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Potent inhibition of both the acute and delayed emetic responses to cisplatin in piglets treated with GR205171, a novel highly selective tachykinin NK_1 receptor antagonist

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1 The effects of GR205171, a selective tachykinin NK_1 receptor antagonist, were investigated on both the acute and delayed phases of cisplatin-induced nausea-like behaviour and vomiting in the conscious piglet.

2 Animals receiving cisplatin (5.5 mg kg^{-1}, i.v.) were observed for 60 h. Fifteen min prior to cisplatin infusion ($T0_{-15\ min}$), eight piglets acting as controls received an intravenous injection of saline solution (1 ml kg^{-1}), whereas experimental animals received a single i.v. administration of GR205171 (1 ml kg^{-1}) at a dose of 0.01 ($n=8$), 0.03 ($n=8$), 0.1 ($n=8$), 0.3 ($n=16$) or 1.0 ($n=13$) mg kg^{-1}. In eight additional piglets, GR205171 (1 mg kg^{-1}) was administered 15 min before the onset of the delayed phase ($T16_{-15\ min}$). A further five piglets received GR205171 (1 mg kg^{-1}) every 6 h throughout the experiment.

3 The latencies of the first emetic episode (EE) and nausea-like behavioural episode (NE) increased in all experimental groups treated at $T0_{-15\ min}$, and the total number of both EE and NE during the 60 h was reduced in a dose-dependent manner. In piglets treated at $T0_{-15\ min}$ with GR205171 1 mg kg^{-1}, eight out of 13 (62%) did not vomit throughout the experiment. Animals treated with GR205171 (1 mg kg^{-1}) at $T16_{-15\ min}$ exhibited an acute response to cisplatin but did not vomit during the delayed phase. The greatest inhibition of both nausea-like behaviour and vomiting was observed in piglets receiving multiple injections of GR205171.

4 These results demonstrate the long-lasting anti-emetic effects of GR205171, and confirm the key role of substance P within the emetic reflex.

Keywords: GR205171; NK_1 antagonist; anti-emetic; acute emesis; delayed emesis; nausea-like behaviour; cisplatin; piglets

Introduction

In the scientific and medical literature, vomiting (or emesis) is generally presented as a primitive reflex protecting the body from the invasion of an accidentally ingested toxin. However, vomiting can also be elicited in a variety of circumstances where the benefit of this response is not well understood (e.g. motion and pregnancy sickness). Nausea and vomiting still remain distressing side-effects associated with various medical practices (e.g. radiotherapy, cancer chemotherapy, medically-induced abortion, post-operative recovery). The essential co-ordinating circuitry for producing the complex act of vomiting is thought to be located within the medulla of the brainstem (review in Grélot and Miller, 1994), and the neurochemicals involved in that circuitry are not clearly identified. Obviously, afferents triggering emesis release various neurotransmitters so that pharmacological agents exhibiting an effective anti-emetic profile against one kind of vomiting could appear ineffective against emesis induced by other stimuli. For instance, in animal models of emesis, compounds such as the $5\text{-}HT_3$ receptor antagonists ondansetron and granisetron exhibit potent anti-emetic activity against acute chemotherapy-induced emesis but do not block the emetic responses to opioid and dopaminergic agonists, copper sulphate or motion. An attractive strategy to block emesis irrespective of its eliciting stimulus would be to administer a pharmacological agent to depress the activity of neurones within the medullary emetic circuitry. Recently, an increasing number of pharmacological studies have clearly demonstrated that selective tachykinin NK_1 receptor antagonists such as CP-99,994 (Bountra *et al*., 1993; Watson *et al*., 1995), CP-122,721 (Kris *et al*., 1996; Gonsalves *et al*., 1996), GR203040 (Gardner *et al*., 1995) or GR205171 (Gardner *et al*., 1996) exhibited potent anti-emetic properties against a wide variety of emetogens acting centrally (e.g. loperamide and apomorphine), peripherally (cisplatin and copper sulphate) or at a mixed site (ipecacuanha). This broad-spectrum anti-emetic profile of the NK_1 receptor antagonists suggest that they might act centrally, probably on neurones triggering emesis in the dorsal vagal complex (specifically in the area postrema and nucleus tractus solitarius). A central site of action is supported by previous studies in the ferret with other selective NK_1 receptor antagonists (Gardner *et al*., 1994; Tattersall *et al*., 1996). In addition, the anti-emetic activity of NK_1 receptor antagonists has been shown to be dependent on brain penetration (Rupniak *et al*., 1997). Furthermore, recent PET studies in rhesus monkeys have demonstrated that peripherally administered ^{11}C-labelled GR205171 distributes into brain regions consistent with specific binding to NK_1 receptors (Fasth *et al*., 1997).

The antineoplastic cytotoxic drug cisplatin induces a biphasic pattern of vomiting in both piglets (Milano *et al*., 1995; Grélot *et al*., 1995, 1996) and human patients (Hesketh, 1996). Vomiting occurring within the 16–18 h period following the administration of cisplatin corresponds to the acute phase of vomiting (Rittenberg *et al*., 1995; Gralla, 1995;

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Gralla *et al*., 1996). Such emesis can be well-controlled with 5-HT_3 receptor antagonists. The less intense but more prolonged bouts of vomiting subsequent to the acute emetic response to cisplatin, which lasts for up to one week in human patients, correspond to the delayed phase of emesis (Fiore and Gralla, 1984). Such a delayed emesis after cisplatin remains less well controlled than the acute phase (Hesketh, 1996), although the 5-HT_3 receptor antagonist ondansetron has been shown to be moderately efficacious especially when combined with dexamethasone (Navari *et al*., 1995; Ossi *et al*., 1996; Olver *et al*., 1996). Hence, the present study in the piglet was designed to investigate the anti-emetic efficacy of GR205171, a novel highly selective tachykinin NK_1 receptor antagonist, against both the acute and the delayed emetic responses to a high dose of cisplatin. Data from the present study, demonstrating the potent and long-lasting high anti-emetic activity of GR205171 against the two components of cisplatin-induced emesis without any toxicity, support further investigation in human patients undergoing anticancer treatment.

Methods

The experimental procedures were carried out in accordance with international guidelines for the care and use of laboratory animals. All efforts were made to minimise animal suffering and to reduce the number of animals used in the experiments. All piglets ($n=75$) included in this experimental series were naive to previous administration of cytotoxic and anti-emetic drugs. The piglets were sacrificed by administering a high dose of sodium pentobarbitone (Nembutal, 60 mg kg^{-1} i.v.) at the end of the experiment.

Animals and surgical preparation

Details of the animal preparation have been described in previous publications (Milano *et al*., 1995; Grélot *et al*., 1996). Briefly, 75 weaned pure-bred (Pietrain, Hampshire) or crossbred (Pietrain *X* Hampshire) piglets (40–70 days old), of either sex, weighing 4.9 to 13.3 kg, were surgically prepared under ketamine (500 mg kg^{-1}) and halothane anaesthesia (i.e. 1 to 2.5% in a mixture of oxygen and room air) with a catheter (Tygon S54-HL) implanted in the left jugular vein for subsequent administration of drugs and saline. The catheter was tunnelled subcutaneously, and was fixed with screws and dental cement on the top of the head. To prevent the development of any infection, the surgery was conducted using aseptic techniques. In addition, all animals were treated immediately after the induction of anaesthesia with a broad spectrum antibiotic (oxytetracyclin, 20 mg kg^{-1} i.m.), and each surgical wound was treated, after suturing, with an ointment (Cortimega) containing antibiotics (benzylpenicillin and dihydrostreptomycin) and an anti-inflammatory agent (prednisolone). Piglets, isolated in individual boxes (0.7 m^3), were then allowed 3–5 days recovery post-surgery before commencement of emesis experiments. Piglets were fed daily with 400–500 g of a solid commercial diet (9500 kJ kg^{-1}; Sofetac, Cenord) and water was provided *ad libitum*.

Experimental protocols

Prior to cytotoxic drug administration, piglets received a sterile intravenous (iv) infusion of isotonic saline (100 ml h^{-1} for 10 h). The antineoplastic agent cisplatin (5.5 mg kg^{-1}, i.e. approximately 100–125 mg/m^2) was then infused as a 2 mg ml^{-1} solution *via* the jugular cannula over 15 min at a rate of 1.7 ml min^{-1} at midnight ($\pm$1.5 h). The animals were then observed continuously throughout the following 60 h to quantify the number of episodes of nausea-like behaviour and vomiting. Throughout the observation period, piglets received intravenous infusions of a sterile isotonic solution of glucose. All pharmacological agents tested in this study were injected through the jugular vein catheter.

In the piglet, episodes of nausea-like behaviour were identified during a period of general prostration by a typical behaviour consisting of a chewing-like activity and the production of a dense saliva (Milano *et al*., 1995). The duration of a single nausea-like episode (NE) ranged from 10–20 s, especially when it was concluded by an emetic episode, to up to a min. A given NE was considered ended, and thus distinct from a subsequent one when the chewing-like activity had stopped for more than 20 s. An emetic episode (EE) was characterised by a partial expulsion of the gastrointestinal contents. In some cases, EE was observed without the preceding signs of nausea-like behaviour. The animals did not have access to solid and liquid food during the observation period, and so the expulsion consisted mainly of a mixture of bile and other gastrointestinal secretions. In piglets, intravenous administration of high-dose cisplatin induces a biphasic pattern of emesis. The initial period of vomiting occurring within the first 16 h following cisplatin injection (T0-T16) corresponds to the acute phase of emesis. Vomiting occurring after this time corresponds to the delayed phase of emesis (T16-T60) (Milano *et al*., 1995; Grélot *et al*., 1996).

Animals receiving a single high dose of cisplatin were divided into eight groups. Eight piglets (group 1) were treated solely with placebo (sterile 0.9% w/v saline) and acted as control animals. In 66 other piglets, the anti-emetic activity of GR205171 (prepared in sterile 0.9% w/v saline) was investigated at doses of 0.01 mg kg^{-1} ($n=8$, group 2), 0.03 mg kg^{-1} ($n=8$, group 3), 0.1 mg kg^{-1} ($n=8$, group 4), 0.3 mg kg^{-1} ($n=16$, group 5) and 1 mg kg^{-1} ($n=13$, group 6). In groups 1-6, the placebo and GR205171 solutions (1 ml kg^{-1}) were administered as a slow intravenous injection over 15–20 s, 15 min before starting the cisplatin infusion ($T0_{-15\ min}$). In group 7 ($n=8$), GR205171 (1 mg kg^{-1}) was administered 15 min before the predicted onset of the delayed phase i.e. at $T16_{-15\ min}$. Piglets of group 8 ($n=5$) received, in addition to an initial dose of GR205171 (1 mg kg^{-1}), further supplementary injections of GR205171 (1 mg kg^{-1}) every 6 h throughout the experiment (i.e. cumulative dose = 10 mg kg^{-1}).

The pharmacokinetics of GR205171

Venous or arterial blood samples (2–3 ml) were taken from four group 6 piglets plus one additional piglet (observed only until the final blood collection at T30) for assessment of the pharmacokinetic parameters: i.e. the half-life ($T_{1/2}$), the area under the plasma drug concentration-time curve (AUC), the total plasma clearance (CLp) and the volume of distribution (Vdss). The pharmacokinetic parameters were determined *via* SIPHAR (version 3.3). GR205171 (1 mg kg^{-1}) was injected in a 10–20 s bolus *via* the jugular vein catheter 15 min prior to cisplatin administration. Blood samples were collected into plastic tubes containing lithium-heparin anticoagulant. Plasma samples were obtained by centrifugation of blood samples taken predose and 5, 10, 20, 40 min and 1, 2, 4, 6, 8 12, 16, 20, 24, 30 h after the end of the infusion for determination of plasma drug concentration. All samples were stored at approximately $-20^{\circ}C$ until analysis. The plasma samples were analysed to determine the amount of GR205171 present. The method involved automated solid phase (Spark Holland

Prospekt) extraction followed by tandem mass spectrometry (Sciex API-3+). The method has been shown to be valid (in human, rat, dog, rabbit and mouse plasma) over a concentration of 1–100 ng ml^{-1}. The limit of quantification is 1 ng ml^{-1} from 0.05 ml of serum or plasma. Data handling was performed using PE-Sciex software. Data was transferred to PRANBAS (version 3.3) using weighted ($1/\times^2$) quadratic regression using the peak area ratio of GR205171 to a mass labelled internal standard.

Drugs

GR205171 dihydrochloride ((2S, 3S)-2-methoxy-5-tetrazol-1-yl-benzyl)-(2-phenyl-piperidin-3-yl)-amine), Glaxo Wellcome); cisplatin (Cisplatin®, Lilly).

Statistical analysis

Data are expressed as the means ± s.e.m. The statistical significance of differences in the latency and incidence of EE and NE of control and treated animals were assessed using analysis of variance (ANOVA) followed by Bonferroni's test. A latency of 60 h was assigned to animals which did not vomit or exhibit nausea-like behaviour during the whole of the observation period. A level of probability of 0.05 or less was accepted as significant.

Results

Cisplatin-induced nausea-like behaviour and vomiting in control piglets

All piglets treated with the placebo exhibited both acute and delayed nausea-like behaviour in response to cisplatin (Table 1). The mean latency of the first NE was 1.72 ± 0.32 h ($n=8$, range 0.33–3.33 h). The mean number of NE during the acute and delayed phases were 26.4 ± 3.6 NE ($n=8$, range 16–48 NE) and 11.4 ± 2.2 NE ($n=8$, range 5–24 NE), respectively. Consequently, the mean number of NE exhibited by control piglets throughout the chemotherapy course (i.e. T0 to T60) reached 37.8 ± 5.5 NE ($n=8$, range 24–72 NE).

Table 1 Effects of GR105171 on nausea-like events (NE) induced by cisplatin in the piglet

Groups	*GR205171 mg/kg*	*Infusion Time (min)*	*Latency of the first NE (h)*	*Number of ANE [% of controls]*	*% of total control in acute phase*	*Number of DNE [% of controls]*	*% of total control in delayed phase*	*Number of CNE [% of controls]*	*% of total during T0-T60*
1 ($n=8$) Controls	0	T0-15	1.72 ± 0.32	26.4 ± 3.6 [100]	0	11.4 ± 2.2 [100]	0	37.8 ± 5.5 [100]	0
2 ($n=8$)	0.01	T0-15	3.83 ± 0.90	18.0 ± 4 [69]	0	15.1 ± 2.5 [133]	13	33.1 ± 5.2 [88]	0
3 ($n=8$)	0.03	T0-15	4.68 ± 1.99	13.0 ± 3.3 [49]	13	12.6 ± 1.8 [111]	0	25.6 ± 3.9 [68]	0
4 ($n=8$)	0.1	T0-15	12.97 ± 3.62	4.4 ± 2.4 [17**]	50	8.6 ± 3.4 [75]	0	13.0 ± 5.4 [35**]	0
5 ($n=8$)	0.3	T0-15	11.91 ± 4.46	7.9 ± 5 [30**]	13	9.1 ± 2.2 [80]	0	17.0 ± 6.8 [45*]	0
6 ($n=13$)	1	T0-15	23.43 ± 4.58 (**)	0.6 ± 0.3 [2***]	62	3.7 ± 1.1 [32]	15	4.3 ± 1.1 [11***]	8
7 ($n=8$)	1	T16-15	1.60 ± 0.21	24.3 ± 3.1 [92]	0	1.1 ± 0.6 [10*]	63	25.4 ± 3.1 [67]	0
8 ($n=5$)	1	T0-15 + each 6 h	14.79 ± 11.35	1.0 ± 0.3 [4**]	20	0.2 ± 0.2 [2*]	80	1.2 ± 0.4 [3**]	20

ANE, DNE and CNE indicate number of acute, delayed and cumulative nausea-like events, respectively. Note that nausea-like events were quoted in only eight piglets in group 5. *$P<0.05$; **$P<0.01$; ***$P<0.001$.

Table 2 Antiemetic effects of GR205171 on cisplatin-induced emetic events (EE) in the piglet

Groups	*Infusion GR205171 mg/kg*	*Latency the Time (min)*	*Number of first EE (h)*	*% of total AEE [% of control]*	*Number of control in acute phase*	*% of total DEE [% of controls]*	*Number of control in delayed phase*	*% of total CEE [% of controls]*	*control during T0-T60*
1 ($n=8$) Controls	0	T0-15	1.95 ± 0.32	21.3 ± 1.9 [100]	0	6.4 ± 1.4 [100]	0	27.6 ± 2.7 [100]	0
2 ($n=8$)	0.01	T0-15	5.47 ± 1.96	9.1 ± 1.9 [43***]	13	8.3 ± 1.5 [130]	13	17.4 ± 2.5 [63*]	0
3 ($n=8$)	0.03	T0-15	6.13 ± 2.72	6.9 ± 1.8 [32***]	13	12.1 ± 2.3 [189*]	0	19 ± 2.9 [69*]	0
4 ($n=8$)	0.1	T0-15	30.83 ± 10.61	1.8 ± 1.1 [9***]	50	1.5 ± 0.8 [23*]	63	3.3 ± 1.3 [12***]	38
5 ($n=16$)	0.3	T0-15	29.06 ± 6.33 (*)	1 ± 0.5 [5***]	56	1.8 ± 0.7 [28*]	44	2.8 ± 0.9 [10***]	25
6 ($n=13$)	1	T0-15	44.15 ± 6.39 (***)	0.2 ± 0.1 [1***]	77	0.9 ± 0.5 [14*]	69	1.2 ± 0.6 [4***]	62
7 ($n=8$)	1	T16-15	1.64 ± 0.2	15.9 ± 1.9 [75]	0	0 ± 0 [0*]	100	15.9 ± 1.9 [58]	0
8 ($n=5$)	1	T0-15 + each 6 h	15.06 ± 11.29	0.8 ± 0.2 [4***]	20	0 ± 0 [0*]	100	0.8 ± 0.2 [3***]	20

AEE, DEE and CEE indicate number of acute, delayed and cumulative emetic events, respectively. *$P<0.05$; **$P<0.01$; ***$P<0.001$.

In response to cisplatin, all control animals experienced both acute and delayed vomiting (Table 2). The first EE occurred with a latency of 1.95 ± 0.32 h ($n=8$, range 1.17–3.85 h) after cisplatin administration. The mean number of vomits during the first 16 h (i.e. severity of the acute phase) was 21.3 ± 1.9 EE ($n=8$, range 14–28 EE). The mean number of vomits during the whole of the delayed phase was 6.4 ± 1.4 EE ($n=8$, range 1–11 EE). Thus, the cumulative (acute + delayed) emetic severity in the control population averaged 27.6 ± 2.7 EE ($n=8$, range 17–39 EE).

Effects of single administration of GR205171 on cisplatin-induced nausea-like behaviour

GR205171 exhibited weak effects on the latency of the first NE (Figure 1A; Table 1). Only the single initial administration of GR205171 at the dose of 1 mg kg^{-1} delayed significantly the occurrence of the first NE (23.43 ± 4.58 h; $n=13$; range 1.32–60 h). In contrast, GR205171 induced a dose-related decrease in the number of acute NE (Figure 1C; Table 1). After the injection of GR205171 at a dose of 0.1, 0.3 and 1 mg kg^{-1}, the

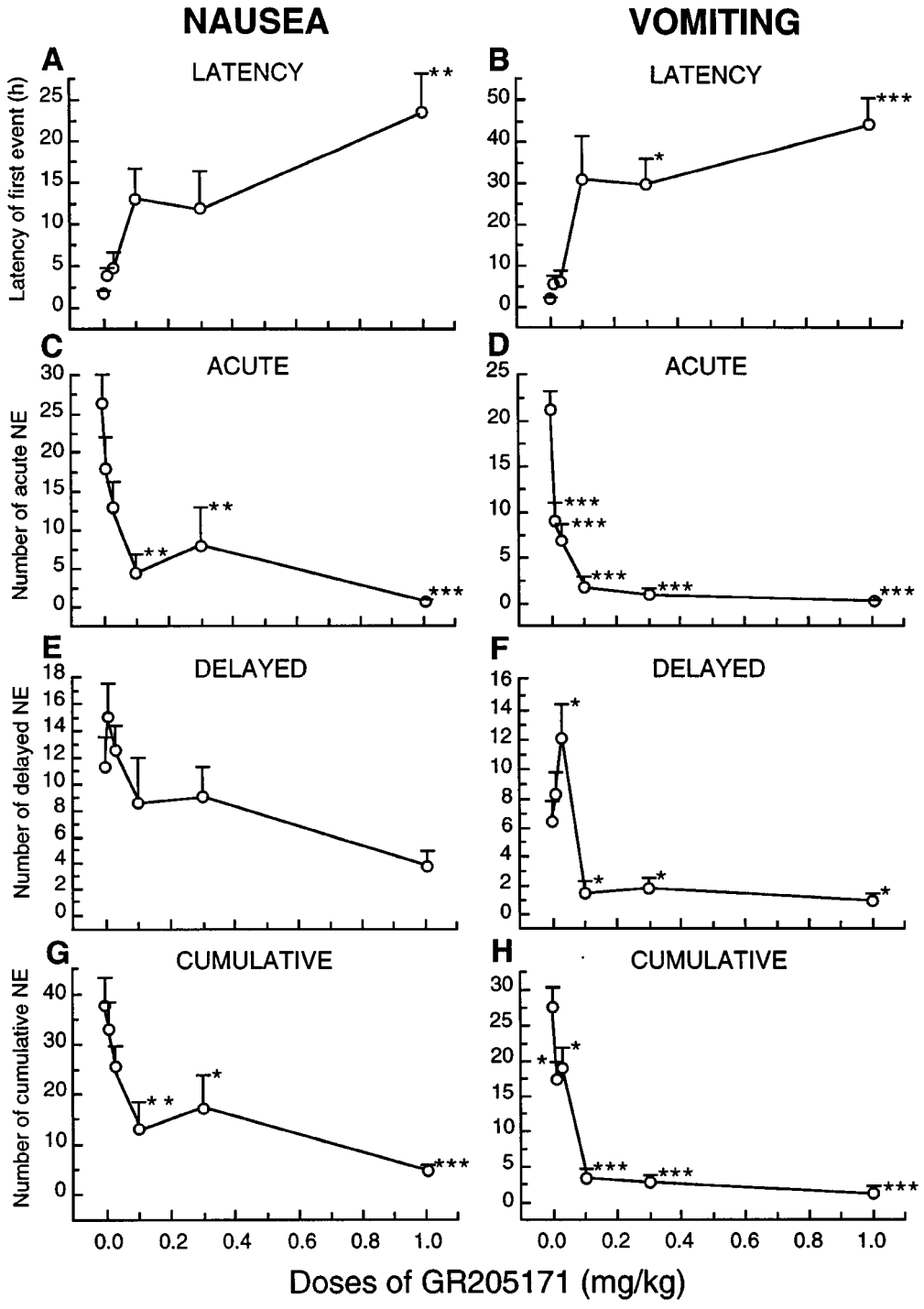

Figure 1 Effects of various single initial doses of GR205171 administered 15 min prior to cisplatin on the latency (expressed in hour) of the first nausea-like behavioural (A) and emetic (B) episodes and on the number of acute (C and D), delayed (E and F) and cumulative (G and H) NE and EE, respectively. Bar above each dot indicates s.e.m.; *, ** and ***, significant difference versus controls at $P<0.05$, $P<0.01$ and $P<0.001$, respectively.

incidence of acute nausea was significantly reduced to 4.4±2.4 (n=8, range 0–18 NE), 7.9±5 (n=8, range 0–42 NE) and 0.6±0.3 (n=13, range 0–3 NE), respectively (Table 1). The production of acute NE was thus attenuated by 83, 70 and 98%, respectively. When administered prior to cisplatin, GR205171, at any dose, did not significantly reduce the incidence of delayed nausea (Figure 1E; Table 1). However, when a high dose of GR205171 (i.e. 1 mg kg^{-1}) was administered at the end of the acute phase, the incidence of nausea was decreased significantly to 1.1±0.6 NE (n=8, range 0–4 NE) during the delayed phase (Table 1). In addition, five out of eight piglets receiving this delayed administration of GR205171 did not exhibit delayed NE (Table 1). GR205171 substantially reduced the incidence of acute nausea in groups 4, 5 and 6, and the number of cumulative NE was also significantly decreased in these groups (Figure 1G; Table 1).

Effects of single administration of GR205171 on cisplatin-induced vomiting

As shown in Table 2, the administration of GR205171 prior to the cisplatin infusion delayed the onset of acute emesis in a dose-dependent manner (Figure 1B). At the lowest (i.e. 0.01 mg kg^{-1}) and highest (i.e. 1 mg kg^{-1}) doses of GR205171, the first emetic event occurred with a mean latency of 5.47±1.96 h (n=8, range 1.00–17.83 h) and 44.15±6.39 h (n=13, range 3.82–60 h), respectively. Statistical evaluation revealed that latencies of the first EE in groups five and six were significantly longer than those observed in the control group 1 animals. In contrast, the incidence of vomiting in the acute phase decreased significantly in groups 2–6 (Figure 1D; Table 2), being 9.1±1.9 EE (n=8, range 0–14 EE, group 2), 6.9±1.8 EE (n=8, range 0–14 EE, group 3), 1.8±1.1 EE (n=8, range 0–9 EE, group 4), 1±0.5 EE (n=16, range 0–7 EE, group 5) and 0.2±0.1 EE (n=13, range 0–1, group 6). The number of animals which produced no vomiting during the first 16 h post-cisplatin increased from 1/8 (13%) in group 2 to 10/13 (77%) in group 6 (Table 2). Animals (group 7) receiving the administration of GR205171 at T16$_{-15\ min}$ exhibited an acute emesis which was statistically similar in both latency (1.64±0.2 h, n=8, range 0.97–2.63 h) and incidence (15.9±1.9 EE, n=8, range 11–26 EE) to that of controls (Table 2).

The incidence of delayed emesis was also affected by the single initial injection of GR205171, but the effect was dose-dependent (Figure 1F; Table 2). At a dose of 0.03 mg kg^{-1}, the severity of vomiting increased significantly to 12.1±2.3 EE (n=8, range 5–21 EE, group 3). In contrast, when increasing the doses to 0.1, 0.3 and 1 mg kg^{-1}, the mean number of EE produced during the delayed phase decreased significantly to 1.5±0.8 EE (n=8, range 0–6 EE, group 4), 1.8±0.7 EE (n=16, range 0–10 EE, group 5) and 0.9±0.5 EE (n=13, range 0–6 EE, group 6), respectively (Table 2). Similarly, when administered just prior to the onset of the delayed phase (i.e. at T16$_{-15\ min}$), GR205171 (1 mg kg^{-1}) still exerted a potent inhibition of emesis by abolishing the delayed emetic response to cisplatin (0±0 EE, group 7; Table 2).

In all experimental groups, treatment with GR205171 administered prior to cisplatin infusion decreased the incidence of acute emesis and, in some of these groups, the incidence of delayed emesis. Therefore, the cumulative severity was attenuated by GR205171 in all experimental groups (Figure 1H; Table 2). The mean number of EE produced during the 60 h observation period decreased by 37% and 96% in animals treated at T0$_{-15\ min}$ with GR205171 0.01 and 1 mg kg^{-1} respectively. Similarly, group 7 animals receiving 1 mg kg^{-1} of GR205171 at the end of the acute phase, exhibited a markedly reduced cumulative severity (15.9±1.9 EE; n=8; range 11–26 EE; Table 2).

Effects of repetitive administration of GR205171 on cisplatin-induced nausea-like behaviour and vomiting

Repetitive administration of GR205171 (1 mg kg^{-1}) every 6 h over 60 h (i.e. cumulative dose of 10 mg kg^{-1}) in group 8 provided the greatest efficacy in the control of both acute and delayed nausea-like behaviour. Multiple injections of GR205171 prevented the occurrence of nausea-like behaviour in one out of five piglets (Table 1). The latency of the first NE was 14.79±11.35 h (n=5, range 1.47–60 h). The mean numbers of NE during the acute and delayed phases were 1.0±0.3 NE (n=5, range 0–2 NE) and 0.2±0.2 NE (n=5, range 0–1 NE), and the total number of NE produced during the whole of the observation period was 1.2±0.4 NE (n=5, range 0–2 NE).

Multiple injections prevented the acute emetic response in only one out of five piglets of group 8 (Table 2). In contrast, all of the piglets (n=5) failed to produce any EE during the delayed phase (Table 2). The latency of the first EE was 15.06±11.29 h (n=5, range 1.48–60 h). The mean number of EE during the 60 h of the observation period which equals that of the acute phase (0.8±0.2 EE, n=5, range 0–1 EE) was significantly reduced compared to that of controls (group 1; Table 2).

The pharmacokinetics of GR205171 in the piglet

Analysis of blood samples provided the following mean (n=5±s.d.) pharmacokinetic parameters: $T_{1/2}$: 3.4±0.8 h, $AUC_{0\text{-}t}$: 401±133 ng b.h ml^{-1}, CLp: 43.1±12.2 ml min^{-1} kg^{-1} and Vdss: 12.1±2.2 l kg^{-1}.

In all of the above experiments, animals treated with GR205171 maintained acceptable physiological conditions (i.e. similar to those of controls) both during and after (up to 1 week) the period of observation. No adverse events were observed other than the nausea-like behaviour and vomiting induced by cisplatin.

Discussion

There have been considerable advances over the past 10 years in the understanding of the peripheral (i.e. in the gut) neurochemical mechanisms responsible for eliciting vomiting in patients undergoing anticancer chemotherapy. In contrast, the central neural and chemical mechanisms responsible for the integration of emetic information and generation of the motor response remain quite obscure. Recently, a substantial breakthrough in the field of emesis research has been the discovery of the unprecedented broad-spectrum anti-emetic activity of NK$_1$ receptor antagonists in a range of species including ferret, dog and house-musk shrew (*Suncus murinus*). The present study, designed in the piglet to investigate the anti-emetic activity of GR205171, demonstrated that a single initial administration of this novel highly selective NK$_1$ receptor antagonist produced a dose-related long-lasting inhibition of nausea-like behaviour and vomiting induced by a high dose of cisplatin.

Anti-emetic profile of GR205171

Recent investigations have clearly demonstrated that antagonists of the NK_1 receptor, specifically CP-99,994 (Bountra *et al*., 1993; Watson *et al*., 1995), CP-122,721 (Gonsalves, 1996) and GR203040 (Gardner *et al*., 1996), possess potent anti-emetic activity in various animal species. GR205171, a trifluoromethyl-substituted analogue of GR203040, appeared as one of the most potent and selective NK_1 receptor antagonists so far reported, possessing a sub-nanomolar affinity ($pK_i = 10.6$) at the human recombinant tachykinin NK_1 receptor (Gardner *et al*., 1996). This novel NK_1 receptor antagonist was demonstrated to have a broad-spectrum anti-emetic activity, inhibiting vomiting induced by anticancer cytotoxic drugs (e.g. cisplatin in ferret and house-musk shrew), morphine (ferret), ipecacuanha (ferret and dog), copper sulphate (ferret), whole-body X-irradiation (ferret) and motion (house-musk shrew) (Gardner *et al*.,1996). Data from the present study show that this novel NK_1 receptor antagonist exhibits an exceptional efficacy against both the acute and the delayed emetic responses to cisplatin in the piglet. Comparison with results from our previous studies in this animal species demonstrated that GR205171 has the highest ratio of anti-emetic activity/dose of any compound ever tested in our experimental model (Milano *et al*., 1995). For instance, a single initial administration of 0.01 mg kg^{-1} of GR205171 attenuated by 57% the incidence of the acute response to cisplatin, whereas granisetron and ondansetron, two 5-HT_3 receptor antagonists considered as effective clinical treatments against acute emesis, administered under the same experimental conditions at a dose of 7 mg kg^{-1} i.v., inhibited acute vomiting by approximately 67 and 32%, respectively (Milano *et al*., 1995; Grélot *et al*., 1995, 1996). This contrasts with the results obtained in the ferret treated with cisplatin (10 mg kg^{-1}, i.p.) in which tropisetron (a 5-HT_3 receptor antagonist; 1 mg kg^{-1}, s.c.) was slightly more effective than CP-99,994 (an NK_1 receptor antagonist, 1 mg kg^{-1}, s.c.) to reduce acute emesis (Watson *et al*., 1995). In the piglet, when the single initial dose was increased to 1 mg kg^{-1}, GR205171 almost abolished the acute response to cisplatin (i.e. inhibition of 99%). Such a level of inhibition or complete abolition of acute vomiting was observed in the ferret under cisplatin (10 mg kg^{-1}, i.v.) following intravenous doses of 3 mg kg^{-1} of CP-99,994 (Tattersall *et al*., 1993) and 0.3 mg kg^{-1} of GR205171 (Gardner *et al*., 1996), and a subcutaneous dose of 0.3 mg kg^{-1} of another NK_1 receptor antagonist, CP-122,721 (Gonsalves *et al*., 1996). However, the short duration of the observation period following cisplatin infusion (i.e. 2 h in Tattersall *et al*., 1993; and 4 h in Gonsalves *et al*., 1996) may have resulted in an overestimate of the efficacy of both CP-99,994 and CP-122,721 against acute cisplatin-induced emesis. Indeed, the primary effects of anti-emetic drugs administered at doses inadequate to reduce the number of EE is to delay the occurrence of the emetic crisis in response to emetogens. This was previously demonstrated in the piglet treated with anti-emetic compounds, such as those acting as 5-HT_3 receptor antagonists (e.g. granisetron and ondansetron; Grélot *et al*., 1995, 1996) or as 5-HT_{1A} receptor agonists (e.g. buspirone and 8-OH-DPAT, unpublished personal observations).

Our data also support previous observations describing the long-lasting anti-emetic activity of GR205171 against cisplatin-induced emesis (Gardner *et al*., 1996). Indeed, a single initial administration of GR205171 at a dose of 1 mg kg^{-1} reduced both the acute and delayed emetic severity in piglet. Consequently, the incidence of vomiting during the whole of the observation period (60 h) decreased by 96%. Moreover, a 1 mg kg^{-1} dose administered at the transition between the acute and delayed phases abolished the delayed emetic response to cisplatin. For comparison, repeated administration of granisetron (1 mg kg^{-1} each 5 h from T0 to T30) and BIMU 1 (a mixed a 5-HT_3 receptor antagonist and 5-HT_4 receptor agonist; 2 mg kg^{-1} each 5 h from T0 to T30) reduced the number of vomiting episodes produced during 60 h by 67 and 30%, respectively (Grélot *et al*., 1995, 1996). A striking feature in the present study was the complete protection induced by the single initial administration of GR205171 (1 mg kg^{-1}) in 62% (8/13) of the animals. Such a complete control of vomiting in response to cisplatin was previously observed only in 38 and 29% of animals treated repeatedly with granisetron and BIMU 1, respectively (Grélot *et al*., 1995, 1996). The long-lasting anti-emetic effect of GR205171 is perhaps surprising, since the pharmacokinetic study revealed that in the piglet GR205171 has a fairly short plasma half-life (3.4 ± 0.8 h) after intravenous administration at a nominal dose of 1 mg kg^{-1}. The discrepancy between the short half-life and the long duration of the anti-emetic activity has also been reported for other anti-emetic agents (e.g. granisetron) in both human patients and animals (Andrews, 1994). This might suggest that GR205171 is rapidly distributed to its sites of action from where it is slowly eliminated. Further investigations are required to determine fully the mechanisms underlying the long-acting anti-emetic effect of GR205171. Finally, the highest control of vomiting produced throughout the whole of the 60 h observation period was obtained in piglets treated by repetitive administration of GR205171 (1 mg kg^{-1}, i.v.) every 6 h. This treatment provided a reduction of 97% of the total number of EE (i.e. CEE in Table 2).

Effect of GR205171 on the nausea-like behaviour

Patients receiving high doses of cisplatin during anticancer chemotherapy experience nausea (Kris *et al*., 1985, 1989). Evidently, this unpleasant sensation of discomfort markedly reduces the patients' quality of life, contributing importantly to non-compliance with potentially life-saving anticancer treatments (for review see Fox, 1992). Animal models investigated for studying nausea have confronted a basic problem, since nausea is a human subjective sensation. Despite this serious limitation, indirect measures have been proposed to identify nausea in animals. In piglets, we previously reported (Milano *et al*., 1995) that emetic episodes were typically preceded by a significant increase in heart rate. These cardiac changes were in every case associated with a typical chewing-like behaviour leading to the abundant production of a viscous saliva. In our previous study, the chewing-like activity was monitored by an EMG bursting activity in the masseter muscle of the jaw, the frequency of which reached a maximum just prior to the emetic event (see Figure 1 in Milano *et al*., 1995). In the present series of investigations, animals were not instrumented, and this chewing-like activity was considered to monitor a nausea-like behaviour in the piglet. Since this chewing-like activity is also exhibited by piglets presenting post-operative vomiting or emesis in response to morphine administered intrathecally (unpublished personal observations), this typical behaviour could be considered as a selective and reliable indicator of nausea in this animal species. Data from the present study revealed that GR205171, in addition to its potent anti-emetic activity, possesses the ability to reduce the incidence of nausea-like behaviour induced by cisplatin, particularly

during the acute phase of emesis. The reduction of the incidence of acute NE reached the level of significance for doses of GR205171 ranging from 0.1 to 1 mg kg^{-1}. At the 1 mg kg^{-1} dose, the incidence of nausea-like behaviour during the acute and delayed phases was attenuated by 98 and 68%, respectively, so that the total number of NE produced during the whole of the observation period was reduced by 89%. GR205171 (1 mg kg^{-1}), administered just prior to the onset of the delayed phase of emesis, decreased by 90% the incidence of nausea-like behaviour during that phase. In total, these data demonstrate the long-lasting effects of GR205171 against the nausea-like behaviour, and also the non-dissociation between the anti-emetic and anti-nausea-like behavioural activities of this compound. As observed for the emetic component of the cisplatin response, repetitive administration of GR205171 provided the greatest control of nausea-like behaviour during the whole of the experiment (i.e. inhibition of 97% of the number of CNE, Table 1).

Conclusion

Data from the present study clearly demonstrate the exceptional efficacy of GR205171 against both nausea-like behaviour and vomiting induced by cisplatin in piglet. This provides additional evidence for a pivotal role of both the neuropeptide substance P and tachykinin NK$_1$ receptors in the emetic reflex. If the anti-emetic profile of NK$_1$ receptor antagonists translates to human patients undergoing anticancer chemotherapy, the present data support further clinical investigation of GR205171.

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