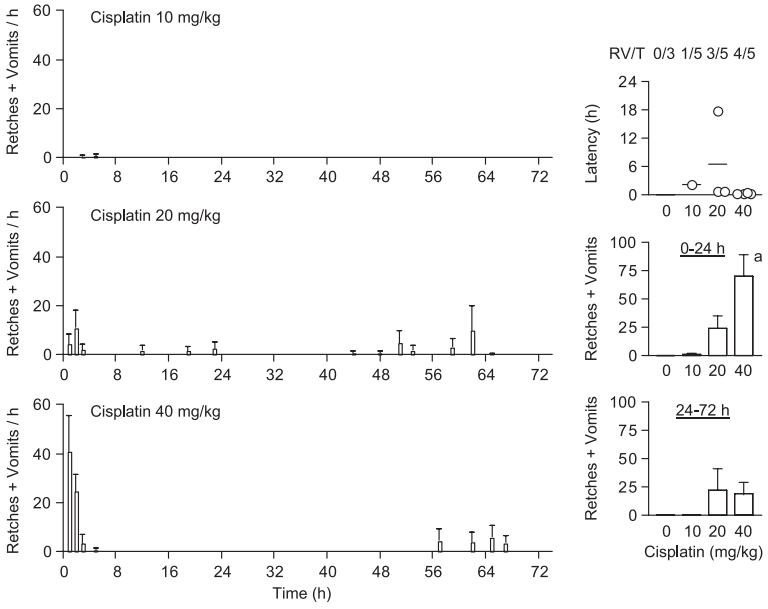

Fig. 1. The profile of cisplatin-induced retching + vomiting in *Suncus murinus* during a 72-h observation period. Cisplatin or vehicle (saline, 0.9% w/v, adjusted to pH 4 with 0.1 N HCl) was administered intraperitoneally. Results represent the mean ± S.E.M. of the total numbers of retches + vomits occurring during 1-, 0–24- and 24–72-h periods. The number of animals retching and/or vomiting out of the number of animals tested (RV/T) is also shown. Individual latencies to the first episode of retching and/or vomiting are shown as open circles (horizontal lines on the latency plot represent the mean latencies of the respective treatment groups). Significant differences relative to vehicle treated animals (actual 72-h profile not shown, since animals did not retch or vomit) are indicated as $^{a}P < 0.05$ (one-way ANOVA followed by a Dunnett's multiple comparison test).

0.1 N HCl and injected at 10 ml/kg did not induce emesis in three animals tested. In the subsequent experiments, it was decided to use cisplatin at 30 mg/kg in an attempt to provide a model of acute and delayed emesis in the absence of lethality.

3.2. Effect of abdominal bilateral vagotomy on cisplatin (30 mg/kg, i.p.)-induced emesis

In sham-operated animals, cisplatin 30 mg/kg, i.p., induced a retching and/or vomiting response following a latency of 0.5 ± 0.9 h and comprised 87.8 ± 19.8 retches + vomits during the 0–24-h period and 40.0 ± 24.1 retches + vomits during the 24–72-h period. Abdominal bilateral vagotomy did not significantly affect the cisplatin-induced retching + vomiting response during the 0–24- ($P>0.05$) and 24–72-h ($P>0.05$) periods but did significantly delay the onset of emesis by approximately 2.1 h ($P<0.05$; see Fig. 2). A more detailed analysis of the data revealed that abdominal bilateral vagotomy reduced significantly the retching + vomiting response by 93.2% during the 0–2-h observation period ($P<0.05$). Further, in the sham-operated animals, no retching or vomiting was seen during the 4–50-h period, but it occurred sporadically in the vagotomised animals ($P>0.05$).

3.3. Effect of ondansetron (1–3 mg/kg, s.c.), administered every 12 h, on cisplatin (30 mg/kg, i.p.)-induced emesis

The profile of cisplatin-induced emesis and the effect of ondansetron (1–3 mg/kg) administered twice per day are shown in Fig. 3. In the vehicle-treated animals, cisplatin induced emesis within 0.8 ± 0.1 h and there were 52.7 ± 8.8 and 53 ± 10.8 retches + vomits during the 0–24- and 24–72-h periods, respectively. Ondansetron produced a trend to reduce emesis during the 0–24- (maximum reduction was 55.2% at 3 mg/kg) and 24–72-h (maximum reduction was 73.0% at 1 mg/kg) periods but the reductions were not statistically significant ($P>0.05$). A more detailed analysis of the data revealed that ondansetron at 1 and 3 mg/kg reduced significantly the retching + vomiting response during the 0–4-h period by 100.0 ($P<0.01$) and 96.9% ($P<0.01$), respectively (Fig. 3; controls exhibited 51.7 ± 9.0 retches + vomits). Ondansetron 3 mg/kg also significantly delayed the latency to onset of cisplatin-induced emesis in the animals that exhibited the retching + vomiting response ($P<0.05$). One of the animals that received cisplatin and ondansetron at 3 mg/kg died unexpectedly during the experiment and the data was excluded from the analysis.

3.4. Effect of granisetron (0.3–1 mg/kg, s.c.), administered every 12 h, on cisplatin (30 mg/kg, i.p.)-induced emesis

The profile of cisplatin-induced emesis and the effect of granisetron (0.3–1 mg/kg) administered twice per day are shown in Fig. 4. In the vehicle-treated animals, cisplatin induced emesis within 0.9 ± 0.2 h and there were 68.4 ± 24.3 and 17.8 ± 17.8 retches + vomits during the 0–24- and 24–72-h periods, respectively. Granisetron produced a trend to reduce emesis during the 0–24-h period (maximum reduction was 75.5% at 0.3 mg/kg) and no animals vomited when granisetron was used at 1 mg/kg during the 24–72-h period, but this was not statistically significant ($P>0.05$) probably because only one out of five control animals exhibited emesis during the same period.

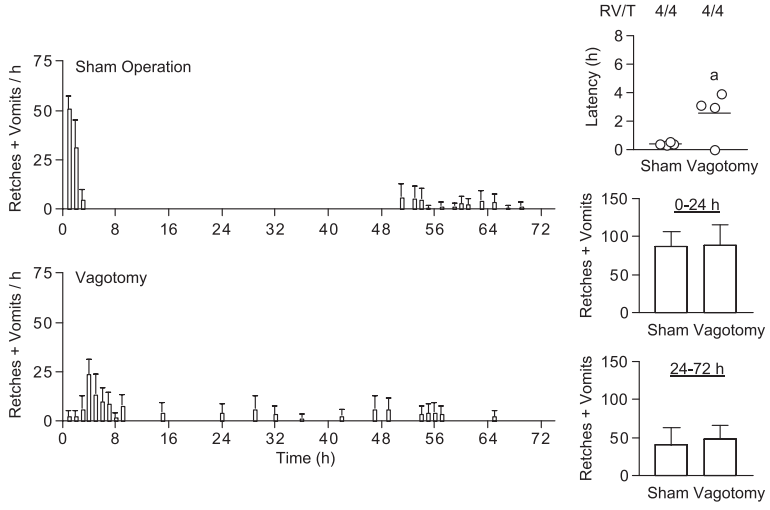

Fig. 2. The effect of abdominal bilateral vagotomy on the profile of cisplatin (30 mg/kg, i.p.)-induced retching + vomiting in *Suncus murinus* during a 72-h observation period. Results represent the mean ± S.E.M. of the total numbers of retches + vomits occurring during 1-, 0–24- and 24–72-h periods. The number of animals retching and/or vomiting out of the number of animals tested (RV/T) is also shown. Individual latencies to the first episode of retching and/or vomiting are shown as open circles (horizontal lines on the latency plot represent the mean latencies of the respective treatment groups). Significant differences relative to sham vagotomized animals are indicated as $^{a}P<0.05$ (unpaired Student's *t*-test).

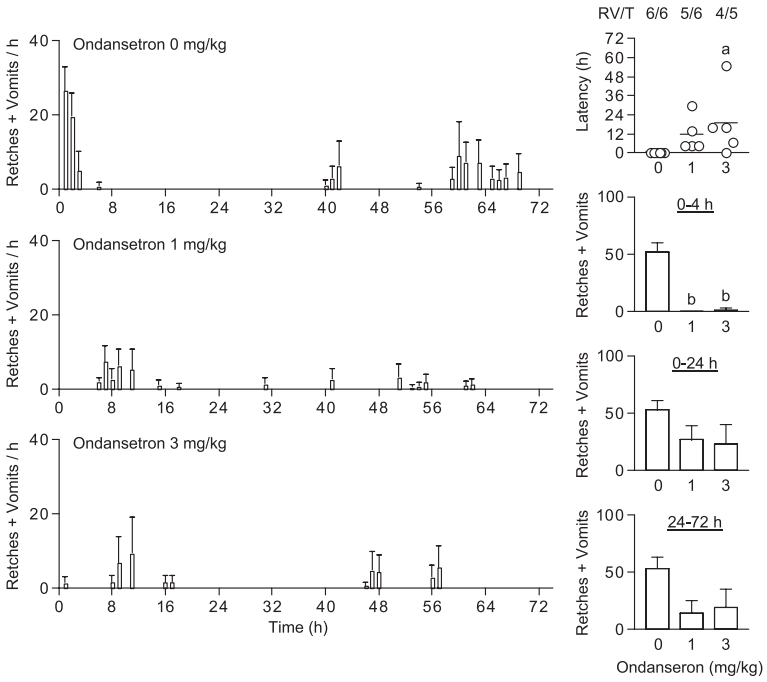

Fig. 3. The effect of ondansetron 1–3 mg/kg, s.c., administered twice per day, on the profile of retching + vomiting in *Suncus murinus* induced by a single injection of cisplatin 30 mg/kg, i.p. Ondansetron or vehicle was administered 30 s following cisplatin injection and then at 12-h intervals for the duration of the experiment. Results represent the mean ± S.E.M. of the total numbers of retches + vomits occurring during 1-, 0–4-, 0–24- and 24–72-h periods. The number of animals retching and/or vomiting out of the number of animals tested (RV/T) is also shown. Individual latencies to the first episode of retching and/or vomiting are shown as open circles (horizontal lines on the latency plot represent the mean latencies of the respective treatment groups). Significant differences relative to the respective vehicle-treated animals are indicated as $^{a}P < 0.05$ or $^{b}P < 0.01$ (one-way ANOVA followed by a Dunnett's multiple comparison test).

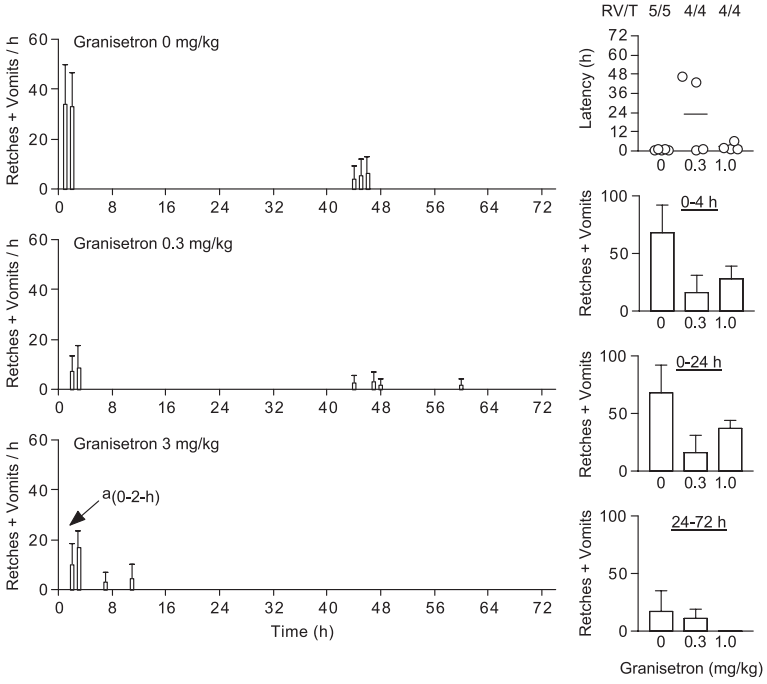

Fig. 4. The effect of granisetron 0.3–1 mg/kg, s.c., administered twice per day, on the profile of retching + vomiting in *Suncus murinus* induced by a single injection of cisplatin 30 mg/kg, i.p. Granisetron or vehicle was administered 30 s following cisplatin injection and then at 12-h intervals for the duration of the experiment. Results represent the mean ± S.E.M. of the total numbers of retches + vomits occurring during 1-, 0–4-, 0–24- and 24–72-h periods. The number of animals retching and/or vomiting out of the number of animals tested (RV/T) is also shown. Individual latencies to the first episode of retching and/or vomiting are shown as open circles (horizontal lines on the latency plot represent the mean latencies of the respective treatment groups). Significant differences relative to the respective vehicle treated animals are indicated as $^{a}P < 0.05$ (one-way ANOVA followed by a Dunnett's multiple comparison test).

Granisetron failed to modify significantly the retching + vomiting response during the 0–4-h period ($P < 0.05$). However, a more detailed analysis of the data revealed that granisetron at 0.3 and 1 mg/kg prevented retching + vomiting response during the 0–2-h period in all animals ($P < 0.05$). Granisetron failed to significantly affect the latency to onset of cisplatin-induced emesis ($P > 0.05$). Two out of six animals that received cisplatin and granisetron at 1 mg/kg died unexpectedly/or were terminated during the experiment and the data was excluded from the analysis.

3.5. Effect of dexamethasone (0.3–1 mg/kg, s.c.), administered every 12 h, on cisplatin (30 mg/kg, i.p.)-induced emesis

The profile of cisplatin-induced emesis and the effect of dexamethasone (0.3–1 mg/kg) administered twice per day are shown in Fig. 5. In the vehicle-treated animals, cisplatin induced emesis within 0.6 ± 0.1 h and there were 62.7 ± 18.2, 63.5 ± 18.8 and 48.7 ± 24.9 retches + vomits during the 0–4-, 0–24- and 24–72-h periods, respectively. Dexamethasone had no action to modify significantly the numbers of retches and/or vomits during the selected observation times ($P > 0.05$) and also failed to modify significantly the latency to onset of emesis ($P > 0.05$). However, in these experiments, three out of nine vehicle-treated animals, three out of eight dexamethasone 0.3 mg/kg treated animals, and four out of nine dexamethasone 1 mg/kg treated animals died unexpectedly/or were terminated during treatment with cisplatin (emetic data was excluded from the analysis).

3.6. The effect of ondansetron (1 mg/kg, s.c.) and/or dexamethasone (1 mg/kg, s.c.), administered every 12 h, on cisplatin (30 mg/kg, i.p.)-induced emesis

In the vehicle-treated animals, cisplatin induced emesis within 8.1 ± 7.1 h and there were 82.0 ± 27.7, 86.9 ± 29.2 and 54.8 ± 25.7 retches + vomits during the 0–4-, 0–24- and 24–72-h periods, respectively. The large variation in the control data was because one of the animals did not exhibit emesis until 57.9 h after cisplatin; the other eight animals in the control group had latencies ranging from 0.5 to 1.5 h (see Fig. 5). Ondansetron (1 mg/kg, s.c.) as a single treatment reduced significantly the retching + vomiting during the 0–4-h period by 95% ($P < 0.01$) but failed to significantly reduce retching + vomiting during the 0–24- and 24–72-h periods ($P > 0.05$). Dexamethasone (1 mg/kg, s.c.) as a single treatment potentiated significantly the retching + vomiting occurring during the 0–4- and 0–24-h periods by 90.7% ($P < 0.05$) and 80.1% ($P < 0.05$), respectively, and produced a 80.3% nonsignificant reduction ($P > 0.05$) of retching + vomiting during the 24–72-h period.

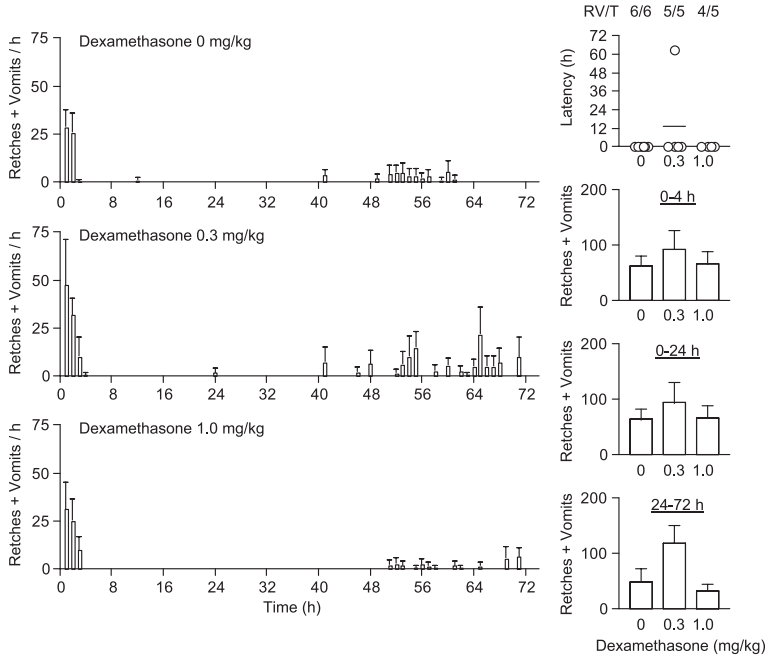

Fig. 5. The effect of dexamethasone 0.3–1 mg/kg, s.c., administered two times per day, on the profile of retching + vomiting in *Suncus murinus* induced by a single injection of cisplatin 30 mg/kg, i.p. Dexamethasone or vehicle was administered 30 s following cisplatin injection and then at 12-h intervals for the duration of the experiment. Results represent the mean ± S.E.M. of the total numbers of retches + vomits occurring during 1-, 0–4-, 0–24- and 24–72-h periods. The number of animals retching and/or vomiting out of the number of animals tested (RV/T) is also shown. Individual latencies to the first episode of retching and/or vomiting are shown as open circles (horizontal lines on the latency plot represent the mean latencies of the respective treatment groups). There were no significant differences relative to the respective vehicle-treated animals ($P > 0.05$; one-way ANOVA).

The combination treatment of ondansetron (1 mg/kg, s.c.) and dexamethasone (1 mg/kg, s.c.) provided an improved control of retching + vomiting compared to dexamethasone as a single regimen during the 0–4- and 0–24-h, but not the 24–72-h, periods (see Fig. 6; $P < 0.001$). There was no additive interaction between ondansetron and dexamethasone to reduce the retching and vomiting response ($P > 0.05$). However, the combination treatment was significantly more effective than the single treatments of ondansetron ($P < 0.05$) and dexamethasone ($P < 0.01$) in delaying the onset of the first episode of retching + vomiting (Fig. 6).

3.7. Effect of ondansetron (3 mg/kg, s.c.), administered at t=30 s and t=6 h, on cisplatin (30 mg/kg, i.p.)-induced emesis

In the previous experiments, ondansetron antagonised emesis for approximately 6 h but emesis appeared during the subsequent 6–12-h period (see Figs. 3 and 6). The administration of ondansetron 3 mg/kg, s.c., at $t = 30$ s and $t = 6$ h delayed emesis for up to 14 h ($P < 0.05$) and effectively reduced the retching + vomiting occurring during the 0–24-h period by 75% ($P < 0.05$). The experiments were terminated at 24 h (Fig. 7).

3.8. Summary of the incidence of mortality (including unexpected deaths and animals terminated humanely) observed during the studies with cisplatin 30 mg/kg, i.p., and drug/vehicle combinations during a 72-h observation period

As some of the animals died unexpectedly/or were terminated during the studies, a retrospective analysis was performed to investigate if drug treatment modified the mortality rate. The mortality rate in animals that received cisplatin and vehicle was 13.3% and only dexamethasone 1 mg/kg, s.c., significantly increased the rate to 44.4% ($P = 0.036$; see Table 1). None of the animals died in the first 24-h period.

Table 1
The effect of cisplatin 30 mg/kg, i.p., and vehicle or drug treatments on mortality rate (including unexpected deaths and animals terminated humanely) during a 72-h observation period

Treatment	Mortality (deaths/ total number tested)
Cisplatin + vehicle	4/30
Cisplatin + ondansetron 1 mg/kg	0/6
Cisplatin + ondansetron 3 mg/kg	2/15
Cisplatin + granisetron 0.3 mg/kg	0/6
Cisplatin + granisetron 1 mg/kg	2/6
Cisplatin + dexamethasone 0.3 mg/kg	3/8
Cisplatin + dexamethasone 1 mg/kg	8/18[a]
Cisplatin + ondansetron 1 mg/kg + dexamethasone 1 mg/kg	2/9

Cisplatin was administered at $t = 0$. Vehicle or drugs were administered subcutaneously at $t = 30$ s and then at regular 12-h intervals. Significant differences relative to cisplatin + vehicle-treated animals are indicated as [a]$P < 0.05$ (Fisher's Exact test).

3.9. Emetic potential of 5-HT, 2-methyl-5-HT and 1-m-chloro-phenylbiguanide in S. murinus

5-HT (10, 20 and 30 mg/kg, i.p.), 2-methyl-5-HT (5, 10 and 20 mg/kg, i.p.) and 1-*m*-chloro-phenylbiguanide (5, 10 and 20 mg/kg, i.p.) failed to induce emesis during a 30-min observation time ($n = 3$).

4. Discussion

Cisplatin (20–80 mg/kg, i.p.) has been used by several investigators to induce emesis in *S. murinus* (see Introduction for references). However, none of the studies has examined the potential of cisplatin to induce emesis over several days nor have they investigated the anti-emetic potential of glucocorticoids that is critical to validating a model of acute and delayed emesis. A new model of cisplatin-induced acute and delayed emesis in a small mammal, such as *S. murinus*, would be valuable to screen for novel anti-emetic drugs that may have activity in man.

At the start of the present studies, we considered that the dose of cisplatin to induce emesis is an important factor that must be investigated. This was based on previous experience using the ferret where the anti-emetic action of glucocorticoids is only reliably seen in models using a lower dose of cisplatin (5 mg/kg) (Rudd et al., 1994; Rudd and Naylor, 1997; Sam et al., 2001). Importantly, dexamethasone also has additive actions to reduce emesis in the lower dose model when combined with 5-HT_3 receptor antagonists (Fukunaka et al., 1998; Rudd and Naylor, 1996).

It was evident from our preliminary studies that cisplatin at doses greater than 10 mg/kg were capable of inducing emesis over a 72-h period, but the use of cisplatin at = 40 mg/kg was associated with fatalities (occurring after 24 h) that we interpreted as an unacceptable toxicity in the model. For these reasons, we decided to concentrate on the mechanism of emesis induced by cisplatin 30 mg/kg: an intermediate dose that we initially considered suitable to induce emesis in most animals over a 3-day period in the absence of lethality. In the initial experiments, cisplatin at 30 mg/kg induced a reliable retching + vomiting response during the first 3–4-h period but emesis rarely occurred during the 24–48-h period and most animals had retching and vomiting during the 48–72-h period. Pooling of the data of all experiments involving cisplatin 30 mg/kg and twice per day injections of saline revealed an incidence of 92% and 76%, respectively (data not shown), for animals to exhibit emesis during the first 24- and 24–72-h period; the incidence rates are similar for cisplatin to induce emesis in man (Hesketh, 1996; Kris et al., 1985).

Our studies with ondansetron and granisetron extend previous work in *S. murinus* demonstrating a role of 5-HT_3

receptors in the emetic reflex (Torii et al., 1991b). It was evident that a single administration of ondansetron and granisetron was capable of delaying emesis for 2–6 h. It was also interesting that ondansetron and granisetron produced a trend to reduce emesis during the 24–72-h period. However, it is important to emphasise that the reductions during the 24–72-h period were not statistically significant, even when using reasonable numbers of animals (e.g. 6–8). This was probably because the actual incidence of emesis in the respective control and treatment groups was variable but we decided not to increase the numbers of animals used in the studies because of toxicity in the model that was identified during the course of the experiments (discussed later).

Previous studies on cisplatin-induced emesis in *S. murinus* have shown that ondansetron is more potent than granisetron in antagonizing the vomiting response in tests lasting up to 180 min and that the order of potency is not predicted from radioligand binding studies or from studies to inhibit emesis in other species (Ito et al., 1995; Torii et al., 1991b). Our studies also found that ondansetron appeared more active than granisetron in preventing the initial emesis induced by cisplatin (granisetron lost activity after approximately 2 h). This was surprising since ondansetron and granisetron are approximately equipotent in the ferret and both can abolish emesis in about 4 h (Kamato et al., 1991; Yoshida et al., 1992). However, it should be noted that the emesis induced by cisplatin on days 2 and 3 was not completely controlled by ondansetron, but was abolished by the higher dose of granisetron. Whilst we do not know the pharmacokinetic profile of the compounds in *S. murinus*, it has been proposed that the pharmacology of the 5-HT_3 receptor is unique in this species (Ito et al., 1995).

It was interesting that emesis returned between the first injection of ondansetron and granisetron and their second administrations at 12 h (possibly because of clearance of the compounds?), suggesting that cisplatin is active in providing a stimulus to activate emetic circuits beyond 180 min in this species and this has not been reported previously. It is likely that the stimulus occurring during the first 24-h period is likely to be one predominantly activating a system involving 5-HT_3 receptors, since the more frequent administration of ondansetron, at 30 s and 6 h, virtually abolished emesis. However, additional factors may contribute to cisplatin-induced emesis, since 5-HT_3 receptor agonists alone in our studies were inactive to induce emesis. Certainly, the lack of emetic activity of 5-HT, 2-methyl-5-HT and 1-*m*-chlorophenylbiguanide in the present studies was unexpected, given the anti-emetic action of ondansetron and granisetron against cisplatin-induced emesis, and that other workers have observed emesis in *S. murinus* with 5-HT_3 receptor agonists (Ito et al., 1995; Javid and Naylor, 2002; Selve et al., 1994; Torii et al., 1991a). In fact, our colony of animals originally had a good emetic response to the doses of the 5-HT_3 receptor agonists used in the present studies when tested under the same experimental conditions in 1994–1995 (Rudd, unpublished data). We believe, therefore, that we have inadvertently bred our animals to be insensitive to the emetic action of 5-HT_3 receptor agonists (but not cisplatin). This seems feasible given that *S. murinus* can loose the emetic response to veratrine, after only five generations of selective breeding (Ebukuro et al., 2000).

A previous study in *S. murinus* demonstrated the effect of bilateral abdominal vagotomy in preventing cisplatin-induced emesis occurring during the first 180 min (Mutoh et al., 1992). Our studies confirmed the original observations but revealed that vagotomy only actually delays emesis for about 2 h. The implication is that the vagus is only involved in the initial mechanism of cisplatin in inducing emesis or that the emetic reflex has been reorganised following the lesion (Andrews et al., 1990). However, vomiting can still occur in *S. murinus* on days 1, 2 and 3, and this is consistent with the effect of vagotomy on cisplatin-induced acute and delayed emesis in the pigeon (Tanihata et al., 2000). Nevertheless, it is interesting that the duration of the anti-emetic action of ondansetron exceeds the block of emesis provided by vagotomy.

It is perhaps important that any new robust animal model of cisplatin-induced acute and delayed emesis must be capable of detecting the anti-emetic action of a glucocorticoid, at reasonable doses, and with the use of a minimum number of animals. We used dexamethasone at doses that are effective at antagonising cisplatin-induced emesis in the ferret (Rudd and Naylor, 1996; Sam et al., 2001) but we failed to see any anti-emetic action to reduce retching and/or vomiting in *S. murinus*. Indeed, in one experiment, dexamethasone actually potentiated cisplatin-induced emesis during the initial 24-h observation period. Moreover, we also studied the potential anti-emetic action of dexamethasone combined with ondansetron in reducing emesis. In these experiments, it was noticeable that the combination provided an interaction to delay the onset of cisplatin-induced emesis, but there was no apparent interaction of the drugs to reduce the total numbers of retches + vomits; a similar situation is seen in the ferret over 24 h, when cisplatin is used at 10 mg/kg (Rudd and Naylor, 1997). Moreover, the action of dexamethasone alone, or combined with ondansetron in *S. murinus*, does not appear to reflect the clinical situation (see Introduction).

There was an unforeseen problem associated with the use of cisplatin and dexamethasone that we uncovered in *S. murinus*. Thus, during the course of the investigations, there were fatalities (unexpected deaths and terminations performed humanely) associated with the use of cisplatin at the dose of 30 mg/kg (incidence rate 13.3%) that we did not predict from the preliminary investigations, where all animals survived (data not show). Moreover, a retrospective analysis of the data revealed that dexamethasone 1 mg/kg, i.p., administered twice per day in combination with cisplatin significantly increased mortality rate. It is not known why dexamethasone contributed to the toxicity but this represents a significant obstacle in refining a model of cisplatin-induced acute and delayed emesis in this species.

We have already discussed that the dose of cisplatin is important when attempting to detect the anti-emetic activity of dexamethasone (see above). It is possible, therefore, that the dose of cisplatin 30 mg/kg that we used in our studies to observe emesis over a 3-day period was too high, and that by lowering the dose of cisplatin we may more closely model the clinical situation. Unfortunately, however, lowering the dose of cisplatin further in this species is not likely to provide a solution to the problem as emesis is expected to be less consistent, particularly over extended observation periods, making the use of the model for delayed emesis and the assessment of the anti-emetic potential of any drug problematic.

In conclusion, cisplatin can induce emesis over a 72-h period in *S. murinus*. However, the failure of dexamethasone alone to antagonise emesis and to have an interaction with ondansetron to reduce the total numbers of retches and vomits over the 72-h period limits the value of the model. It is also a limit that dexamethasone contributed to unacceptable toxicity when combined with cisplatin in this species. The failure of bilateral abdominal vagotomy to prevent cisplatin-induced emesis and the inability of 5-HT_3 receptor agonists to induce emesis in these studies highlights the complexity of the emetic reflex with regard to the action of cisplatin and the 5-HT_3 receptor. We believe that *S. murinus* is not suitable to model cisplatin-induced acute and delayed emesis, but we recommend extending observation periods to at least 16 h when examining the anti-emetic potential of new drug candidates in this species.

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