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

SU-ER W. HUSKEY, BRIAN J. DEAN, RAY BAKHTIAR, ROSA I. SANCHEZ, F. DAVID TATTERSALL, WAYNE RYCORFT, RICHARD HARGREAVES, ALAN P. WATT, GARY G. CHICCHI, CAROLANN KEOHANE, DONALD F. HORA, AND SHUET-HING L. CHIU

Departments of Drug Metabolism (S.-E.W.H., B.J.D., R.B., R.I.S., C.A.K., S.-H.L.C.), Comparative Medicine (D.F.H.), and Immunology and Rheumatology (G.G.C.), Merck Research Laboratories, Rahway, New Jersey; and Departments of Medicinal Chemistry (A.P.W.) and Pharmacology (F.D.T., W.R., R.H.), Merck Research Laboratories, Terlings Park, Essex, United Kingdom

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ABSTRACT:

The pharmacokinetics, metabolism, and brain penetration of the neurokinin 1 (NK₃) receptor antagonist (substance P receptor antagonist), aprepitant (MK-0869), were examined in ferrets. This species exhibits human-type NK₃ 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]aprepi tant 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 NK₃ 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 H₂-blockers (Bergman and Spealman, 1988; Tomato et al., 1994), D₂-blockers (Jovanovic-Micic et al., 1995; Mystakidou et al., 1998), 5-hydroxytryptamine₃ blockers (Gregory and Ettenger, 1998; Ye et al., 2001), and cannabinoids (Abramov et al., 1995; Mechoulam and Hanu, 2001). More recently, efforts have been made to determine the efficacy of NK₃ receptor 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 NK₃ 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).

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-
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 NK1 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-2-14C]aprepitant (specific activity 29.83 μCi/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 [14C]aprepitant (3 mg/kg; specific activity 18 μCi/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 = 3–4 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 Vacutainer blood collection tubes at 1, 2, 4, 6, 8, 10, 24, 32, 48, 56, and 72 h after dosing. 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 [14C]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 μl of M(14C)-aprepitant (3 μCi) in 1 ml of ethanol, 3 ml of water, 1 ml of water, and 0.5 ml of methanol. The i.v. samples were mixed with 20 μl of M(14C)-aprepitant (3 μCi) in 1 ml of ethanol, 3 ml of water, 1 ml of water, and 0.5 ml of methanol. The samples were vortexed, incubated for 1 h, and centrifuged for 10 min at 3000g. The supernatants were collected and stored at −70°C until analysis.
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 C_{18} (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 µl of mobile phase (see below), and analyzed by LC-MS/MS.

Alternatively, aliquots of the plasma samples (50 µl) 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 µl of acetonitrile. After vortex-mixing, samples were spun in a centrifuge at 3000g for 10 min. Aliquots (5 µl) 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 [^{14}C]-aprepitant. After centrifugation at 3,000g, the supernatants were transferred to clean tubes and evaporated to dryness under N$_2$. The residues were redissolved in 1 ml of 40% aqueous methanol and 250 to 400 µl 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 µl) were transferred to a 96-well plate and 100 µl of acetonitrile was added. The samples were vortex-mixed and spun in a centrifuge at 3000g for 10 min. Aliquots (25 µl) of the supernatants were subjected to LC-MS/MS analysis.

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 30 min. The supernatants were transferred to clean glass test 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 C_{18} 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/water. The eluents collected after sample loading and during the cartridge wash were combined and evaporated to dryness under N$_2$. 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 test tubes and evaporated to dryness under a stream of N$_2$. The residues were redissolved in 1 ml of 40% aqueous methanol, and aliquots (400 µl) 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 × 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 C_{18} column (4.6 × 50 mm; 5 µm; 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 → product ion combinations of m/z 535→179 and 517→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 µl) or control brain cortex extract (50 µl) was spiked with increasing concentrations of aprepitant (0.25–1000 ng) and 20 or 100 ng of internal standard. A power regression fit, [Y = Y^0 + X^z], 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 $Dose × \frac{AUC}{AUMC}$, where AUMC is the area under the first moment of the product ion combinations of $m/z 179$ and $m/z 161$ was used for quantification of 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 → product ion combinations of m/z 535→179 and 517→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 µl) or control brain cortex extract (50 µl) was spiked with increasing concentrations of aprepitant (0.25–1000 ng) and 20 or 100 ng of internal standard. A power regression fit, [Y = Y^0 + X^z], 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.

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 1×10^5 receptors per cell (Casciere et al., 1992; Fong et al., 1992). Briefly, cells were grown in monolayer culture, detached from the plate using Enzyme-free Cell Dissociation Solution (SPECIALT Media, A Division of Cell & Molecular Technologies Inc., Phillipsburg, NJ) and washed prior to the assay. 125I-Tyr-substance P (0.1 mM; 2200 Ci/nmol; PerkinElmer Life Sciences) and the compound under investigation (in 5 µl of dimethyl sulfoxide) were incubated with 5×10^4 Chinese hamster

<table>
<thead>
<tr>
<th>Dose Route</th>
<th>(AUC)_{f+inf}</th>
<th>C_{max}</th>
<th>t_{1/2}</th>
<th>t_{max}</th>
<th>% F</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/kg</td>
<td>(ng·h/ml)</td>
<td>(ng/ml)</td>
<td>(h)</td>
<td>(h)</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>i.v.</td>
<td>5619 ± 449</td>
<td>1.5 ± 0.1</td>
<td>1.3 ± 0.1</td>
<td>9.7 ± 0.9</td>
</tr>
<tr>
<td>1</td>
<td>p.o.</td>
<td>5151 ± 1660</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>
Concentrations of aprepitant in plasma and brain of ferrets dosed orally at 1 or 2 mg/kg.
The values are the mean ± S.D. of n = 3 or 4 ferrets at each time point.

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Ferret</th>
<th>Time (h)</th>
<th>Plasma (ng/ml)</th>
<th>Brain (ng/g tissue)</th>
<th>Brain-to-Plasma Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>1</td>
<td>237 ± 11</td>
<td>29 ± 2</td>
<td>0.12</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>2</td>
<td>190 ± 68</td>
<td>39 ± 11</td>
<td>0.21</td>
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<tr>
<td>1</td>
<td>3</td>
<td>4</td>
<td>339 ± 67</td>
<td>136 ± 10</td>
<td>0.40</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>10</td>
<td>353 ± 68</td>
<td>160 ± 5</td>
<td>0.51</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>24</td>
<td>213 ± 37</td>
<td>84 ± 10</td>
<td>0.39</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>1</td>
<td>613 ± 222</td>
<td>82 ± 12</td>
<td>0.13</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>2</td>
<td>498 ± 97</td>
<td>91 ± 15</td>
<td>0.18</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>4</td>
<td>401 ± 144</td>
<td>118 ± 31</td>
<td>0.29</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>10</td>
<td>995 ± 167</td>
<td>384 ± 34</td>
<td>0.39</td>
</tr>
</tbody>
</table>

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 ± 37 ng/ml; 2 mg/kg, 266 ± 24 ng/ml) and also in the brain (1 mg/kg, 84 ± 10 ng/g of tissue; 2 mg/kg, 145 ± 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. The concentrations of total radioactivity in 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 ~600 and ~450 ng Eq/ml in plasma 24 and 48 h post dose, whereas levels in the brain were quite similar at ~450 and ~350 ng Eq/g of tissue, respectively. The mean brain-to-plasma ratio of total radioactivity was ~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. Bailie, and S. H. L. Chiu, manuscript submitted for publication), aprepitant and its hydroxy-lactam metabolite M-4, coeluted at 27 min. Therefore, the concentrations of aprepitant were estimated to be ~360 ng Eq/ml (Table 3). This profile was qualitatively identical with ovary cells in 50 mM Tris-HCl, pH 7.5, containing 5 mM MnCl₂, 150 mM NaCl, 0.02% bovine serum albumin, 40 μg/ml bacitracin, 4 μg/ml leupeptin, and 10 μM phosphoramidon. Compound titrations typically consisted of 10 half-log doses up to 0.1, 1, or 10 μM, 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 compound. Incubations were carried out at room temperature until equilibrium (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

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 (V_d), and terminal T_{1/2} values were 1.5 ml/min/kg, 1.3 l/kg, and ~10 h, respectively. The mean oral bioavailability of aprepitant was ~46%, with a range of 35 to 63% (Table 1).
that obtained 24 h postdose (data not shown), and the concentrations of aprepitant were estimated to be \( \approx 530 \text{ ng Eq/ml} \) 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).

TABLE 3

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

<table>
<thead>
<tr>
<th>Ferret</th>
<th>Time</th>
<th>Total Radioactivity</th>
<th>Total Radioactivity as Aprepitant*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Plasma</td>
<td>Brain</td>
</tr>
<tr>
<td>h</td>
<td>ng Eq/ml</td>
<td>ng Eq/g tissue</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>24</td>
<td>610</td>
<td>469</td>
</tr>
<tr>
<td>2</td>
<td>24</td>
<td>570</td>
<td>460</td>
</tr>
<tr>
<td>3</td>
<td>48</td>
<td>393</td>
<td>370</td>
</tr>
<tr>
<td>4</td>
<td>48</td>
<td>493</td>
<td>332</td>
</tr>
</tbody>
</table>

* 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.

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
eight hours after a single oral dose of [14C]aprepitant, the major ferrets in an attempt to verify whether aprepitant is the major component postdose. Subsequently, radiolabeled aprepitant was administered to the levels of aprepitant were high in both plasma and brain at 24 h plasma and the brain tissue of ferrets were analyzed initially for contributed to its excellent duration of action in the ferret. Since the and vomiting for 72 h in all ferrets challenged with cisplatin (5 mg/kg in ferrets. Furthermore, in a model of acute and delayed emesis, inhibited completely the emetic response to cisplatin (10 mg/kg i.v.) observation period, aprepitant (0.3, 1, or 3 mg/kg i.v. or 3 mg/kg p.o.) animal model (Bountra et al., 1993; Tattersall et al., 1994; Beattie et al., 1997; Zaman et al., 2000). Cisplatin administration causes vomiting in their penetration and subsequent binding in the brain (Watson 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 antiepileptics 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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**References**


