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The tachykinin NK_1 receptor antagonist PD 154075 blocks cisplatin-induced delayed emesis in the ferret

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Abstract

The activity of a selective tachykinin NK_1 receptor antagonist, PD 154075 ([(2-benzofuran)-CH_2OCO]-(*R*)-α-MeTrp-(*S*)-NHCH(CH_3)Ph), was examined in radioligand binding studies, in a [Sar^9,$Met(O_2)^{11}$]substance P-induced foot-tapping model in the gerbil, and in cisplatin-induced acute and delayed emesis in the ferret. In radioligand binding studies, PD 154075 showed nanomolar affinity for the human, guinea-pig, gerbil, dog and ferret NK_1 receptors with an approximate 300 times lower affinity for the rodent NK_1 receptor. Using NK_2, NK_3 receptors and a range of other receptor ligands, PD 154075 was shown to exhibit a high degree of selectivity and specificity for the human type NK_1 receptor. Following subcutaneous administration PD 154075 dose dependently (1–100 mg/kg) antagonised the centrally mediated [Sar^9,$Met(O_2)^{11}$] substance P-induced foot tapping in the gerbil with a minimum effective dose (MED) of 10 mg/kg. The ability of PD 154075 to readily penetrate into the brain following oral administration was confirmed by its extraction and high performance liquid chromatography assay from the rat brain. PD 154075 was shown to achieve a relatively fast and sustained brain concentration (brain/plasma ratios ranged from 0.27 to 0.41 during the time period of 0.25–12 h). Further pharmacokinetic studies revealed that the absolute oral bioavailability of PD 154075 in the rat was (mean ± S.D.) 49 ± 15%. PD 154075 (1–30 mg/kg, i.p.) dose dependently antagonised the acute vomiting and retching in the ferret measured for 4 h following administration of cisplatin (10 mg/kg, i.p.) with a MED of 3 mg/kg. The administration of a lower dose of cisplatin (5 mg/kg, i.p.) in the ferret induces both an acute (day 1) and delayed (days 2 and 3) phase of emesis. The i.p. administration of PD 154075, 10 mg/kg three times a day for 3 days, almost completely blocked both the acute and delayed emetic responses. In the same study, the $5\text{-}HT_3$ receptor antagonist ondansetron (1 mg/kg, i.p., t.i.d.) was also very effective against the acute emetic response observed during the first 4 h following cisplatin, but it was only weakly active against the delayed response. In conclusion, PD 154075 is a selective and specific high affinity NK_1 receptor antagonist with good oral bioavailability which is effective against both acute and delayed emesis induced by cisplatin in the ferret.

Keywords: Radioligand binding; Oral bioavailability; $t_{1/2}$; Brain penetration; Vomiting; Retching; Nausea; Ondansetron

1. Introduction

Nausea and vomiting are distressing symptoms associated with a variety of conditions such as motion sickness, pregnancy, pain following surgery, release of endogenous pro-emetic agents, disturbance of the vestibular system and a number of gastrointestinal disorders. Emesis following cancer chemotherapy and radiation may be particularly severe, greatly reducing the quality of life and effecting compliance in some patients (Hickok and Morrow, 1993). The chemotherapy-induced emesis in man may last several days, depending on the chemotherapeutic agent and the dose regimen used, and has been classified as either acute, delayed or anticipatory (Fiore and Gralla, 1984). The 'acute' nausea and vomiting is generally accepted to occur within the first 24 h period; delayed emesis occurs after this period. Anticipatory nausea and vomiting usually develops within four courses of chemotherapy in patients whose emesis was not controlled in previous cycles. It is

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thought to be a conditioned response provoked by factors such a sight, sound and smell of the chemotherapy clinic (Morrow, 1989).

The discovery of the 5-HT_3 receptor antagonists has dramatically improved the treatment of emesis induced by anti-cancer therapy (Aapro, 1991; for review, see Morrow et al., 1995). Their major beneficial effect is observed in the acute phase of chemotherapy-induced emesis in both animal models (Bermudez et al., 1988; Higgins et al., 1989; Rudd and Naylor, 1996) and in humans (for review, see Morrow et al., 1995). However, 5-HT_3 receptor antagonists fail to completely control the delayed nausea and vomiting associated with chemotherapy in man and there remain some patients who are resistant to 5-HT_3 receptor antagonists (Gandara et al., 1993; Kris et al., 1992).

The recognition that the vagus nerve plays an important role in emesis induced by cytotoxic drugs prompted research into the possibility that drugs targeted at vagal afferent transmitter systems (e.g., acetylcholine, cholecystokinin, substance P) may possess antiemetic activity (for review, see Andrews et al., 1988). These studies led to the demonstration that direct administration of substance P into the hindbrain, in the region of the nucleus tractus solitarii, induces emesis (Gardner et al., 1994). These preliminary findings were extended by the recent discovery of selective and potent non-peptide NK_1 receptor antagonists. It was shown that this class of compounds possess a broad spectrum of antiemetic activity, blocking emesis induced by a wide range of both peripheral and centrally mediated emetic stimuli including apomorphine, morphine, nicotine, copper sulphate, ipecacuanha, radiation and cisplatin (Bountra et al., 1993; Tattersall et al., 1993; Gardner et al., 1995; Watson et al., 1995). Recently, a cisplatin-induced model of acute and delayed emesis has been described in the ferret (Rudd et al., 1994). In the present study we examine the antiemetic action of the NK_1 receptor antagonist PD 154075 (Boyle et al., 1994) in this model, the selectivity and specificity of its actions at the NK_1 receptor, and its ability to antagonise substance-P-mediated effects.

2. Materials and methods

2.1. Animals

Mongolian gerbils (40–70 g) of either sex (Bantin and Kingman, UK) were housed in groups of 8. Adult male albino Fitch ferrets (> 1 kg weight) were individually housed. Male Wistar rats (300–350 g) were housed in groups of six. All animals had free access to food and water (SDS Diet 'C', Special Diet Services, Essex, UK).

2.2. Receptor binding assays

2.2.1. Tachykinin receptor binding assays

Tachykinin NK_1 receptor binding assays were carried out as described previously (Boyle et al., 1994). Human lymphoma IM9 cells were grown in RPMI 1640 culture medium supplemented with 10% foetal calf serum and 2 mM glutamine and maintained under an atmosphere of 5% CO_2. Cells were harvested for experiments by centrifugation at $1000 \times g$ for 3 min. Pelleted cells were washed once by resuspension into assay buffer (50 mM Tris-HCl pH 7.4, 3 mM $MnCl_2$, 0.02% bovine serum albumin, 40 μg/ml bacitracin, 2 μg/ml chymostatin, 2 μM phosphoramidon, 4 μg/ml leupeptin) and repeating the centrifugation step before resuspending at a concentration of 2.5×10^6 cells/ml assay buffer. Cells (200 μl) were incubated with [^{125}I]Bolton-Hunter Substance P (0.05–0.1 nM) in the presence and absence of varying concentrations of test compounds for 50 min at 21°C. Non-specific binding (10% of the total binding observed under these conditions) was defined by 1 μM [Sar^9,Met(O_2)^{11}]substance P. Reactions were terminated by rapid filtration under vacuum onto GF\C filters presoaked in 0.2% PEI for 1–2 h, using a Brandel cell harvester. Filters were washed with 6×1 ml ice-cold Tris HCl (50 mM, pH 7.4) and bound radioactivity determined using a gamma counter. Results were analysed using iterative curve fitting procedures in RS1 or Graphpad Inplot.

For tachykinin NK_1 receptor binding assays using membranes prepared from brain tissues, P2 tissue was prepared by initially homogenising brain tissue in 10 volumes 0.32 M sucrose, and centrifuging at $1000 \times g$ for 10 min. The supernatant was recentrifuged at $40\,000 \times g$ for 15 min, the pellet washed twice by resuspension and centrifugation before resuspending at a concentration appropriate for adding 2–5 mg wet weight of P2 pellet per tube, depending on the species.

Binding assays for tachykinin NK_2 receptors were carried out as described previously (Guard et al., 1993b) using [^{125}I]neurokinin A and membranes prepared from hamster urinary bladder.

Tachykinin NK_3 receptor binding assays were carried out as described previously (Suman-Chauhan et al., 1994) using [^{125}I][MePhe^7]neurokinin B and membranes prepared from Chinese Hamster Ovary-K1 cells stably expressing cloned human NK_3 receptors.

2.2.2. Non-tachykinin receptor binding assays

In order to determine selectivity, 32 binding assays for non-tachykinin receptors or binding sites were carried out using standard methodology as described in the literature. The species and radioligands used are shown in Table 1.

2.3. Brain penetration and bioavailability

2.3.1. Induction of [Sar^9,Met(O_2)^{11}]substance P-induced foot tapping

Gerbils were briefly anaesthetised with an isoflurane O_2/NO_2 mixture. An incision was made into the scalp to expose the skull. [Sar^9,Met(O_2)^{11}]substance P (1–100 nmol/ animal in 5 μl) was administered intracerebroven-

Table 1
Receptor binding assays used to determine the selectivity profile of PD 154075

Receptor	Ligand	Tissue
Cholecystokinin CCK_A	[^{125}I]BHCCK8s	Rat pancreas
Cholecystokinin CCK_B	[^{125}I]BHCCK8s	Mouse cortex
Neuromedin B	[^{125}I][Tyr^4]bombesin	Rat olfactory bulb
Gastrin releasing peptide	[^{125}I][Tyr^4]bombesin	Rat cortex
Galanin	[^{125}I]Galanin	Rat basal forebrain
Somatostatin	[^{125}I]Somatostatin	Rat cortex
Neurotensin	[^{125}I]Neurotensin	Neonatal mouse forebrain
Opiate κ	[^{3}H]U69593	Guinea-pig forebrain
Opiate μ	[^{3}H]DAGOL	Guinea-pig forebrain
Opiate δ	[^{3}H]DPEPE	Guinea-pig forebrain
Na/K ATPase	[^{3}H]Ouabain	Rat cortex
Dopamine D_1	[^{3}H]SCH23390	Pig striatum
Dopamine D_2	[^{3}H]Spiperone	Pig striatum
5-HT	[^{3}H]5HT	Guinea-pig cortex
5-HT_{1A}	[^{3}H]8OH-DPAT	Guinea-pig cortex
5-HT_2	[^{3}H]Ketanserin	Rat cortex
5-HT_3	[^{3}H]GR65630	Rat cortex
K^+ channel ATP sensitive	[^{3}H]Glibenclamide	Pig brain
Benzodiazepine	[^{3}H]Flunitrazepam	Mouse brain
Cocaine	[^{3}H]Cocaine	Rat cortex
L-type Ca^{2+} channel	[^{3}H]Nimodepine	Rat cortex
L-type Ca^{2+} channel	[^{3}H]Diltiazem	Pig cortex
Histamine H_1	[^{3}H]Pyrilamine	Pig cortex
Adrenoceptor α_1	[^{3}H]Prazosin	Rat cortex
Muscarinic M_1	[^{3}H]QNB	Pig brain
$GABA_A$	[^{3}H]GABA	Rat cortex
$GABA_B$	[^{3}H]GABA	Rat cortex
Gabapentin	[^{3}H]gabapentin	Pig cortex
Glutamate	[^{3}H]glutamate	Rat cortex
Glycine	[^{3}H]Glycine	Rat cortex
NMDA	[^{3}H]MK801	Rat cortex
NMDA	[^{3}H]CGP39635	Rat cortex

List of non-tachykinin receptor binding assays performed to determine selectivity of PD 145075 for tachykinin NK_1 receptors. Assays were carried out using standard methodology as described in the literature.

tricularly (i.c.v.) by vertical insertion of a cuffed 27-gauge needle to a depth of 4.5 mm below bregma. Animals were placed individually into observation boxes and duration of hindpaw tapping was recorded for 5 min immediately following recovery of the animals righting reflex (within 1 min of injection). For antagonism studies PD 154075 was administered s.c. at 30 min before the injection of [Sar^9,Met(O_2)^{11}]substance P.

2.3.2. Bioavailability of PD 154075

PD 154075 dissolved in ethanol/PEG-400/water (1:2:1) was administered either p.o. by gavage (20 mg/kg) or i.v. via the jugular vein (5 mg/kg) in fasted rats. Blood samples were drawn from the jugular vein cannulae at various time intervals up to 24 h. Plasma samples were analysed using a validated high-performance liquid chromatography (HPLC) method described below.

2.3.3. Brain penetration of PD 154075

PD 154075 (21.4 mg/kg) dissolved in ethanol/PEG-400/water (1:2:1) was administered p.o. by gavage in fasted rats. Animals were anaesthetised with ethyl ether prior to sample collection. Blood and brain samples were collected simultaneously up to 12 h postdose, with three rats contributing to each time point. Blood samples were drawn by cardiac puncture, and whole brains were harvested after a trans-cardiac perfusion with 0.9% NaCl. Plasma and brain samples were analyzed using separate validated HPLC methods described below.

2.4. HPLC assays for PD 154075

2.4.1. Plasma

Plasma samples (100 μl) were precipitated with acetonitrile (250 μl) after addition of an internal standard. Samples were then vortexed, centrifuged, and supernatants evaporated. The extracts were reconstituted in 200 μl of acetonitrile/water (1:1) and 150-μl samples were chromatographed on a 4.6 mm × 150 mm, 5 μm Beckman Ultrasphere ODS column. The mobile phase consisted of 47% 20 mM sodium citrate (pH 3.5) and 53% acetonitrile. The analytes were detected using fluorescence monitoring at excitation of 278 nm and emission of 342 nm. The assay was validated over the concentration range of 5–1000 ng/ml of PD 154075.

2.4.2. Brain tissue

Brain tissue samples were homogenised with water (1:2) using a Kinematica homogenizer. Aliquots of 250 μl homogenate were precipitated after addition of an internal standard (PD 154073) with acetonitrile (750 μl). Thereafter, extracts were treated and chromatographed in the same manner as that for the assay of plasma PD 154075 as described above.

2.5. Pharmacokinetic analysis

Pharmacokinetic parameters for plasma and brain profiles were calculated using a non-compartmental method as follows: $t_{1/2} = 0.693/\lambda$ where $t_{1/2}$ is the terminal elimination half life of the drug and λ is the rate constant; peak times (t_{max}) where peak concentrations (C_{max}) occurred were recorded from the observed plasma profiles; intravenous plasma clearance $CL_{tot} = Dose/AUC_{0-}$ is the area under the plasma concentration-time curves from zero to infinite time. $V_{ss} = CL_{tot} \cdot MRT$, where V_{ss} is the estimated steady-state volume of distribution and MRT is the mean residence time of the drug after i.v. administration; and %F (oral bioavailability) is the ratio of oral AUC_{0-} to intravenous AUC_{0-} corrected for the doses. Data represent means (± S.D.).

2.6. Induction and measurement of emesis

The ability of PD 154075 (dissolved in PEG-200) to block cisplatin-induced emesis was examined in the ferret.

Briefly, 30 min prior to the commencement of the studies, ferrets were presented with 100 g of commercially available tinned cat food. For the acute emesis studies cisplatin (10 mg/kg, i.p.) was administered 1 h after PD 154075 and the animal behaviour was recorded by video camera and visual observation over a 4 h period. The tapes were subsequently read to measure the number of episodes, retches and vomits made by each animal and the time of their occurrence. To induce the full profile of both acute and delayed emesis a lower dose of cisplatin (5 mg/kg) was administered i.p. and the animal behaviour was video taped for the following 72-h period (Rudd et al., 1994). PD 154075 or ondansetron was administered 1 h before cisplatin and drug treatment continued at regular 8-h intervals. The emesis parameters were scored as for the acute response.

2.7. Drugs and solutions

PD 154075 ([(2-benzofuran)-CH_2OCO]-(*R*)-α-MeTrp-(*S*)-NHCH(CH_3)Ph) was synthesised at Parke-Davis Neuroscience Research Centre, Cambridge, UK. Unless stated otherwise PD 154075 was dissolved in PEG-200. [Sar^9,$Met(O_2)^{11}$]substance P (Sigma, Poole, UK) was dissolved in 0.01% acetic acid. Cisplatin (Lederle) was prepared in saline (0.9%, w/v) at 70–75°C followed by gradual cooling to 40–50°C and administered immediately. Ondansetron dihydrochloride (Glaxo) was dissolved in water. Cisplatin was administered in a volume of 5 ml/kg and administrations in the gerbil were made in the volume of 2 ml/kg. Unless stated otherwise, other drugs were administered in a volume of 1 ml/kg.

2.8. Statistical analysis

The significance of difference between treatments was assessed by one way analysis of variance (ANOVA) followed by Dunnett's *t*-test.

3. Results

3.1. Binding studies

PD 154075 possessed high affinity for human, guinea pig, hamster, ferret, dog, lamb and gerbil tachykinin NK_1 receptors (respective IC_{50} values and ranges in parantheses, $n = 3$–6) 0.84 (0.45–1.3), 2.5 (1.2–6.9), 5.1(4.7–5.4), 3.4 (3.1–3.7), 0.65 (0.42–0.96), 0.58 (0.23–1.1) and 6.2 (4.0–8.9) nM. In contrast, PD 154075 exhibited significantly lower affinity for NK_1 receptors present in rat and mouse cortex (respective IC_{50} values and ranges in parantheses, $n = 3$) 302 (193–444) and 479 (399–620) nM.

PD 154075 exhibited a high degree of selectivity for the human tachykinin NK_1 receptor, showing $< 50\%$ inhibition of specific binding at 10 μM against a wide range of receptors, including tachykinin NK_2. The exceptions (CCK_B, human NK_3, human NMB, rat NMB and human GRP) yielded IC_{50} values in the range 1–10 μM. Amongst the assays profiled, PD 154075 possessed the highest affinity for the CCK_A receptor (IC_{50} 322 nM; range 271–431; $n = 3$).

3.2. Brain penetration and bioavailability

3.2.1. Effect of PD 154075 on [Sar^9,$Met(O_2)^{11}$]substance-P-induced foot tapping in the gerbil

Intracerebroventricular injection of [Sar^9,$Met(O_2)^{11}$]substance P dose dependently (1–100 nmol/animal) induced a species-typical foot-tapping response in the gerbil with a minimum effective dose of 10 nmol (Fig. 1a). The dose of 100 nmol produced almost constant foot tapping during the 5 min observation period (Fig. 1a). The foot tapping response was not observed following administration of vehicle (Fig. 1a). The dose of

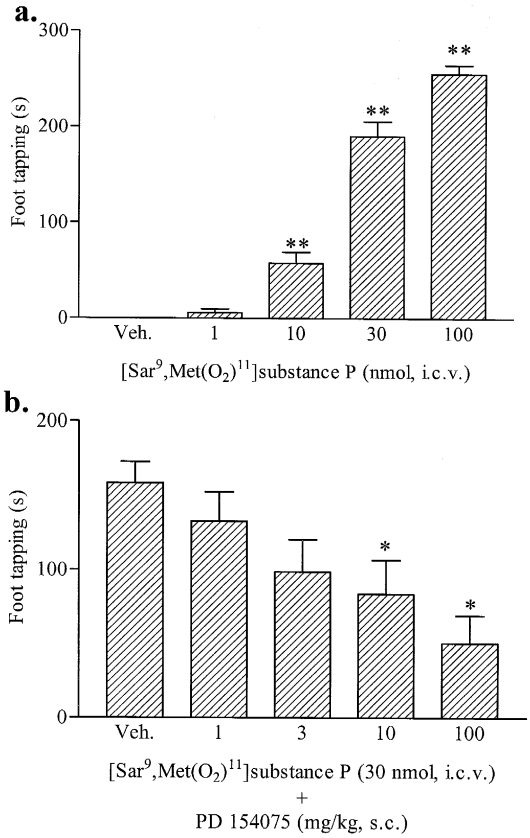

Fig. 1. Effect of PD 154075 on [Sar^9,$Met(O_2)^{11}$]substance-P-induced foot tapping in the gerbil. (a) Top panel shows dose response to [Sar^9,$Met(O_2)^{11}$]substance P following i.c.v. administration. The duration of foot tapping was recorded for 5 min immediately following recovery of the animals righting reflex. (b) Lower panel shows the effect of PD 154075 on this response following s.c. administration 30 min before [Sar^9,$Met(O_2)^{11}$]substance P (30 nmol/animal). Results are shown as the mean (vertical bars show ±S.E.M.) of 8–10 animals per group. * $P < 0.05$, ** $P < 0.01$, Significantly different from the vehicle (Veh.)-treated control group.

Table 2
Pharmacokinetic parameters in male Wistar rats following a single i.v. or p.o. dose of PD 154075

Parameter	i.v.	p.o.
Dose (mg/kg)	5.0	20
C_{max} (ng/ml)	—	774 (±220)
t_{max} (h)	—	5.0 (±1.2)
$t_{1/2}$ (h)	3.5 (±1.0)	3.0 (±0.4)
AUC_{0-} (ng·h/ml)	3556 (±771)	6956 (±1450)
CL_{tot} (ml/min per kg)	23.9 (±4.4)	—
V_{dss} (l/kg)	3.0 (±1.3)	—
%F	—	49 (±15)

Data are shown as means ± S.D. of 4 rats per group.

30 nmol [Sar^9,$Met(O_2)^{11}$]substance P was chosen for antagonism studies as it represented a submaximal dose. Preliminary results showed that PD 154075 (10 mg/kg, s.c.) induced maximum inhibition of gerbil foot tapping when administered 30 min before [Sar^9,$Met(O_2)^{11}$]substance P. The administration of PD 154075 at the peak pretreatment time dose dependently (1–100 mg/kg, s.c.) antagonised the [Sar^9,$Met(O_2)^{11}$]substance P foot tapping response with a minimum effective dose (MED) of 10 mg/kg (Fig. 1b).

3.2.2. Plasma concentrations of PD 154075

Following i.v. dosing, PD 154075 plasma concentrations declined multiexponentially with a terminal elimination half life of (mean ± S.D.) 3.5 ± 1.0 h (Table 2). Following p.o. administration, a relatively fast but sustained absorption profile was observed, as evident from a plasma concentration of 246 ± 106 ng/ml at 30 min and a mean peak concentration (C_{max}) of 774 ± 220 ng/ml at 5 h (t_{max}). It is of interest to note that relatively high drug concentrations persisted for up to 12 h: 751 ± 193, 545 ± 186, and 202 ± 79 ng/ml at 6, 8, and 12 h postdose, respectively. The absolute p.o. bioavailability was estimated to be 49 ± 15%.

3.2.3. Brain penetration of PD 154075

Following p.o. administration of PD 154075, a relatively fast and sustained brain/plasma profile was observed (Table 3). Plasma and brain profiles were parallel, with both brain and plasma concentrations of PD 154075 peaking at 4 h. This is consistent with the plasma PD 154075 t_{max} from the bioavailability study (Table 2). Brain/plasma concentration ratios ranged from 0.27 to 0.41 during the time period of 0.25–12 h, indicating that a substantial proportion of PD 154075 readily penetrated into tissue and the proportion was sustained for a long period of time after a single p.o. dose (Table 3). The respective AUC_{0-12} values for brain and plasma were estimated to be 1039 and 3137 ng · h/ml, giving an AUC ratio of 0.33.

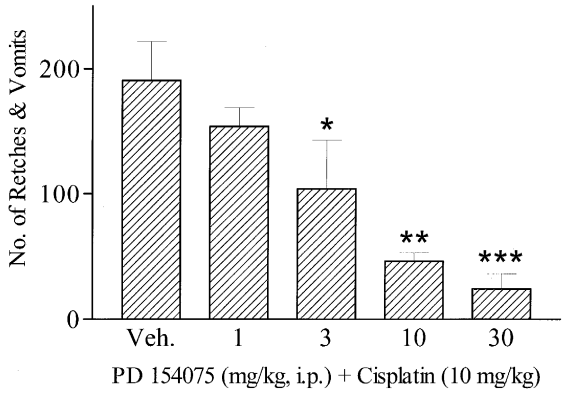

Fig. 2. Effect of PD 154075 on cisplatin-induced acute emesis in the ferret. Cisplatin (10 mg/kg, i.p.) was administered 1 h after PD 154075 (i.p.) or vehicle (PEG-200). Results are shown as the mean number of vomits and retches for the first 4 h observation period (vertical bars show ±S.E.M.) in 4 animals per group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ Significantly different from the vehicle-treated control group (ANOVA followed by Dunnett's *t*-test).

3.3. Effect of PD 154075 on cisplatin-induced acute and delayed emesis

3.3.1. The acute response induced by cisplatin (10 mg / kg)

PD 154075 administered i.p. 1 h before cisplatin (10 mg/kg, i.p.) dose dependently (1–30 mg/kg) reduced the frequency of vomiting and retching during the 4 h observation period with a minimum effective dose of 3 mg/kg (Fig. 2). At 30 mg/kg PD 154075 produced a complete antagonism of the emetic response (Fig. 2).

3.3.2. The acute and delayed response induced by cisplatin (5 mg / kg)

The 3 times daily injection of PD 154075 using a regimen of 10 mg/kg, i.p. administered every 8 h caused a major antagonism or abolition of cisplatin emesis in all animals studied (Fig. 3). During days 1 ('acute'), 2 and 3 (delayed), and using the values obtained during the entire

Table 3
Brain and plasma PD 154075 concentrations and ratios in rats after a p.o. dose of 21.4 mg/kg

Time (h)	Brain conc. (ng/g)	Plasma conc. (ng/ml)	Brain/plasma conc. ratio	Amount in brain (ng)
0.25	31 (±17)	126 (±92)	0.27 (±0.06)	57 (±31)
2	96 (±79)	301 (±244)	0.30 (±0.03)	189 (±156)
4	212 (±168)	642 (±433)	0.29 (±0.09)	410 (±333)
8	41 (±38)	108 (±80)	0.33 (±0.11)	81 (±73)
12	15 (±5)	45 (±20)	0.41 (±0.27)	30 (±11)

Data are shown as means ± S.D. of 3 rats per time point.

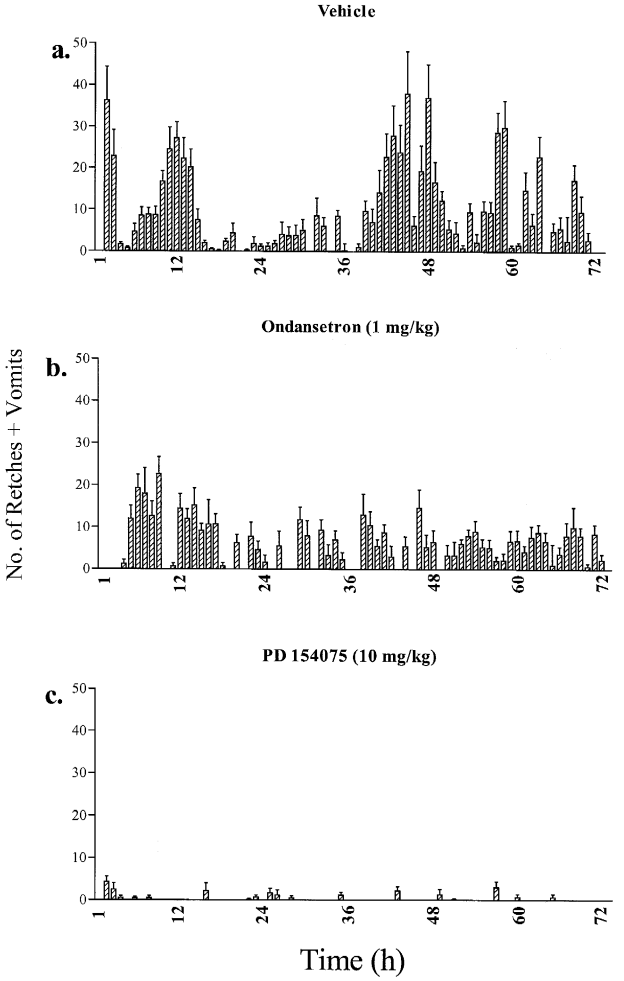

Fig. 3. Effect of PD 154075 on cisplatin-induced delayed emesis in the ferret. Cisplatin (5 mg/kg) was administered i.p. 1 h before either (a) PEG-200 (vehicle), (b) ondansetron (1 mg/kg, i.p.) or (c) PD 154075 (10 mg/kg, i.p.) Drug administration was continued every 8 h for the 3 days. Results are shown as the mean number of vomits and retches (vertical bars show ±S.E.M.) in 6 animals per group.

24 h periods, PD 154075 reduced retching and vomiting by 95.7, 97.3 and 97.6%, respectively. Such antagonisms were highly significant ($P < 0.0001$) and were achieved in the absence of sedation, motor impairment or any other overt changes in behaviour.

The results obtained during the first 24 h period were further delineated into two groups: data obtained during the periods 0.0–2.9 and 3.0–23.9 h. This was done to maximise the efficacy of the comparator drug ondansetron during the first 3 h period (see below). During the 0.0–2.9 h period, PD 154075 reduced retching and vomiting by 88.5% ($P < 0.0001$) and by 98.3% ($P < 0.0001$) during the remaining 21 h period of the first day.

During each of the 24-h periods, the 8 hourly administrations of ondansetron (1 mg/kg, i.p.) caused modest reductions of emesis 41% ($P < 0.001$), 49% (0.0001) and 24% ($P < 0.005$) on days 1, 2 and 3, respectively (Fig. 3). However, analysis of the data during the 0.0 to 2.9 h and 3.0 to 23.9 h during the first day of treatment indicated an abolition of retches and vomits during the first time period ($P < 0.0001$). However, no reduction was observed during the period 3.0 to 23.9 h. Again, animals treated with ondansetron remained in good health.

4. Discussion

PD 154075 is a highly selective tachykinin NK_1 receptor antagonist, shown in radioligand binding studies to possess nanomolar affinity for the human, guinea-pig, dog and ferret NK_1 receptors with a 300 times reduced affinity for the rodent NK_1 receptor. This confirms previous studies which demonstrate that guinea-pig and human tachykinin NK_1 receptors are similar to each other but different to rodent NK_1 receptors. A high degree of selectivity for the human type NK_1 receptor is exhibited by PD 154075. It was found to be inactive in most binding assays (< 50% inhibition of specific binding at 10 μM). A modest affinity was observed for the CCK_A receptor (322 nM). It should be noted that unlike some of the piperidine-based NK_1 receptor antagonists (Schmidt et al., 1992; Guard et al., 1993a), PD 154075 up to high concentrations of 10 μM failed to interact with calcium channels.

Functional studies have shown that PD 154075 is a competitive NK_1 receptor antagonist (Boyle et al., 1994). It has been reported that the intracerebroventricular administration of NK_1 receptor agonists induce a characteristic foot tapping response in the gerbil (Graham et al., 1993; Bristow and Young, 1994; Rupniak and Williams, 1994). It has been suggested that this model measures brain penetration of NK_1 receptor antagonists and the present study indicates that the systemic administration of PD 154075 is able to block [Sar^9,$Met(O_2)^{11}$]substance P induced behaviour in the gerbil. The ability of PD 154075 to penetrate into the brain is further supported by a HPLC assay of brain concentration of PD 154075. These data show that following oral administration, PD 154075 rapidly penetrates into the brain and that high concentrations are maintained for several hours.

Previous studies have reported that piperidine-based NK_1 receptor antagonists CP-99,994 and GR203040 possess a broad spectrum of antiemetic activity against cisplatin, apomorphine, copper sulphate, ipecacuanha, radiotherapy, morphine and motion sickness (Bountra et al., 1993; Gardner et al., 1995; Watson et al., 1995). It is known that $5\text{-}HT_3$ receptor antagonists have a more limited spectrum of antiemetic activity, being active mostly against emesis induced by chemotherapy (acute response), radiotherapy and ipecacuanha (for reviews, see Aapro, 1991; Andrews, 1994). The results presented here show that PD 154075, which is structurally distinct from CP-99,994 and GR203040, can block the cisplatin-induced acute emetic response in the ferret. This supports the hypothesis that the NK_1 receptor plays an important role in

mediating chemotherapy-induced emesis. It has been reported that compounds such as L-743,310 and GR 82334 have poor CNS penetration and do not block cisplatin-induced emesis, unless administered directly into the hindbrain, in the region of the nucleus tractus solitarii (Gardner et al., 1994; Hargreaves et al., 1994). These studies indicate that brain penetration of a NK_1 receptor antagonist is of paramount importance for the treatment of acute phase of chemotherapy-induced emesis.

The major finding of the present study is that PD 154075 almost completely blocked the delayed phase of cisplatin-induced emesis. In the present study PD 154075 was administered every 8 h to block the delayed emetic response. However, the antiemetic effect of PD 154075 was still apparent after 8 h indicating that PD 154075 has a long duration of action. This is consistent with pharmacokinetic studies indicating that it has a long $t_{1/2}$ in the rat of 3.0–3.5 h. Further studies are required to assess dose-response and duration of action of PD 154075 in the ferret delayed emesis model. It will be interesting to see whether blockade of NK_1 receptors by administration of one single dose of PD 154075 before the start of chemotherapy is sufficient or whether repeated dosing is required to provide protection against both the acute and delayed phases of emesis. Recently, it has been reported that CP-99,994 is also effective against cisplatin-induced delayed emesis in the ferret (Watson et al., 1996). This study taken together with the present data provide strong evidence for the involvement of the NK_1 receptor in cisplatin-induced delayed emesis.

The mechanism involved in the initiation of delayed emesis is currently unknown but the present results do suggest that chemotherapy-induced delayed emesis is not wholly dependent on the acute response. Thus, pronounced delayed emesis was observed in ferrets given ondansetron to completely abolish the acute response, with a reduction of only 24–49% by the continued treatment with ondansetron. This is consistent with the clinical data showing that 5-HT_3 receptor antagonists induce a modest reduction of vomiting and nausea after the first day of chemotherapy (for review, see Morrow et al., 1995). Recently, it has been shown that dexamethasone improves the control of emesis by ondansetron in the ferret model (Rudd and Naylor, 1996). This is also consistent with the clinical profile of these agents in the treatment of chemotherapy-induced emesis. These observations appear to suggest that the ferret model may be useful in the detection of antiemetic action for treatment of chemotherapy-induced acute and delayed emesis in man.

There is currently no satisfactory treatment that can eliminate severe chemotherapy-induced delayed emesis in man. It is noteworthy that side effects associated with dexamethasone (fluid retention and perineal irritation) has severely limited its use as an antiemetic agent given in combination with 5-HT_3 receptor antagonists. The good pharmacokinetic profile of PD 154075 suggests that it may be suitable for oral administration to control chemotherapy-induced delayed emesis. It should be noted that PD 154075 showed antiemetic activity in the absence of any side effects at the doses studied. Clinical use of 5-HT_3 receptor antagonists has revealed that this class of antiemetic agents is more effective at controlling vomiting than nausea (for review, see Morrow et al., 1995). It will be interesting to evaluate the efficacy of NK_1 antagonists against nausea in man. It has been shown that NK_1 receptor antagonists block emesis regardless of the cause in several species. If substance P also plays an important role in the induction of emetic reflex in humans, then NK_1 receptor antagonists could have much improved therapeutic potential than 5-HT_3 receptor antagonists in the treatment of chemotherapy-induced nausea and vomiting.

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