

BRAIN PENETRATION OF APREPITANT, A SUBSTANCE P RECEPTOR ANTAGONIST, IN FERRETS

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ABSTRACT:

The pharmacokinetics, metabolism, and brain penetration of the neurokinin 1 (NK₁) receptor antagonist (substance P receptor antagonist), aprepitant (MK-0869), were examined in ferrets. This species exhibits human-type NK₁ receptor pharmacology and is of proven value in the identification of clinically useful drugs for the treatment of chemotherapy-induced nausea and vomiting in humans. After a single p.o. dose of aprepitant at 1 or 2 mg/kg, plasma levels of the compound were between ~200 and 270 ng/ml, 24 h after dosing. In the brain cortex, concentrations of aprepitant reached between ~80 and 150 ng/g of tissue 24 h after dosing. The predominant radioactive component present in the plasma and the

brain of ferrets at 24 or 48 h after a single oral dose of [¹⁴C]aprepitant at 3 mg/kg was the parent compound itself. The slow plasma clearance of aprepitant (~1.5 ml/min/kg) and its abundance in ferret brain were in accord with its efficacy in blocking the retching and vomiting at 24 and 48 h postdose when ferrets were challenged with the emetic anticancer drug, cisplatin. When aprepitant and some of its metabolites were assessed for their in vitro binding affinity to the human NK₁ receptor, aprepitant demonstrated the highest affinity. Collectively, these data suggested that aprepitant, rather than its metabolites, was responsible, primarily, for the antiemetic activity of this compound in the male ferret.

One of the unpleasant side effects associated with chemotherapy is the intense feeling of nausea often accompanied by vomiting. Among the agents used to alleviate these conditions are H₁-blockers (Bergman and Spealman, 1988; Tonato et al., 1994), D₂-blockers (Jovanovic-Micic et al., 1995; Mystakidou et al., 1998), 5-hydroxytryptamine₃ blockers (Gregory and Ettinger, 1998; Ye et al., 2001), and cannabinoids (Abrahamov et al., 1995; Mechoulam and Hanu, 2001). More recently, efforts have been made to determine the efficacy of NK₁ receptor¹ antagonists

against chemotherapy-induced nausea and vomiting (Bountra et al., 1993; Tattersall et al., 1994; Beattie et al., 1995; Gardner et al., 1995, 1996; Harrison et al., 2001). Using the ferret, it has been established that brain penetration of NK₁ antagonists is essential to prevent drug-induced (cisplatin) emesis, and several compounds have been shown to be efficacious in this regard (Watson et al., 1995; Gonsalves et al., 1996; Rudd et al., 1996; Tattersall et al., 1996; Rupniak et al., 1997; Singh et al., 1997; Zaman et al., 2000; Harrison et al., 2001). One of these compounds is aprepitant (MK-0869; 5-[[[(2R,3S)-2-[(1R)-1-[3,5-bis(trifluoromethyl)phenyl]ethoxy]-3-(4-fluorophenyl)-4-morpholinyl]methyl]-1,2-dihydro-3H-1,2,4-triazol-3-one) (Hale et al., 2000; Tattersall et al., 2000; Harrison et al., 2001).

¹ Abbreviations used are: NK₁, neurokinin 1; MK-0869, aprepitant, 5-[[[(2R,3S)-2-[(1R)-1-[3,5-bis(trifluoromethyl)phenyl]ethoxy]-3-(4-fluorophenyl)-4-morpholinyl]methyl]-1,2-dihydro-3H-1,2,4-triazol-3-one); M-1, (2R,3S)-2-[(1R)-1-[3,5-bis(trifluoromethyl)phenyl]ethoxy]-3-(4-fluorophenyl)morpholine; M-2, (2R)-2-[(1R)-1-[3,5-bis(trifluoromethyl)phenyl]ethoxy]-3-(4-fluorophenyl)-5,6-dihydro-2H-1,4-oxazine; M-3, (5S,6R)-6-[(1R)-1-[3,5-bis(trifluoromethyl)phenyl]ethoxy]-5-(4-fluorophenyl)-3-morpholinone; M-4, (6R)-6-[(1R)-1-[3,5-bis(trifluoromethyl)phenyl]ethoxy]-5-(4-fluorophenyl)-5-hydroxy-morpholinone; M-5, [(1R)-1-[(1R)-1-[3,5-bis(trifluoromethyl)phenyl]ethoxy]-2-(4-fluorophenyl)-2-oxoethoxy]acetic acid; M-6, [(1R)-1-[(1R)-1-[3,5-bis(trifluoromethyl)phenyl]ethoxy]-2-(4-fluorophenyl)-2-hydroxyethoxy]acetic acid; LC-MS/MS, liquid chromatography-tandem mass spectrometry; HPLC, high-performance liquid chromatography; AUC, area under the plasma concentration versus time curve; AUMC, total area under the first moment of the drug concentration versus time curve from time 0 to infinity; C_{max}, maximal concentration of aprepitant observed in plasma; T_{max}, time required to reach C_{max} values; CL_{cr}, mean plasma clearance; Vd_{ss}, volume of distribution at steady state.

Aprepitant exhibited a protracted effect in blocking the cisplatin-induced emesis in ferrets (Tattersall et al., 2000; Harrison et al., 2001). This is a crucial effect because, in humans undergoing treatment with chemotherapeutic agents such as cisplatin, the nausea and vomiting may persist for many days after the initial cancer treatment (Navari et al., 1999; Campos et al., 2001; Cocquyt et al., 2001). Brain penetration and subsequent receptor occupancy are important factors in mediating the beneficial effects of aprepitant in the vomiting induced in the ferret by cisplatin. However, it is not known whether the extended antiemetic activity resides with aprepitant or with its metabolites or, indeed, with both parent compound and metabolites.

For this reason, the pharmacokinetics of aprepitant and the metabolism of radiolabeled aprepitant were examined in ferrets after a single oral dose of the compound. In addition, the brain levels, as well as the identification of metabolites in the ferret brain, were evaluated. Previously, several metabolites of aprepitant were identified in pri-

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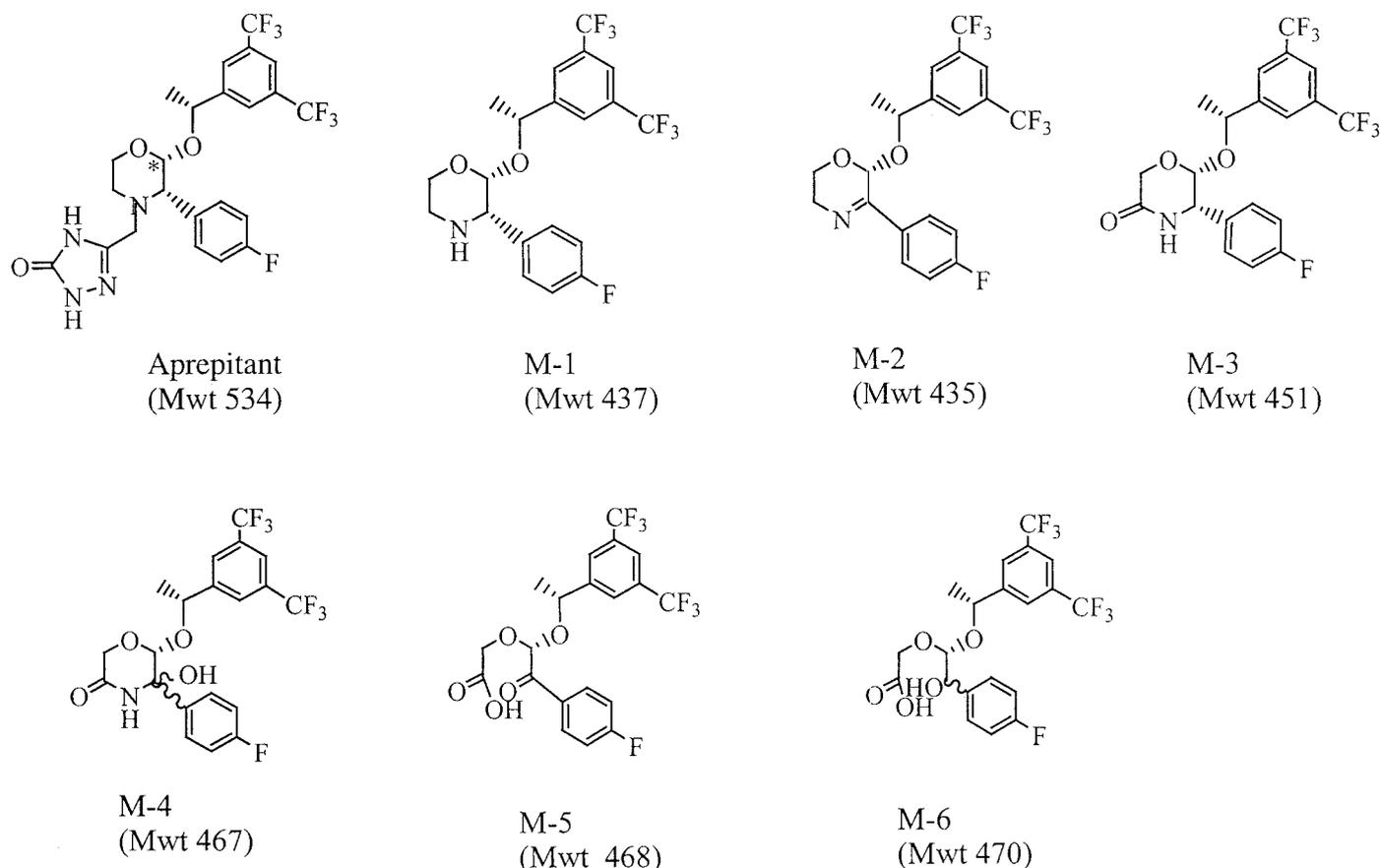


FIG. 1. Structures of aprepitant and its metabolites.

* denotes the positions of ^{14}C label.

mary rat hepatocyte cultures (S. E. Huskey, R. I. Sanchez, G. A. Doss, B. H. Arison, B. J. Dean, J. Pang, K. Leung, B. Zhu, M. P. Braun, P. E. Finke, D. Luffer-Atlas, T. A. Baillie, and S. H. L. Chiu, manuscript submitted for publication), and this information was used to facilitate the identification of the *in vivo* metabolites in ferret, as described herein. Furthermore, to gain insight into the pharmacologically active entities in the ferret brain, the NK_1 receptor binding affinities of aprepitant and authentic metabolites were evaluated. These experiments led to the conclusion that the major entity responsible for the beneficial, antiemetic effects of aprepitant in the ferret was the parent molecule itself.

Materials and Methods

Chemicals. Aprepitant (Fig. 1) was prepared by Merck Process Research (Rahway, NJ). [Morpholine-2- ^{14}C]aprepitant (specific activity 29.83 $\mu\text{Ci}/\text{mg}$) was synthesized by the Merck Labeled Compound Synthesis Group (Rahway, NJ). The desfluoro derivative of aprepitant and several metabolites, including M-1, M-3, M-4, M-5, and M-6 (Fig. 1) were synthesized by Merck Medicinal Chemistry (Rahway, NJ). Metabolite M-2 was synthesized by Merck Medicinal Chemistry (Terlings Park, UK).

Dose Preparation. For pharmacokinetic studies, the *i.v.* dose of aprepitant (0.5 mg/kg) was administered in a vehicle consisting of ethanol/propylene glycol/water (1:6:3 by volume, 0.5 ml/kg) and the oral dose (1 mg/kg) in a suspension in 0.5% aqueous methylcellulose containing 0.02% sodium lauryl sulfate (1 ml/kg). For the brain penetration studies, two oral doses of aprepitant (1 and 2 mg/kg) were prepared in ethanol/propylene glycol/water (1:6:3 by volume, 1 ml/kg), and one oral dose of [^{14}C]aprepitant (3 mg/kg; specific activity 18 $\mu\text{Ci}/\text{mg}$) was formulated in a suspension in 0.5% aqueous methylcellulose containing 0.02% sodium lauryl sulfate (1 ml/kg). The *i.v.* solutions were passed through a sterile 0.45- μm filter before dosing.

Pharmacokinetic Studies. Male ferrets (~1–2 kg) were purchased from Marshall Farms USA Inc. (North Rose, NY) or a registered breeder of ferrets in the UK. The animals were housed under standard conditions, with a 12-h light/dark cycle, in the Comparative Medicine facilities of Merck Research Laboratories, Rahway, NJ, or Merck, Sharp and Dohme Laboratories, Terlings Park, UK. Animals were allowed access to commercial chow and water *ad libitum*. They were not fasted overnight, but food was withdrawn before dosing and then returned 4 or 6 h after dosing. Animals were allowed unrestricted access to water during the study period.

For the pharmacokinetic studies, three male ferrets were used in a crossover design. They were dosed with aprepitant at 0.5 mg/kg by bolus injection via a percutaneously placed cephalic vein catheter. After a 2-week washout period, the same three ferrets were dosed orally by gavage at 1 mg/kg. Blood samples (1 ml) were obtained by venipuncture from the jugular vein into heparinized Vacutainer blood collection tubes at predose, 5 (*i.v.* only), 15, or 30 min, and 1, 2, 4, 6, 8, 10, 24, 32, 48, 56, and 72 h after dosing. Plasma was prepared by centrifugation of the blood and stored at -20°C until analysis.

Brain Penetration Studies. Adult male ferrets ($n = 3\text{--}4$ per time point), weighing ~1.0–2.0 kg, were dosed with aprepitant orally, by gavage, at a dose of 1 or 2 mg/kg. Terminal blood samples were collected from the abdominal aorta of anesthetized ferrets into heparinized tubes at 1, 2, 4, 10, or 24 h post dose. Plasma was prepared by centrifugation at 3000g for 10 min. Ferret brain cortices were collected and stored at -70°C until analysis.

Another group of male ferrets ($n = 2$ per time point), weighing ~2 kg, received a single oral dose of [^{14}C]aprepitant, by gavage, at a dose of 3 mg/kg. Terminal blood samples were collected by cardiocentesis from anesthetized ferrets at 24 or 48 h post dose. Plasma was prepared by centrifugation at 3000g for 10 min. Entire ferret brains were collected and stored at -70°C until analysis.

Preparation of Plasma Samples for LC-MS/MS Analysis. Plasma samples (0.2 ml), from ferrets in the pharmacokinetic study, were mixed with 20

ng of internal standard, a desfluoro derivative of aprepitant, and diluted with 1.7 ml of deionized water followed by 0.5 ml of acetonitrile. This solution was transferred to a Bond Elut C₁₈ (500 mg) cartridge (Varian; Harbor City, CA). Following percolation through the cartridge, the solid phase was washed with 6 ml of water and eluted with 3 ml of methanol. The methanol eluent was evaporated to dryness under a stream of nitrogen, resolubilized in 300 μ l of mobile phase (see below), and analyzed by LC-MS/MS.

Alternatively, aliquots of the plasma samples (50 μ l) from ferrets, in the brain penetration study, were transferred to a 96-well plate, mixed with 5 ng of internal standard, and precipitated with 100 μ l of acetonitrile. After vortex-mixing, samples were spun in a centrifuge at 3000g for 10 min. Aliquots (5 μ l) of the supernatants were subjected to LC-MS/MS analysis.

Preparation of Plasma Samples for HPLC Analysis. Plasma proteins were precipitated by the addition of 2 volumes of acetonitrile to 3 ml plasma samples obtained from animals in the brain penetration study using [¹⁴C]aprepitant. After centrifugation at 3,000g, the supernatants were transferred to clean tubes and evaporated to dryness under N₂. The residues were redissolved in 1 ml of 40% aqueous methanol and 250 to 400 μ l aliquots of these solutions were analyzed by HPLC (see below).

Extraction of Brain Homogenates for LC-MS/MS Analysis. Half of the brain cortex obtained from ferrets in the brain penetration studies were weighed and homogenized with 1 ml of deionized water. To each homogenate was added 100 ng of internal standard in methanol, and the suspension was subjected to sonication for 5 min. Aliquots (50 μ l) were transferred to a 96-well plate and 100 μ l of acetonitrile was added. The samples were vortex-mixed and spun in a centrifuge at 3000g for 10 min. Aliquots (25 μ l) of the resulting supernatants were subjected to LC-MS/MS analysis (see below).

Extraction of Brain Homogenates for HPLC Analysis. Entire brains from ferrets were weighed and homogenized with 3 volumes of deionized water. The proteinaceous material in each homogenate (10 ml) was precipitated by the addition of 9 volumes (v/v) of acetonitrile. These suspensions were thoroughly vortex-mixed, sonicated for 5 min, and spun in a centrifuge at 3000g for 10 min. The supernatants were transferred to clean glass tubes, and the pellets were re-extracted with methanol (10 ml). The methanolic extracts were combined with the acetonitrile supernatants prior to loading onto Varian Bond Elut C₁₈ cartridges adapted with Acrodisc glass filters (Gelman Instrument Co., Ann Arbor, MI). After sample application, the cartridges were washed with 5 ml of a 2:1:1 mixture, by volume, of methanol/acetonitrile/distilled water. The eluents collected after sample loading and during the cartridge wash were combined and evaporated to dryness under N₂. The residues were redissolved in 5 ml of methanol, vortex-mixed, subjected to sonication, and spun in a centrifuge as described previously; and the supernatants were transferred to clean glass tubes and evaporated to dryness under a stream of N₂. The residues were redissolved in 1 ml of 40% aqueous methanol, and aliquots (400 μ l) were analyzed by HPLC (see below).

Radioactivity Measurements. For an estimate of total radioactivity, duplicate aliquots of plasma (0.1–0.5 ml) were counted directly by liquid scintillation counting following the addition of scintillation cocktail (Ultima-FLO M; PerkinElmer Life Sciences, Boston, MA). Triplicate aliquots of brain homogenates (0.4–0.7 g), prepared as described previously, were transferred by pipette into paper combustion cones and air-dried overnight prior to combustion. These samples were combusted in an oxidizer (model 307; PerkinElmer Life Sciences) and their radioactive content was estimated by liquid scintillation counting (Beckman Coulter, Inc., Fullerton, CA).

HPLC Analysis with Radiometric Detection. A Shimadzu HPLC system (Shimadzu Scientific Instruments Inc., Columbia, MD), consisting of two pumps (LC-10AD), a controller (SCL-10A), an autosampler (SIL-10A), a radiomonitor (INUS Systems Inc., Tampa, FL), and a fraction collector (model

FC 204; Gilson Medical Electronics, Middletown, WI), was used for all analyses.

Chromatography was performed on a Zorbax RX-C8 (4.6 \times 250 mm; MAC-MOD Analytical Inc., Chadds Ford, PA) column for both plasma and brain extracts. **Method A.** The mobile phase consisted of solvent A (10 mM ammonium acetate in water) and solvent B (7.2 mM ammonium acetate in 7.2% methanol and 92.8% acetonitrile, v/v). **Method B.** Elution solvents C (10 mM ammonium acetate in water containing 0.1% trifluoroacetic acid) and D (7.2 mM ammonium acetate in 7.2% methanol, 92.7% acetonitrile, and 0.1% trifluoroacetic acid, by volume) were used in this method. The eluent flow rate was 1 ml/min using a linear gradient from 35 to 80% B or 35 to 80% D in 40 min for methods A and B. Radioactivity profiles were obtained by on-line profiling using a radiomonitor (INUS Systems Inc.) connected to the column.

LC-MS/MS Analysis of Plasma and Brain Samples. Quantification of aprepitant in plasma and brain was performed on a tandem mass spectrometer (Sciex API III⁺; Applied Biosystems, Foster City, CA) using a heated nebulizer interface. Chromatographic separation was achieved on a Spherisorb C₈ column (4.6 \times 50 mm; 5 μ m; Waters, Milford, MA) using a mobile phase consisting of 55% acetonitrile, 45% 10 mM ammonium acetate, and 0.1% formic acid, by volume. The flow rate was 1 ml/min. In this system, aprepitant and the internal standard coeluted at approximately 1.5 min. The dwell time for the multiple reaction monitoring was 450 ms. A peak was defined typically by 10 to 12 data points. The positive ion mode was used. Multiple reaction monitoring using the precursor \rightarrow product ion combinations of *m/z* 535 \rightarrow 179 and 517 \rightarrow 161 was used for quantification of aprepitant and the internal standard, respectively. The levels of aprepitant in plasma or brain extract were determined by using standard curves in which control plasma (50 or 200 μ l) or control brain cortex extract (50 μ l) was spiked with increasing concentrations of aprepitant (0.25–1000 ng) and 20 or 100 ng of internal standard. A power regression fit, [Y = kXⁿ], was used to quantify the unknowns. Standards and samples were extracted concurrently. The limits of quantification for aprepitant were 5 ng/ml in plasma and 5 ng/g of tissue in brain, respectively.

Determination of Pharmacokinetic Parameters. The areas under the plasma concentration versus time curves (AUCs) were calculated using the linear trapezoidal method for the ascending portion of the curve and the logarithmic method for the descending portion of the curve. The area from the last measurable concentration to infinity was calculated by dividing the last measurable plasma concentration by the elimination rate constant, which was estimated from the plasma concentration versus time curve by linear regression at the terminal phase of the semilogarithmic plot. The plasma clearance of aprepitant was calculated from the dose divided by the total AUC. The apparent volume of distribution at steady state was determined as Dose \times AUMC/(AUC)², where AUMC is the total area under the first moment of the drug concentration versus time curve from time 0 to infinity. The *t*_{1/2} was calculated by dividing 0.693 (ln 2) by the elimination rate constant. For the oral dose, the *C*_{max} and *T*_{max} values represented the maximal concentration of aprepitant observed in plasma and the time required to reach *C*_{max} values, respectively. Oral bioavailability (% F) was calculated from the mean AUC values, corrected for dose, from the oral and i.v. doses.

In Vitro Binding Affinity of Aprepitant and Metabolites to Human NK₁ Receptor. The human NK₁ receptor was cloned and stably expressed in Chinese hamster ovary cells at a level of 1 \times 10⁵ receptors per cell (Cascieri et al., 1992; Fong et al., 1992). Briefly, cells were grown in monolayer culture, detached from the plate using Enzyme-free Cell Dissociation Solution (Specialty Media, A Division of Cell & Molecular Technologies Inc., Phillipsburg, NJ) and washed prior to the assay. [¹²⁵I]-Tyr⁸-substance P (0.1 nM, 2200 Ci/mmol; PerkinElmer Life Sciences) and the compound under investigation (in 5 μ l of dimethyl sulfoxide) were incubated with 5 \times 10⁴ Chinese hamster

TABLE 1

Pharmacokinetics of aprepitant in male ferrets following single i.v. or p.o. administration

The values are the mean \pm S.D. of *n* = 3 ferrets (crossover study design, with 2-week washout period).

Dose	Route	(AUC) _{0-∞}	CL _p	Vd _{ss}	<i>t</i> _{1/2}	<i>C</i> _{max}	<i>T</i> _{max}	% F
mg/kg		ng · h/ml	ml/min/kg	l/kg	h	ng/ml	h	
0.5	i.v.	5619 \pm 449	1.5 \pm 0.1	1.3 \pm 0.1	9.7 \pm 0.9			
1	p.o.	5151 \pm 1660				326.7 \pm 69.2	3.3 \pm 1.2	45.4 \pm 11.3

TABLE 2

Concentrations of aprepitant in plasma and brain of ferrets dosed orally at 1 or 2 mg/kg

The values are the mean \pm S.D. of $n = 3$ or 4 ferrets at each time point.

Dose	Ferret	Time	Concentrations of Aprepitant ^a		
			Plasma	Brain	Brain-to-Plasma Ratio
mg/kg	<i>n</i>	<i>h</i>	ng/ml	ng/g tissue	
1	4	1	237 \pm 11	29 \pm 2	0.12
1	3	2	190 \pm 68	39 \pm 11	0.21
1	3	4	339 \pm 67	136 \pm 10	0.40
1	4	10	353 \pm 68	160 \pm 5	0.51
1	4	24	213 \pm 37	84 \pm 10	0.39
2	3	1	613 \pm 222	82 \pm 12	0.13
2	3	2	498 \pm 97	91 \pm 15	0.18
2	3	4	401 \pm 144	118 \pm 31	0.29
2	4	10	995 \pm 167	384 \pm 34	0.39
2	4	24	266 \pm 24	145 \pm 17	0.55

ovary cells in 50 mM Tris-HCl, pH 7.5, containing 5 mM MnCl₂, 150 mM NaCl, 0.02% bovine serum albumin, 40 μ g/ml bacitracin, 4 μ g/ml leupeptin, and 10 μ M phosphoramidon. Compound titrations typically consisted of 10 half-log doses up to 0.1, 1, or 10 μ M, depending on the affinity of the compound. Incubations were carried out at room temperature until equilibrium was achieved (1 h), and then the receptor-ligand complex was harvested by filtration over PerkinElmer Unifilter plates presoaked in polyethyleneimine using a 96-well harvester. Nonspecific binding was determined using excess substance P (1 μ M; Peninsula Laboratories, Belmont, CA) and was less than 10% of total binding to cells. Some ¹²⁵I-substance P binding experiments were performed using the human NK₁ receptor transiently transfected into COS cells.

Results

Pharmacokinetics of Aprepitant in Ferret. The pharmacokinetics and oral bioavailability of aprepitant were determined in three male ferrets dosed i.v. at 0.5 mg/kg and p.o. at 1 mg/kg in a crossover design. The mean plasma clearance (CL_p), volume of distribution at steady state (Vd_{ss}), and terminal $t_{1/2}$ values were 1.5 ml/min/kg, 1.3 l/kg, and \sim 10 h, respectively. The mean oral bioavailability of aprepitant was \sim 46%, with a range of 35 to 63% (Table 1).

Concentrations of Aprepitant in Ferret Plasma and Brain. After p.o. administration to ferrets at 1 or 2 mg/kg, levels of aprepitant were high in the plasma and brain (Table 2, Fig. 2). The observed plasma C_{max} values were 353 and 995 ng/ml, and T_{max} occurred at 10 h post dose for both the 1 and 2 mg/kg doses. Similarly, the brain C_{max} values were 160 and 384 ng/ml at 10 h post dose. Of note, at 24 h post dose, high levels of aprepitant were still present in the plasma (1 mg/kg, 213 \pm 37 ng/ml; 2 mg/kg, 266 \pm 24 ng/ml) and also in the brain (1 mg/kg, 84 \pm 10 ng/g of tissue; 2 mg/kg, 145 \pm 17 ng/g of tissue). The brain-to-plasma ratios of aprepitant, ranging from 0.12 to 0.55 at selected time points at either dose level, increased with time and reached their highest ratios at 10 or 24 h postdose. Collectively, the data suggested that aprepitant was abundant in brain, especially at 10 and 24 h post dose.

Brain Penetration of [¹⁴C]Aprepitant in the Ferret. Total radioactivity in ferret plasma and brain of ferrets, following an oral dose of [¹⁴C]aprepitant at 3 mg/kg, are shown in Table 3. The levels of radioactivity were \sim 600 and \sim 450 ng Eq/ml in plasma 24 and 48 h post dose, whereas levels in the brain were quite similar at \sim 450 and \sim 350 ng Eq/g of tissue, respectively. The mean brain-to-plasma ratio of total radioactivity was \sim 0.8, ranging from 0.67 to 0.94 at 24 or 48 h post dose.

Metabolite profiles and concentrations of [¹⁴C]aprepitant in ferret plasma and brain. The radioactive metabolite profile of plasma, at 48 h postdose, is shown in Fig. 3, panel A. [¹⁴C]Aprepitant was the major component, accounting for 70–80% of plasma radioactivity at the 48-h time point after dosing. As described previously (S. E. Huskey, R. I. Sanchez, G. A. Doss, B. H. Arison, B. J. Dean, J. Pang, K. Leung, B. Zhu, M. P. Braun, P. E. Finke, D. Luffer-Atlas, T. A. Baillie, and S. H. L. Chiu, manuscript submitted for publication), aprepitant and its hydroxy-lactam metabolite M-4, coeluted at 27 min using HPLC Method A. However, in this instance, it was confirmed, using HPLC Method B, that [¹⁴C]aprepitant, and not M-4, was the chemical species present in ferret plasma and brain since the retention time of aprepitant was 27 min, whereas that of M-4 was \sim 24 to 25 min. Therefore, the concentrations of aprepitant were estimated to be \sim 360 ng Eq/ml (Table 3). This profile was qualitatively identical with

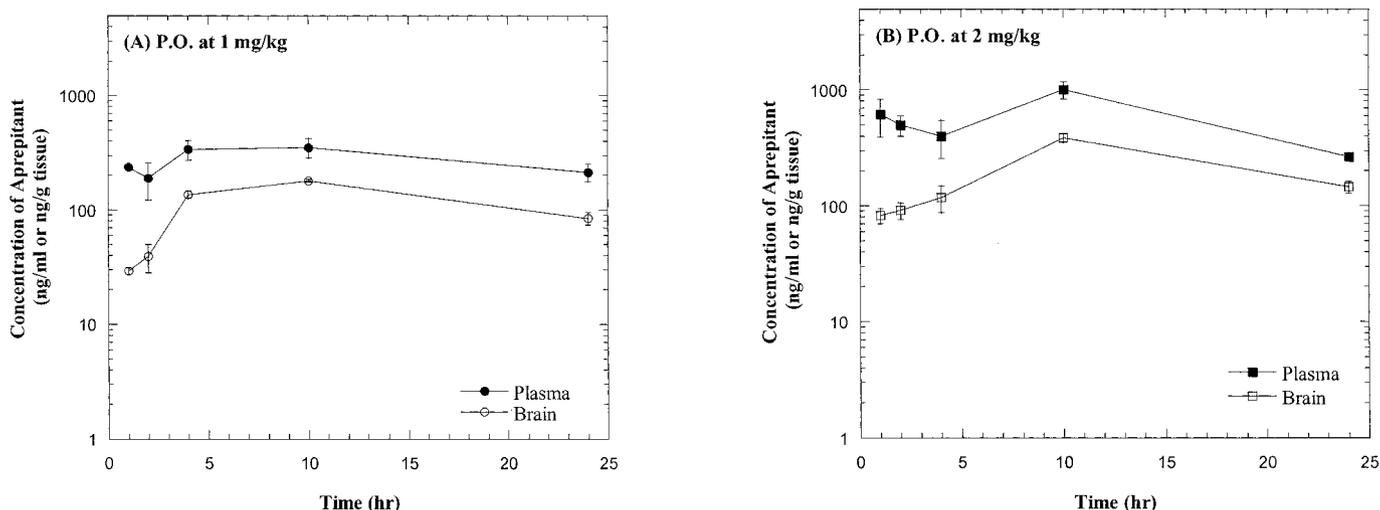


FIG. 2. Concentrations of aprepitant in plasma and brain of ferrets following p.o. dosing with aprepitant at 1 or 2 mg/kg.

At selected time intervals, blood samples and brain were collected from ferrets. Plasma samples were obtained by centrifugation of blood samples, and brain cortex was homogenized with water and extracted by acetonitrile. Concentrations of aprepitant at 1 mg/kg (A) (●, plasma; ○, brain) and 2 mg/kg (B) (■, plasma; □, brain) were determined by LC-MS/MS. The limits of quantification for aprepitant were 5 ng/ml in plasma and 5 ng/g tissue in brain. The concentrations of aprepitant were plotted against time.

TABLE 3
Distribution of [^{14}C]aprepitant and its metabolites in plasma and brain of ferrets dosed orally with 3 mg/kg

Ferret	Time h	Total Radioactivity			Total Radioactivity as Aprepitant ^a		
		Plasma ng Eq/ml	Brain ng Eq/g tissue	Brain-to-Plasma Ratio	Plasma ng Eq/ml	Brain ng Eq/g tissue	Brain-to-Plasma Ratio
1	24	610	469	0.77	586	375	0.64
2	24	570	460	0.81	487	324	0.67
3	48	393	370	0.94	362	229	0.63
4	48	493	332	0.67	360	227	0.63

^a Estimated from chromatographic profile, as described under *Materials and Methods*. Two ferrets were used at each time point.

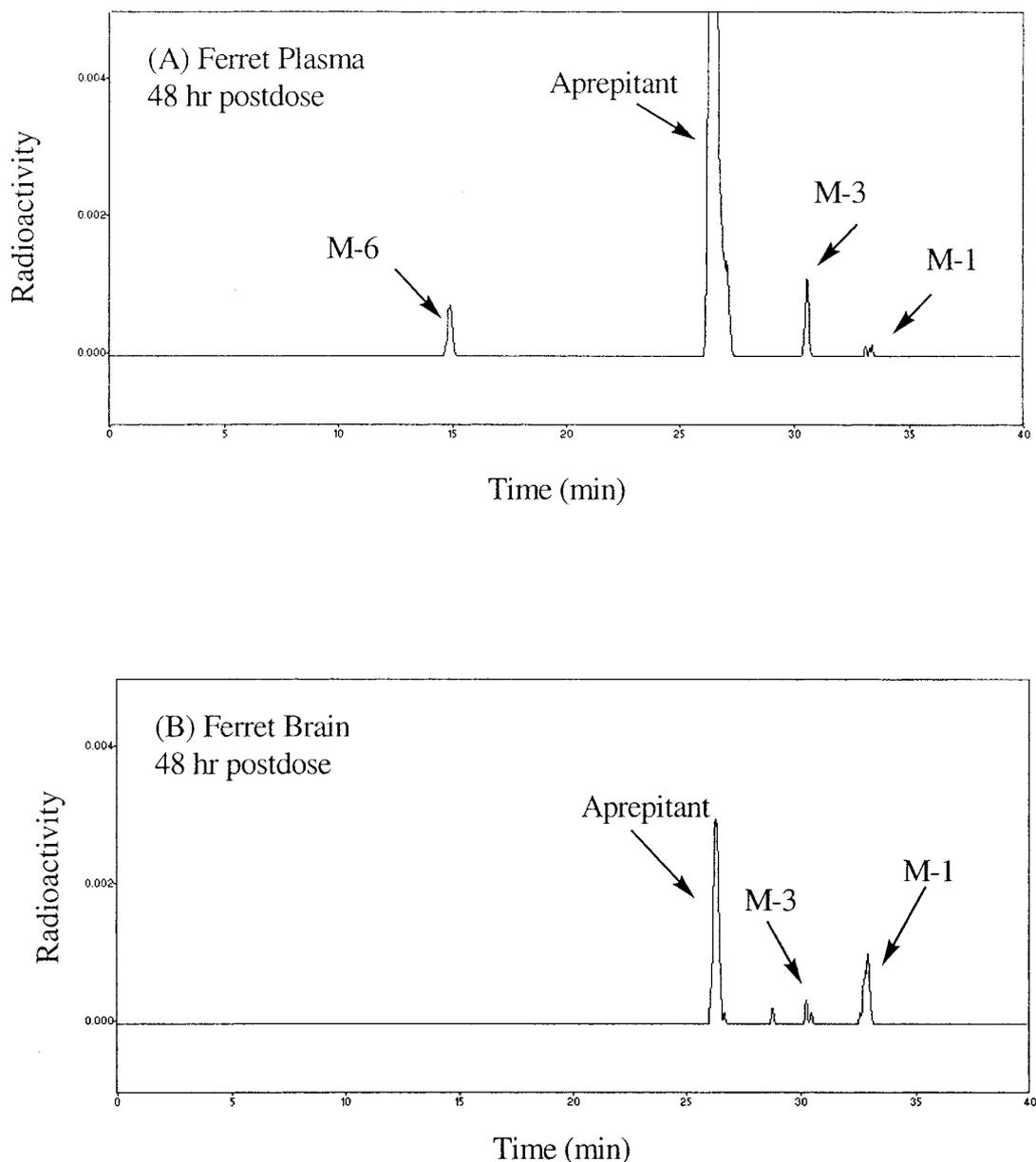


FIG. 3. Representative radioactivity profiles of aprepitant from plasma (A) and brain (B) of ferrets following *p.o.* dosing with [^{14}C]aprepitant at 3 mg/kg.

At 48 h postdose, plasma samples and brain extract from ferrets were subjected to HPLC analysis. Chromatography was carried out on a Zorbax RX-C8 column, as described under *Materials and Methods*.

that obtained 24 h postdose (data not shown), and the concentrations of aprepitant were estimated to be ~ 530 ng Eq/ml at the 24-h time point (Table 3). Several metabolites, M-1, M-3, and M-6 (Fig. 1), were detectable at low concentrations at the 48-h time point (Fig. 3).

Brain extracts, obtained from the same ferrets used for the 24- and 48-h plasma studies, were analyzed by HPLC. As shown in Fig. 3, panel B, the radioactivity profile of the extracts revealed a major component that eluted with the same retention time as aprepitant (27

TABLE 4

Binding affinities (mean \pm S.D.) of aprepitant and its metabolites in human NK₁ receptor binding assay

Parent/Metabolite	IC ₅₀ nM
Aprepitant	0.12 \pm 0.08
M-1	0.5 \pm 0.07
M-2	10 \pm 1
M-3	1.7 \pm 1.6
M-4	30 \pm 5
M-5	880 \pm 360
M-6	18 \pm 5

min). Based on the radiochromatograms, [¹⁴C]aprepitant accounted for >60% of the radioactivity recovered from brain at 24 and 48 h, and the concentrations of aprepitant were estimated to be ~350 and ~230 ng Eq/g tissue (Table 3). The brain-to-plasma ratios of total radioactivity or aprepitant were estimated to be ~0.64 (Table 3). In addition, two minor radioactive components, M-1 and M-3, were identified by their coelution with authentic materials by HPLC (Fig. 3).

Biological Activity of Metabolites of Aprepitant. The potential of metabolites of aprepitant (M-1, M-2, M-3, M-4, M-5, and M-6; Fig. 1) to serve as NK₁ receptor antagonists was evaluated using the human NK₁ receptor binding assay. When compared with aprepitant (mean IC₅₀ = 0.12 nM), all of the metabolites showed much reduced binding affinities, ranging from ~4-fold for M-1 to ~14-, ~250-, and ~7000-fold for M-3, M-4, and M-5, respectively (Table 4).

Discussion

There is strong evidence to suggest that the beneficial effects of substance P antagonists (NK₁ receptor antagonists) on nausea and vomiting observed after chemotherapy treatment are dependent on their penetration and subsequent binding in the brain (Watson et al., 1995; Gonsalves et al., 1996; Tattersall et al., 1996; Rupniak et al., 1997; Zaman et al., 2000). Cisplatin administration causes vomiting in ferrets, and this observation has led to the testing of antiemetics in this animal model (Bountra et al., 1993; Tattersall et al., 1994; Beattie et al., 1995; Gardner et al., 1995, 1996; Harrison et al., 2001). In a 4-h observation period, aprepitant (0.3, 1, or 3 mg/kg i.v. or 3 mg/kg p.o.) inhibited completely the emetic response to cisplatin (10 mg/kg i.v.) in ferrets. Furthermore, in a model of acute and delayed emesis, aprepitant (1 or 2 mg/kg once daily) completely prevented retching and vomiting for 72 h in all ferrets challenged with cisplatin (5 mg/kg i.p.) (Tattersall et al., 2000).

The question therefore arose whether metabolites of aprepitant contributed to its excellent duration of action in the ferret. Since the pharmacological effect is correlated with brain penetration, both the plasma and the brain tissue of ferrets were analyzed initially for aprepitant following a single oral dose at 1 or 2 mg/kg. As expected, the levels of aprepitant were high in both plasma and brain at 24 h postdose. Subsequently, radiolabeled aprepitant was administered to ferrets in an attempt to verify whether aprepitant is the major component present in both plasma and the target organ, the brain. Forty-eight hours after a single oral dose of [¹⁴C]aprepitant, the major radioactive component in the plasma was parent drug. Three other minor metabolites (M-1, M-3, and M-6) also were detected. In the ferret brain at this same time point, [¹⁴C]aprepitant was the predominant radioactive component; metabolites M-1 and M-3 were detected at lower levels, and the ratios of aprepitant to M-1 and aprepitant to M-3 were ~4 and ~4 at 48 h postdose. The mean brain-to-plasma ratio of aprepitant was estimated to be ~0.64 in ferrets 24 and 48 h

postdose (Table 3), suggesting that aprepitant penetrated the brain readily and was retained there for at least 48 h. Furthermore, the binding of aprepitant and its metabolites to the human NK₁ receptor demonstrated that aprepitant was more avidly bound than the metabolites M-1 through M-6. In our experience with numerous NK₁ antagonists from several structural classes, the compound binding affinities for the ferret NK₁ receptor consistently approximate those of the human NK₁ receptor. Collectively, these findings indicate that aprepitant is primarily responsible for preventing cisplatin-induced acute and delayed retching and vomiting in the ferret described by Tattersall et al. (2000).

In humans, aprepitant has proven to be efficacious against chemotherapy-induced nausea and vomiting (Navari et al., 1999; Campos et al., 2001) and is a low-clearance compound (Dr. A. Majumdar, Department of Drug Metabolism, Merck Research Laboratories, West Point, PA, personal communication). The in vitro binding affinities of the metabolites (M-1 through M-6) to the human NK₁ receptor showed them to have a much reduced binding affinity (~4 to ~7000-fold) when compared with aprepitant (Table 4). Therefore, although the metabolite profiles in human brain are not available, it is likely that aprepitant is responsible for the antiemetic activity observed in humans.

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