ABSTRACT:

A water-soluble phosphoramidate prodrug (L-758,298, compound I) of the potent and selective human Substance P receptor antagonist L-754,030 (compound II) is under development as an i.v. drug for treatment of emesis, migraine, and chronic pain. Compound I undergoes hydrolysis readily to II under acidic conditions. In the studies reported herein, we investigated the stability of I in blood and hepatic subcellular fractions from rats, dogs, and humans as well as the conversion of I to II in rats and dogs after i.v. dosing. Compound I was converted to II rapidly in rat blood but was stable in dog and human blood. However, the conversion was rapid in liver microsomes prepared from dogs and humans. As expected from the results of in vitro studies, the in vivo conversion of I to II was rapid after i.v. dosing of I to rats and dogs. The relative extent of exposure of II after i.v. dosing of I was estimated by comparing the dose-adjusted area under the plasma concentration versus time curve values of II after i.v. dosing of I with those after i.v. dosing of II. In rats, the extent of exposure was estimated to be ~90% and ~100% at 1 and 8 mg/kg, respectively; in dogs, that was ~59% at 0.5 mg/kg. A nonproportional increase in the area under the concentration versus time curve value of II with dose was observed after i.v. administration of I in dogs from 0.5 to 32 mg/kg, suggesting that the elimination of II might have been saturated at higher doses.

In recent years, the successful cloning and expression of human tachykinin receptors have initiated an intensive search for selective nonpeptide receptor antagonists in the pharmaceutical industry using in vitro receptor binding assays (Takeda et al., 1991; Cascieri et al., 1996, 1998). L-754,030 (II) 1 (Fig. 1) is a very potent reversible NK₁ receptor antagonist (M.A. Cascieri, unpublished data) (Kᵦ = 86 pM) of the morpholine series (Cascieri et al., 1997); however, II exhibits limited solubility in aqueous buffers (J.V. Pivinichney and D.A. Levorse, unpublished data; H. Jahansouz and M.L. Bray, unpublished data) (~8 µg/ml at pH 8), which presents a challenge for formulation as an i.v. drug for antiemesis (Rupniak et al., 1997). A prodrug approach was thus taken in chemical synthesis to increase the solubility of II (Benkovic and Sampson, 1971; Anderson et al., 1985), and among prodrug derivatives synthesized, L-758,298 (I), a phosphoramidate prodrug of II, exhibits the best overall profile of a prodrug including solubility and in vivo rate of conversion. Compound I is a relatively weak antagonist for human NK₁ receptor (M.A. Cascieri, unpublished data) (Kᵦ = 4 nM); it is freely soluble in aqueous buffers at a solubility of ~55 mg/ml (free acid equivalents at pH 8) (J.V. Pivinichney and D.A. Levorse, unpublished data; H. Jahansouz and M.L. Bray, unpublished data), which is about a 7000-fold increase in aqueous solubility compared with II. Moreover, compound I can be hydrolyzed to II chemically under mild acidic conditions.

1 Abbreviations used are: L-754,030 (II), [2R]-[(1R)-3,5-bis(trifluoromethyl)pheny1](ethoxy)-3-(4-fluorophenyl)-4-(3-o xo-4H,1,2,4-triazolo)methylmorpholine; L-758,298 (I), [(2R)-{(1)-3,5-bis(trifluoromethyl)pheny1}(ethoxy)-3-(4-fluorophenyl)-4-(3-1,2,4-triazolo)-5-oxo-5-oxo-4H,1,2,4-triazolo)methylmorpholine; Compound III, [(2R)-{(3,5-bis(trifluoromethyl) benzoyl)}(3S)-phenyl]-4-(3-[1-phosphonyl]-5-oxo-4H,1,2,4-triazolo)methylmorpholine; Compound IV, [(2R)-{(1R)-3,5-bis(trifluoromethyl)pheny1}(ethoxy)-3-(3)-(5-oxo-1H,4H,1,2,4-triazolo)methylmorpholine; AUC, area under the plasma concentration versus time curve; PEG400, polyethylene glycol; SD, Sprague-Dawley; LC-MS/MS, liquid chromatography-tandem mass spectrometry; SRM, selected reaction monitoring; V₅₀, volume of distribution at steady state.

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Metabolic Stability of I in Subcellular Fractions of Dog and Human Liver. All liver microsomes and cytosolic fractions were prepared using the following procedure. Thawed livers were homogenized with 2 volumes of 50 mM Tris-buffer (pH 7.5) containing 1.15% KCl. For microsomal and cytosolic preparations, the homogenate was centrifuged for 20 min at 9000g and the resulting supernatant was centrifuged for 60 min at 105,000g. The resulting cytosolic fractions were recentrifuged at 105,000g for 60 min. Fractions of cytosol were aliquoted into small tubes and stored at −70°C. Subsequently, the microsomal pellets were washed with 10 mM EDTA containing 1.15% KCl and were centrifuged at 105,000g for 60 min. Washed microsomes were resuspended in 10 mM potassium phosphate buffer (pH 7.4) containing 250 mM sucrose, aliquoted into small tubes, and stored at −70°C. Protein concentrations were determined by a modified Lowry assay (Smith et al., 1985). The specific cytochrome P-450 content in each microsomal preparation was measured as described by Omura and Sato (1964).

Frozen cytosolic and microsomal fractions (stored at −70°C) of dog (preparation 116) and human livers (Nos. 113, 115, and 118) were used in the in vitro studies. Human liver was obtained from Professor W.G. Levine (Department of Molecular Pharmacology, Albert Einstein College of Medicine, Yeshiva University, Bronx, NY). The organ donors were a 46-year-old male (113), a 59-year-old male (115), and a 43-year-old male (118), all with no known drug history. The microsomal or cytosolic fractions from human liver were combined with equal amounts of proteins from three subjects (Nos. 113, 115, and 118).

Compound I (final concentration 8.1 μM) was incubated in triplicate with cytosolic or microsomal fractions (0.5 mg/ml) of dog or human liver at 37°C for 15, 30, 60, and 120 min. The reaction was quenched by the addition of acetonitrile (0.5 ml) and two internal standards, kept on ice, followed by the addition of 1.7 ml of water (final concentration of solvent ~18%). The mixture was processed immediately by solid-phase extraction and analyzed by LC-MS/MS for I and II simultaneously. The storage time for samples was less than 1 week.

Dose Preparation. The doses for compound I were prepared by dissolving I (the bis-N-methyl-D-glucamine salt, molecular weight 1004.9) in a solution of lactose (50 mg/ml), potassium carbonate (1.38 mg/ml), citric acid monohydrate (0.85 mg/ml), and sodium chloride (4 mg/ml) (pH 7.0). Doses were filtered through a 0.45 μM filter before dosing. The doses for II were prepared in a solution of ethanol/propanol glycol/water (15:60:25, v/v/v) or in a solution of ethanol/PEG400/water (20:60:20, v/v/v). Doses of II were prepared and stirred constantly at 25°C overnight before dosing.

Pharmacokinetics in Rats. Male SD rats were obtained from Charles River Breeding Laboratories (Wilmington, MA) or Taconic Laboratories (German-town, NY). They were housed under standard conditions and were maintained under a 12-h light/dark cycle in the Laboratory Animal Resources facilities, Merck Research Laboratories, Rahway, NJ. They were allowed access to commercial rodent chow and water ad libitum. Rats were fasted overnight before dosing and then until 1 h after dosing. Water was allowed ad libitum during the fasting period.

Rats were cannulated at the femoral vein for serial bleeding and jugular vein for dosing. The b.w.t. of individual rats, ranging from 0.3 to 0.4 kg, were determined on the morning of the study. Four male rats per group were dosed i.v. with I or II by bolus injection into the jugular vein at 1, 8, or 25 mg/kg body weight or at 0.2, 2, or 5 mg/kg b.wt., respectively. After dosing, 0.5-ml specimens of blood were collected by serial sampling from the femoral cannula at 2 to 3, 5, 15, and 30 min, and 1-ml specimens of blood were drawn at 1, 2, 4, 6, 8, 10, 14, 20, 30, 48, and 72 h. After 1 h, blood was replaced with an equal volume of sterile heparinized saline and donor blood.

Pharmacokinetics in Dogs. Six male Beagle dogs were housed under standard conditions and were maintained under a 12-h light/dark cycle in the Laboratory Animal Resources facilities, Merck Research Laboratories, Rahway, NJ. They were allowed access to water ad libitum. Dogs were fasted overnight before dosing and then until 4 h after dosing. Water was allowed during the fasting period. The b.w.t. of the individual dogs ranged from 9.6 to 14.3 kg.

The dogs were dosed i.v. with I or II by bolus injection into the cephalic vein via an indwelling vascular catheter at 0.5 or 2 mg/kg b.wt. or at 0.2, 0.5, and 2 mg/kg body weight, respectively. At the 32 mg/kg dose of I, the dogs were dosed by infusion into the cephalic vein via an indwelling vascular catheter for 45 s at 3.2 mg/kg followed by a saline flush for 15 s. After dosing, heparinized blood samples

### Tables

| Compounds |  
| --- | --- |  
| I | CH₃ | F |  
| II | CH₃ | F |  
| III | H | H |  
| IV | CH₃ | H |  

**Fig. 1.** Structures of compounds I, II and two internal standards, III and IV.
Sample Preparation. To minimize enzymatic hydrolysis of I in blood during sample preparation, 12.5 to 125 μl of 200 mM vanadate in saline (final concentration 5 mM) was added immediately to fresh blood samples, which were kept on ice. Plasma was obtained by centrifugation at 4°C within 30 min. Each plasma sample (0.2 ml for rats and 0.5 ml for dogs) was added to a test tube containing 60 to 250 ng of III and 60 to 250 ng of IV, the respective internal standards for I and II, followed by 1.7 ml of water and 0.5 ml of acetonitrile. The mixture then was loaded onto a Varian BondElut C18 cartridge (500 mg). The cartridge was washed with ~6 ml of water followed by elution with ~3 ml of methanol. The methanol eluant was evaporated to dryness and stored at 4°C (storage time less than 1 week) before analysis using a LC-MS/MS assay.

Quantification of I and II in Plasma by LC-MS/MS. The quantification of I and II in plasma was performed on a SCIEX API III tandem mass spectrometer using the ionspray interface. The collision gas used for collision-induced dissociation was argon. The HPLC system consisted of two Shimadzu 10AD pumps, SCL-10A controller and SIL-10A autoinjector. Chromatographic separation was performed on a BDS-Hypersil C18 column (4.6 mm × 250 mm) using a mobile phase consisting of 72% acetonitrile and 28% 10 mM ammonium acetate (adjusted to pH 7.4 with HPLC grade triethylamine). The flow rate was 1.05 ml/min and the effluent was split such that 5% of the flow entered the ionspray interface. In this system, I and its internal standard, III, eluted at approximately 2.2 min; II and its internal standard, IV, eluted at approximately 4.4 and 4.6 min, respectively.

A two-period approach was used due to the significant difference in retention time and peak shape of the two sets of compounds. The first period used a dwell time for selected reaction monitoring (SRM) of 475 ms with a 10-ms pause; the dwell time for the second period was 400 ms with a 5-ms pause. Positive ion detection was used during data acquisition for I and its internal standard; however, both positive and negative ion detection were used in different cases for the detection of I and its internal standard.

A two-period SRM assay (method 1) was developed, with negative ion detection for I and III in the first period, followed by positive ion detection for II and IV in the second period. In the first period, the negative precursor/product ion pairs at m/z ~ 613/79 and 581/79 were used for quantification of I and III, respectively; in the second period, the positive precursor/product ion pairs at m/z ~ 535/277 and 517/259 were used for quantification of II and IV, respectively.

Alternatively, a two-period positive ion SRM assay (method 2) was used subsequently to eliminate cross talk between the channels. In the first period, the precursor/product ion pairs at m/z ~ 615/277 and 583/259 were used for quantification of I and III, respectively; in the second period, the precursor/product ion pairs at m/z ~ 535/179 and 517/161 were used for quantification of II and IV, respectively.

Two standard curves were generated for each assay by plotting the peak area ratio of the response for either I or II to that of its respective internal standard versus the amount of compound added to the control plasma sample. The range of concentrations used to define the standard curve was dependent on the expected plasma levels, and the amount of internal standard used was chosen to be roughly at the mid-point of the standard curve. An average of three replicates at each concentration over the entire range was used in rat and dog plasma to establish the standard curves. A power fit regression [Y = kX^b] was used to quantify the unknowns. The limits of quantification for I were 6.25 to 62.5 ng/ml of rat plasma and 25 to 100 ng/ml of dog plasma; limits of quantification for II were 6.25 to 12.5 ng/ml of rat plasma and 5 to 20 ng/ml of dog plasma.

Calculations of Pharmacokinetic Parameters. The area under the plasma concentration versus time curve (AUC) was determined by the UNICUE program with linear trapezoidal interpolation in the ascending slope and logarithmic trapezoidal interpolation in the descending slope (Yeh and Small, 1989). The portion of the AUC from the last measurable concentration of II in plasma to infinity was estimated by C₀/λ, where C₀ represents the last measurable concentration in plasma and λ is the terminal rate constant determined from the plasma concentration versus time curve by linear regression at the elimination phase of the semilogarithm plot. Concentrations below the quantifiable level were treated as zero for the purpose of calculating mean concentrations.

Results

Validation of a Procedure for Blood Sample Preparation. To determine whether vanadate can prevent the ex vivo conversion of prodrug (compound I) to II in blood samples, compound I (25 ng/ml–25 μg/ml) was added to rat or dog blood in the presence of saline or vanadate (5 mM, an inhibitor for alkaline phosphatase) and the formation of II was quantified by LC-MS/MS. No significant increase of concentrations of II was detected in rat blood while stored on ice up to 1 h (data not shown). When rat blood samples were treated with saline, 18 to 1800 ng/ml of II (or 10–13% of I added) was detected in plasma. In comparison, only 16 to 170 ng/ml of II (or 1–3% of conversion) was detected when vanadate was added immediately to rat blood samples after mixing with I at various concentrations to rat blood (data not shown). In dog blood, the ex vivo conversion of I to II was low (~1–3%), and the addition of vanadate did not achieve significant reduction of the conversion.

In Vitro Conversion of Prodrug I to II. When the prodrug, compound I, was added to blood at concentrations of 1 and 10 μg/ml, incubations were carried out immediately and processed as described in Materials and Methods. Vanadate was included in the sample preparation procedure after incubation to minimize the ex vivo conversion of I to II. The concentrations of both compounds in plasma at selected time intervals were determined simultaneously by LC-MS/MS. The conversion of I to II in rat, dog, or human blood was expressed by plotting the increase in molar concentration of II against the decrease in molar concentration of I in plasma versus time (Fig. 2). The concentrations of I in rat plasma diminished rapidly with a half-life of ~30 min when 10 μg/ml of I was added (Fig. 2A). Similar results were obtained from incubations with I at 1 μg/ml (data not shown). Compound I was more stable in dog blood than in rat blood with a half-life (in dog blood) of ~230 and ~350 min for I and 10 μg/ml, respectively; only 20 to 30% of I was converted to II during the 2-h incubation period (Fig. 2B). Compound I was very stable in human blood with less than ~15% conversion observed during the 2-h incubation period (Fig. 2C).

Metabolic Stability of Compound I in Subcellular Fractions of Dog and Human Liver. Compound I (5 μg/ml) was incubated with microsomal and cytosolic fractions followed by simultaneous quantification of compound I and its dephosphorylated product (compound II) by LC-MS/MS. The decline of its concentrations with the concomitant increase of the levels of compound II with time is shown in Figs. 3 and 4. In dog liver microsomes, the conversion was nearly complete in 30 min with only ~5% of the substrate remaining at that time. The conversion of I to II was rapid in human liver microsomes such that only ~2% of I was detectable after the first time point (15 min); the conversion was complete at 30 min. The rate of conversion was slower in cytosolic fractions for both species (55% remaining at 30 min in dog; 78% remaining in human). After the 2-h incubation period, conversion by hepatic cytosolic fractions was nearly complete (~6% remaining) in dog, and ~65% complete (~35% remaining) in human.

In Vivo Conversion of I in Rats. The conversion of I to II was studied in rats dosed i.v. at 1, 8, and 25 mg/kg b.wt. The concentrations of I and II in plasma samples were determined simultaneously by LC-MS/MS (method 2). The concentrations of intact I were quantifiable only at the earliest time points (2–5 min) for the lowest dose, and up to 1 h for the highest dose. After the 25 mg/kg dose, the concentrations of I in plasma declined rapidly from 1117 to 75 ng/ml between 3 and 60 min (data not shown).
The concentration of II in plasma was maximal at the first sampling time point (2–3 min) after i.v. dosing of I at all three dose levels (Fig. 5). Ten hours after dosing with I at 1, 8, and 25 mg/kg, the plasma concentrations of II were 14, 140, and 620 ng/ml, respectively; at 24 h, the levels were measurable only in one rat given the highest dose (25 mg/kg).

A near proportional increase in the AUC values of II with dose was observed after i.v. administration of I at 1 and 8 mg/kg (Table 1). At 25 mg/kg, the AUC value increased 4-fold over that at 8 mg/kg. The elimination curve (Fig. 5) showed a convex phase (2–10 h) at the highest concentration, and the factors contributing to this phenomenon are uncertain.

Pharmacokinetics of II also were studied in rats dosed i.v. with II at 0.2, 2, and 5 mg/kg. As shown in Fig. 6, a steady decline of II was observed after all three doses. The kinetics appeared to be linear over the dosing range, with an increase in plasma AUC values nearly proportional to the 10- and 25-fold increase in dose from 0.2 to 2 and 5 mg/kg, respectively. Plasma clearance was 15 ml/min/kg, the volume of distribution at steady state (V_{dss}) was 3 liters/kg, and the terminal half-life was 3 h (Table 1).

The AUC values of II in rat plasma after dosing of I or II were compared to estimate the relative extent of exposure of II. Due to the large differences in the molecular weights (I, mw 1004.9 salt; II, mw 534.4 free base), the AUC values were normalized to per mole of dose. When rats were dosed with I at 1 mg/kg, the relative extent of exposure of II in plasma was estimated to be 91% by comparison with the average AUC calculated from the 0.2 and 2 mg/kg i.v. doses of II. Similarly, when the dose was 8 mg/kg, relative extent of exposure of II in plasma was 100%, estimated by comparing the average AUC values calculated from the 2 and 5 mg/kg i.v. doses of II. At the highest dose (25 mg/kg), the relative extent of exposure was not determined because plasma concentrations and AUC values of II exceeded those from the highest i.v. dose (5 mg/kg) of II and therefore could not be compared with one another.

**In Vivo Conversion of I in Dogs.** The in vivo conversion and pharmacokinetics of I were studied in beagle dogs dosed i.v. at 0.5, 2,
and 32 mg/kg. The concentrations of I and II in plasma samples were determined simultaneously by LC-MS/MS (method 1). After i.v. dosing of prodrug I to dogs, conversion to II was very rapid and intact I levels were measurable only at 2 to 3 min for the two lower doses. For the highest dose (32 mg/kg), concentrations of I ranged from 32 to 77 mg/ml (mean 57 mg/ml) and declined rapidly to 1.3 mg/ml by 5 to 6 min. The corresponding concentrations of II were 20 and 17 mg/ml, indicating a much slower decline compared with compound I. For all three doses studied, II levels in plasma were the highest at the first time point and declined slowly thereafter (Fig. 7). At 24-h post dosing, plasma levels of II were 7 to 155 ng/ml for the two lower doses; at 72 h, the levels of II (~2.6 µg/ml) remained in the quantifiable range only in dogs treated with the highest dose.

A nonproportional increase in AUC values of II with dose was observed after i.v. administration of I. For the doses of 2 and 32 mg/kg, the AUC value was estimated only through 48 or 72 h due to nonlinear kinetics and the uncertainty involved in extrapolation from the last measurable time point to infinity. The AUC value of II exhibited an 8-fold increase as the dose increased 4-fold from 0.5 to

### Table 1

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>1</th>
<th>8</th>
<th>25</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dose</strong> (µmol/kg)</td>
<td>1.00</td>
<td>7.96</td>
<td>24.88</td>
</tr>
<tr>
<td><strong>AUC of II (ng/h/ml)</strong></td>
<td>568 ± 121</td>
<td>6249 ± 1373</td>
<td>22392 ± 1158</td>
</tr>
<tr>
<td><strong>Exposure of II (%)</strong></td>
<td>~91%</td>
<td>~100%</td>
<td>NCb</td>
</tr>
<tr>
<td><strong>Dose of Compound II (mg/kg)</strong></td>
<td>0.2</td>
<td>2</td>
<td>5</td>
</tr>
</tbody>
</table>

a Exposure of II was estimated by comparing the AUC values of II in plasma after dosing of I with that after dosing of II to rats. At 1 mg/kg, the relative exposure was calculated from the 0.2 and 2 mg/kg i.v. doses of II. At 8 mg/kg, the relative exposure was calculated from the 2 and 5 mg/kg i.v. doses of II. Calculations were normalized for dose.
b NC: not calculated.

c For T½ calculations, the terminal rate constant was estimated by linear regression of the last three to four data points: 6 to 24 h for 5 mg/kg, 6 to 10 h for 0.2 mg/kg, and 6 to 24 h for 2 mg/kg.

Male SD rats (n = 4) were i.v. dosed with II (mw 534.4 free base) prepared in a solution of ethanol/propylene glycol/water (15:60:25, v/v/v) or in a solution of PEG400/water/ethanol (60:20:20, v/v/v). Plasma samples (0.2 ml) were processed by solid-phase extraction and analyzed for II by LC-MS/MS. The limit of quantification for II was 0.5 to 1.25 ng/ml.
quantification for I and II were 25 to 100 and 5 to 20 ng/ml, respectively. Plasma samples (0.5 ml) were processed by solid-phase extraction and analyzed simultaneously for I and II by LC-MS/MS. The limit of quantification for II was 1.0 to 2.5 ng/ml.

Three male beagle dogs were dosed i.v. with I (bis-N-methyl-o-glucamine salt; mw 1004.9 as salt) prepared in a solution of lactose, potassium carbonate, citric acid monohydrate, and sodium chloride (pH 7.0). Plasma samples (0.5 ml) were processed by solid-phase extraction and analyzed simultaneously for I and II by LC-MS/MS with III and IV as the respective internal standards. The limits of quantification for I and II were 25 to 100 and 5 to 20 ng/ml, respectively.

2 mg/kg, and a greater than 40-fold increase between 2 and 32 mg/kg (Table 2). These results suggest that the elimination of II might have been saturated at the higher doses.

Pharmacokinetics of II also were studied in dogs dosed i.v. with II at 0.2, 0.5, and 2 mg/kg b.wt. A steady decline in the concentration of II in plasma was observed after i.v. dosing at 0.2 or 0.5 mg/kg (Fig. 8). The kinetics appeared to be linear over the 0.2 to 0.5 mg/kg dosing range with a nearly proportional increase in AUC values when the dose increased 2.5-fold. The half-life was 6 to 7 h, plasma clearance was ~2.5 ml/min/kg, and the Vd was ~1 liter/kg for the two lower doses (Table 2). When plasma concentrations from the 2 mg/kg dose were plotted as a function of time, a convex phase was detected, indicating that the rate of elimination of II in this species at this dose (Fig. 8). The increase in the plasma AUC over the 0.2 mg/kg dose was ~10-fold, and the decrease in clearance was ~2-fold when the dose was increased from 0.5 to 2 mg/kg (Table 2). These results suggest that the elimination of II may have been saturated at the 2 mg/kg dose. The relative extent of exposure of II in plasma was ~59% at the 0.5 mg/kg dose, as determined by comparing the plasma AUC value with the average AUC value obtained from i.v. doses of II at 0.2 to 0.5 mg/kg, at which linear kinetics is followed. Due to nonlinear kinetics at high concentrations, the relative extent of exposure of II in plasma at the higher doses (2 and 32 mg/kg) could not be determined (Table 2).

**Discussion**

To establish assay methods for I in plasma, a procedure was validated for the preparation of blood samples from rats and dogs. Vanadate, an inhibitor of alkaline phosphatase, was used to inhibit the ex vivo hydrolysis of I to II by alkaline phosphatase (Hagerstrand et al., 1976; Hatoff and Hardison, 1982). The addition of vanadate was effective in reducing the ex vivo conversion of I to II from 10 to 13% to 1 to 3% in rat blood, however, its use did not eliminate the conversion of I to 3% in dog blood. Therefore, under the sample preparation conditions used in our studies, about 1 to 3% conversion of I to II is expected to take place in both rat and dog blood.

The conversion of compound I, a phosphoramidate prodrug, to the potent NK\(_1\) receptor antagonist, II, was essential for its in vivo biological activity as a prodrug. This conversion was studied in rat, dog, and human blood. The conversion was rapid in rat blood, somewhat slower in dog blood, and very slow in human blood. The conversion of I to II was further investigated in subcellular fractions from dog and human liver. Results indicate that compound I was hydrolyzed rapidly in human and dog liver microsomes. Based on the above in vitro results, it is anticipated that the conversion of I to II also will be rapid in preclinical species (rat and dog) and humans, when compound I is administered i.v.

As expected, the conversion of I to II in vivo was rapid in rats. A near proportional increase in the AUC values of compound II with increase in doses of I was observed after i.v. administration to rats at 1.8, and 25 mg/kg. Pharmacokinetics of compound II appeared to be linear in rats when it was dosed at 0.2, 2, and 5 mg/kg. As shown in Table 1, the relative extent of exposure of II in plasma after i.v. dosing...
of I to rats was ~91 to 100% of that after i.v. dosing of II at two lower doses. The results indicate that I is a suitable prodrug that is effectively converted to II in vivo in the rat.

Likewise, the conversion of I to II in vivo was rapid in dogs such that the level of I was not quantifiable 15 min post dosing of compound I at two dose levels studied. However, a nonproportional increase in AUC values of II with dose was observed after i.v. administration of I at 2 and 32 mg/kg, suggesting the elimination of II might have been saturated at the high doses (Table 2). In comparison, pharmacokinetics of II appeared to be linear in dogs from 0.2 to 0.5 mg/kg. Deviation from linear kinetics was observed at 2 mg/kg, in that the apparent plasma clearance of II was decreased (from 2.3–0.9 ml/min/kg). The relative extent of exposure of II in plasma was less than 59% at the 0.5 mg/kg dose. Due to nonlinear kinetics observed in dogs at high concentrations, the relative extent of exposure of II after i.v. dosing of I at higher doses could not be determined. Taken together, the results suggest that I was effectively converted to II in vivo despite an apparent saturation of the elimination of II.

Based on the results of in vitro stability of I, it is anticipated that the conversion of I to II will be rapid in preclinical species (rat and dog) and humans when I is administered i.v. as illustrated in this report, the conversion of I to II in vivo was rapid in rats and dogs, therefore, it is feasible to predict that the conversion of I to II will be rapid in humans as well. This remains to be seen in clinical trials of I in the near future.

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